Plant Biosecurity Cooperative Research Centre

Final Report

Managing myrtle rust in Australia

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1. Executive Summary

Austropuccinia psidii, commonly known as myrtle, eucalyptus and guava rust, has long been considered a significant threat to Australian plant industries and ecosystems. In April 2010, *A. psidii* was detected for the first time in Australia on the central coast of New South Wales. The impact *A. psidii* would have on plant industries reliant on Myrtaceae and native species was unknown. This project aimed to deliver a standardised method for assessing myrtle rust susceptibility and impact on Myrtaceae in Australia, and in doing so, identify species and plant communities at greatest risk, while at the same time identifying possible management options to minimise the impact of the disease on plant industries and the environment.

The geographic distribution of *A. psidii* in Australia continues to expand with detections now extending from Tasmania, along the entire east coast of Australia as far north as Bamaga at the tip of Cape York Peninsula and most recently in the Tiwi Islands and Darwin in the Northern Territory. Reports from west of the Great Dividing Range still remain low and restricted to nurseries and urban gardens. The current host range in Australia includes 347 species from 57 different genera, of which 242 species have been identified from infections under field conditions. A total of 180 species have now been rated for susceptibility to *A. psidii* and detailed impact data for more than 20 species provides important information for species conservation planning. *Austropuccinia psidii* infection has been identified on flowering and fruiting structures of 32 species of Myrtaceae. We also provide additional evidence of the effects repeated infection indirectly has on flower and fruit production.

In just the short time that *A. psidii* has been established in Australian natural ecosystems, we have observed significant damage and tree mortality. There are few exotic diseases in Australia that threaten such a wide range of Australian flora. Our studies, while currently limited, have shown that *A. psidii* is severely affecting key species in natural ecosystems, and likely to be significantly affecting a much wider range of species. We have identified significant impacts caused by *A. psidii* on threatened species with restricted natural ranges as well as those with a broad native range and considered widespread. Outcomes from this study have resulted in applications to have *Rhodomyrtus psidioides* and *Rhodamnia rubescens* listed as Critically Endangered species.

Impacts on plant communities have now become more apparent with changes in host density due to myrtle rust related dieback within species rich environments likely to impact on the long term survival of species. *Austropuccinia psidii* has caused significant disturbance in wet sclerophyll environments where Myrtaceae dominate the rainforest understorey. Significant dieback caused by repeated *A. psidii* infection has seen once dominant species in severe decline with little evidence of potential for regeneration. Impacts on keystone species such as *Melaleuca quinquenervia* include tree death, decline in tree vigour and reduced flowering rates with additional decline found to be associated with interactions with insect damage. Using glasshouse screening methods developed in this project we were able to study populations of *M. quinquenervia* and other broad-leaved *Melaleuca* spp. providing a better understanding of resistance patterns as well as identifying populations at greatest risk of significant impact.

The impacts of *A. psidii* on plant industries reliant on Myrtaceae are variable, with the nursery and lemon myrtle industries most affected. Our studies have helped identify host species and their relative susceptibility levels to aid in species selection for future commercial development. Additionally, we have examined resistance/susceptibility patterns in *Eucalyptus* and *Corymbia* species of commercial significance with selections at the family level possible. However, it was also identified that resistance to *A. psidii* often resulted in susceptibility to endemic pathogens.

Our studies have demonstrated, at a species and plant community level, the potential for *A. psidii* to negatively affect Australia's biodiversity in the short- and long-term. Continued monitoring programs are required to identify species and plant communities at greatest risk as well as identifying resistance for potential use in regeneration programs. The implementation of a disease screening and tree breeding program may be required for some species as it may be the case that without human intervention, regaining lost genetic diversity within these species populations may not be possible. To date, only two species have been recommended for legislative listing. We recommend that other species of Myrtaceae be considered and that conservation strategies be developed to ensure help manage myrtle rust in Australia.

2. Introduction

Austropuccinia psidii, previously *Puccinia psidii* and commonly known as myrtle, eucalyptus and guava rust, has long been considered a significant threat to Australian plant industries and ecosystems. In April 2010, *A. psidii* was detected for the first time in Australia on the central coast of New South Wales. Globally *A. psidii* is spreading rapidly impacting on Myrtaceae of both commercial and ecological significance. It was first reported from Brazil in 1884 infecting *Psidium guajava* (Winter 1884). It has since been identified from the USA (Hawaii, Florida, California) (Marlett & Kimbrough 1979; Uchida *et al*. 2006; Mellano, 2006), New Caledonia (Giblin 2013; Machado *et al*. 2015), South Africa (Roux *et al*. 2013), Indonesia (McTaggart *et al*. 2016) and more recently Singapore (du Plessis *et al*. 2017). The implications of this global spread are still largely undetermined.

Although originally described from Brazil, it can be assumed that *A. psidii* is endemic to neighbouring countries. Its detection in countries in South and Central America and the Caribbean can be tracked via publication reporting (Simpson *et al*., 2006): Paraguay (1884), Uruguay (1889), Ecuador (1891), Colombia (1913), Puerto Rico (1913), Cuba (1926), Dominican Republic (1933), Jamaica (1933), Venezuela (1934), Argentina (1946), Dominica (1948), Trinidad and Tobago (1951), Guatemala (1968), El Salvador (1987) and Costa Rica (1998). It is likely that *A. psidii* was present in El Salvador and Costa Rica for some time prior to being reported. In North America, *A. psidii* was reported in Florida in 1977 (Marlatt & Kimbrough, 1979), Mexico in 1981 (Léon-Gallegos & Cummins, 1981), Hawaii in 2005 (Killgore & Heu, 2007) and California in 2006 (Mellano, 2006), although likely present there prior to 2006. The introduction into California is likely to have been from the live plant trade or foliage trade from Florida, based on data on interceptions and nursery detections (Zambino & Nolan, 2012). The introduction to Hawaii is also likely to have been from the live plant trade or foliage trade (Loope *et al*., 2007; Loope & Rosa, 2008), most likely from mainland USA. Currently *A. psidii* is restricted to the south-east of Florida, has a restricted distribution in California, but has spread throughout the islands in Hawaii.

In 2007, *A. psidii* was detected on rooted cuttings of *Metrosideros polymorpha* in Japan (Kawanishi *et al*. 2009), again most likely imported in the live plant trade. No further reference to its distribution in Japan has been found however. In 2011, *A. psidii* was reported from southern China from collections in 2009 (Zhuang & Wei 2011). In 2010, *A. psidii* reached Australia (Carnegie *et al*. 2010), and is now widespread along the east coast (Carnegie & Cooper 2011; Pegg *et al*. 2014). There is no indication of the pathway of entry into Australia. In 2013, *A. psidii* was reported from both South Africa, where its distribution was originally restricted (Roux *et al*. 2013) but is now considered widespread (Roux et al. 2016). In New Caledonia (IPPC 2013) *Austropuccinia psidii* has spread throughout the islands (DAVAR Nouvelle-Calédonie 2014). More recent detections include Indonesia (McTaggert *et al*. 2015), Sumatra (M. Purcell pers. Comm.), (impacting on *Rhodomyrtus tomentosa* within its native range), and street plantings in Singapore (du Plessis et al. 2017).

Rating systems

There have been various approaches to rating disease incidence and severity, in both seedling/sapling glasshouse situations and in seedling/sapling/mature field scenarios. Complexities arise due to the huge range of differing leaf morphologies and subsequent differing symptom presentations. Developing a standardised rating system is difficult as a mature tree with large leaves and a dense canopy is very different to a small shrub with tiny leaves and a sparse canopy. Early pustule symptoms can range from tiny pale yellow dots on a leaf to large waxy vivid yellow clusters on others. Older symptoms are an ash grey to brown lesion resembling damage caused by chewing insects and, thus, difficult or impossible to differentiate with the naked eye.

In Hawaii, disease indexes for rust on *Syzygium jambos* (Anderson & Uchida 2008) and on *Metrosideros polymorpha* (Uchida *et al*. 2008) were developed to show symptoms of disease with varying degrees of severity. These were done to assist with the identification and reporting of disease in a standard way. A disease index category was developed for *Syzygium jambos* using the following:

- 0 - No symptoms found, or symptoms found but no rust spores confirmed
- 1 – 1 to 5 spots, yellow or white urediniospores confirmed
- 2 – 3 to 7 large or about 10 to 15 small spots, with a moderate level of disease; yellow or white urediniospores confirmed
- 3 - Severe disease levels; stems with pustules and/or no leaves
- 4 - Dead apical tips and numerous defoliated tips

In Australia, Morin *et al*. 2012 carried out inoculation experiments in the glasshouse and rated disease incidence and severity on the 3 most affected leaves. They devised a 1 to 5 rating system where 1 indicated no visible symptoms and 5 indicated abundant sporulation with 25% of the leaf surface covered by fully-developed uredinia.

Also in Australia, Sandhu *et al*. 2013 developed a scale based on different infection types produced by a range of highly resistant to highly susceptible genotypes. The rating system was:

- **Highly resistant** – no visible signs of infection
- **Resistant** – mild hypersensitivity/flecks/dark flecks/necrosis
- **Moderately resistant** – Restricted pustule/dark grey surrounding/chlorosis/necrosis
- **Moderately susceptible** - Small to medium sized pustules low in frequency and may be with some chlorosis present
- **Susceptible** - Fully developed pustules on leaves and medium to high in frequency
- **Very susceptible** - Abundance of fully developed pustules on leaves, twigs and buds

Strains of Austropuccinia psidii

Austropuccinia psidii is native to South America and has not co-evolved with Myrtaceae in Australia, therefore, it cannot be determining impacts on species and ecosystems and associated industries is difficult. Usually in a co-evolved host/pathogen interaction, there will be minimal impact to ensure the survival of the pathogen. At the time of writing this report, only one strain of *Austropuccinia psidii* is present in Australia but there are up to seven potential strains/biotypes of the fungus described worldwide. It cannot be predicted whether or not other strains of the fungus will be more or less virulent than the strain found here now. It is generally accepted that not all strains of the fungus will affect all hosts the same (da Silva *et al*. 2013).

Multiple strains of *A. psidii* have been identified from Brazil. A single strain has been recorded on multiple myrtaceous hosts in Hawaii since 2005 but only causes mild symptoms on the widespread and significant native ‘ohia (*Metrosideros polymorpha*). Of five Brazilian strains tested on ‘ohia seedlings in Brazil, three strains were found to be highly virulent and two strains less so (da Silva *et al.* 2013). No resistance was observed. On the other hand, the strain found in Hawaii has been highly virulent on the non-native *Syzygium jambos* populations, causing significant mortality. This strain of the fungus is not reported in Brazil and unfortunately couldn’t be compared in their studies. In 2016, Roux *et al*. reported that the isolate detected in South Africa is different from what is present in Australia and is in fact unique. The isolate from Indonesia was identified as being identical to what is present in Australia (McTaggart *et al.* 2015.).

Host range and spread

Austropuccinia psidii affects plants in the Myrtaceae family, which includes many Australian natives including eucalypt, paperbark, bottlebrush, tea tree and lilly pilly. The fungus spread rapidly along the east coast and in December 2010 was found in Queensland followed by Victoria a year later. More recently (January 2015) myrtle rust was detected in Tasmania and the Northern Territory. *Austropuccinia psidii* was initially restricted to the south-eastern part of Queensland but spread as far north as Mossman and then to Bamaga at the tip of Cape York Peninsula. In Queensland 48 species of Myrtaceae are considered highly or extremely susceptible to the disease (Pegg *et al*. 2014). The impact of *A. psidii* on individual trees and shrubs has ranged from minor leaf spots, foliage, stem and branch dieback to reduced fecundity. Tree death, as a result of repeated infection, has been recorded for *Rhodomyrtus psidioides*. Rust infection has also been recorded on flower buds, flowers and fruits of 28 host species.

The perceived threat to Australian biodiversity and industry is now being realised. Severe damage to key species has been observed in native environments, including rainforest understorey species such as *Rhodamnia rubescens* and *Rhodomyrtus psidioides* and the keystone wetland species *Melaleuca quinquenervia* (Carnegie & Cooper 2011; Carnegie & Lidbetter 2012; Pegg *et al.* 2014). The essential oil industry is being impacted, particularly lemon myrtle (*Backhousia citriodora*), and although *A. psidii* has been found in eucalypt plantations, the forest industry has not yet seen significantly damage (Carnegie 2014). The disease has seen an increase in reliance on regular chemical applications in the nursery industry and in some cases resulted in a removal of the more susceptible species from production and on-sale.

Austropuccinia psidii is now identified from a range of native forest ecosystems including coastal heath (*Austromyrtus dulcis*, *Homoranthus* spp.), coastal and river wetlands (*Melaleuca quinquenervia*, *M. viridiflora*), sand island ecosystems of Moreton, Stradbroke and Fraser Islands, and littoral, montane, subtropical and tropical rainforests (*Syzygium* spp., *Rhodamnia* spp., *Rhodomyrtus* spp.) (Pegg *et al*. 2014). The disease is prevalent in urban and peri-urban environments around major cities and towns, commonly reported from botanic gardens and nature reserves with disease impacts ranging from minor leaf spots to severe dieback and infection, and premature senescence of flowers and fruits (Pegg *et al*. 2014).

Austropuccinia psidii has been listed as a key threatening process to the natural environment in NSW (http://www.environment.nsw.gov.au/determinations/exoticrustfungiFD.htm), and was recently nominated as a key threatening process at the federal level (Makinson 2014). However, there is a paucity of studies on the impact of *A. psidii* in the native environment in Australia. This is surprising considering the heightened publicity *A. psidii* received prior to arriving and during the emergency response, and the perceived threat to native Myrtaceae and biodiversity.

Symptoms of infection by *A. psidii* range from minor leaf spots to severe foliage and stem blight, as well as infection of flowers and fruit of some species. Of the highly or extremely susceptible species, several have importance economically, e.g. *Backhousia citriodora* and *Chamelaucium uncinatum*, and environmentally, e.g. *Melaleuca quinquenervia*. The level of natural resistance within species populations in Australia is unknown. Field observations indicate variability in susceptibility to the disease within some species (Pegg *et al*. 2014).

Currently, there is no nationally agreed method in Australia for scoring the susceptibility of myrtaceous hosts to myrtle rust, either in the glasshouse or in the field. A standardised method of disease rating has been specifically requested by the Nursery and Garden Industry in order to enable trade of disease free species and to provide clients, such as councils, with advice on the levels of myrtle rust susceptibility or tolerance for particular species and/or varieties assisting them in their planting schemes. In addition, a standardised scoring system will be an invaluable tool to determine levels of susceptibility for the resistance breeding program conducted under the National Transition to Management Program (T2M). A sound understanding of the biology of the disease, including disease epidemiology and factors influencing disease development, host-pathogen interactions and disease impacts for a range of myrtaceous species will underpin the development of this disease rating system.

3. Aims

This project is aimed at delivering a standardised method for assessing myrtle rust susceptibility and impact in Australia, in both the glasshouse or in the field. The outcomes from this project will benefit the Nursery and Garden Industry and other plant industries that rely on myrtaceous plants, and other stakeholders, including businesses, local governments and the community to help them manage myrtle rust and limit its impacts on plant production, trade and the environment in Australia. The outcomes of this project will also benefit ongoing research and development into myrtle rust management, such as resistance breeding or selection programs. The research also provides baseline data on the impact myrtle rust is having and likely to have on species and plant communities in natural ecosystems.

A standardised disease rating system for a range of Myrtaceae will provide plant producers, nurseries and other stakeholders with a valuable tool to assist in species selection, as well as identify potential variability in resistance within host species for further development. This includes:

- **Screening methodologies for**:
    - Selecting resistance to *Austropuccinia psidii*
        - within genera and species
        - within breeding populations
    - Examining resistance patterns, heritability and resistance mechanisms
- **Field assessment methodologies for**:
    - Rating susceptibility of Myrtaceae to *Austropuccinia psidii*

4. Materials and Methods

Host range and susceptibility

Susceptibility of Myrtaceae to *A. psidii* and impact of infection under natural conditions was determined through surveys in botanic gardens, public gardens and private botanical collection as well as in native ecosystems. Public reports continued to be received in Queensland and photos were examined for presence of *A. psidii* symptoms and host identification.

Susceptibility assessments, primarily focussed on new growth (shoots, juvenile stems and expanding foliage) were made using the following categories (Figure 1):

- **Relatively tolerant** (RT) = restricted leaf spot or spots only;
- **Moderate susceptibility** (MS) = blight symptoms on new shoots and expanding foliage;
- **High susceptibility** (HS) = blight symptoms on new shoots and expanding foliage and juvenile stems;
- **Extreme susceptibility** (ES) = death of new shoots and severe blighting on all foliage types, shoot and stem dieback.

Identification of new host species was confirmed by botanists and samples collected and submitted into the BRIP (Brisbane Plant Pathology) collection. Locations of these detections were recorded along with all public reports made to Biosecurity Queensland.

Figure 1 (Pegg et al. 2014) *Austropuccinia psidii* severity levels Relatively tolerant (a, b): sori present on <10% of expanding leaves and shoots; limited number sori per infected leaf; Moderate susceptibility (c, d): sori present on 10–50% of expanding leaves and shoots; limited–multiple number sori per infected leaf; High susceptibility (e, f): sori present on 50–80% expanding leaves and shoots; some evidence of disease on juvenile stems; evidence of disease on older leaves and stems; multiple sori per leaf/stem causing blight and leaf/stem distortion; Extreme susceptibility (g, h): sori present on all expanding leaves and shoots and juvenile stems; shoot, stem and foliage dieback; evidence of older stem/shoot dieback.

Data collected from these surveys was used to identify species of Myrtaceae of greatest risk of significant impact to assist:

- The nursery industry identify resistant or more tolerant species for commercial production
- Planning of urban garden planting program through removal of highly susceptible species and selection of more resistant species

- Identification of species and plant communities requiring more detailed research into the short and long term impacts of *A. psidii*
- Planning of species conservation programs

Impact of *Austropuccinia psidii* on species of Myrtaceae

Selected study species

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We selected two rainforest species to quantify the impact of *A. psidii* in natural ecosystems in Australia and to illustrate the potential for *A. psidii* to affect similarly susceptible Myrtaceae. The two species, *Rhodamnia rubescens* and *Rhodomyrtus psidioides,* are listed as highly to extremely susceptible to *A. psidii*, including fruit infection, based on field observations in Australia (Carnegie & Cooper 2011; Carnegie & Lidbetter 2012; Pegg *et al*. 2014). *Rhodamnia rubescens* (brush turpentine) is a common pioneer species in subtropical, cool and warm temperate rainforests, with a coastal distribution from Batemans Bay in southern NSW to Gympie in southern Queensland (Floyd 1989). It is an understorey shrub to small tree with dense foliage, and although reported to reach heights of 25 m (Floyd 1989), we rarely observed trees over 15 m. *Rhodomyrtus psidioides* (native guava) is an understorey shrub to small tree (to 12 m) found in littoral rainforests and wet sclerophyll forests with a coastal distribution from Gosford on the Central Coast of NSW to Gympie in southern Queensland (Floyd 1989). It is known as a pioneer species in disturbed environments (Williams & Adam 2010). *A. psidii* is known to have been established across the range of these species since mid-2011 (www.bionet.nsw.gov.au/; Carnegie & Lidbetter 2012; Pegg *et al*. 2014). There is a paucity of botanical or ecological research on these two species: both are known to be susceptible to drought and frost, but have few natural enemies, and are often described as good "screen" trees for their dense foliage (Floyd 1986; www.noosanativeplants.com.au; www.brushturkey.com.au). Neither species was considered as either rare or of conservation concern prior to 2010 and are still currently listed as 'Least Concern' under state and federal legislation (http://www.environment.nsw.gov.au/threatenedspecies/; http://www.ehp.qld.gov.au/wildlife/threatened-species/; http://www.environment.gov.au/biodiversity/threatened/species)

Effect of repeated damage by *Austropuccinia psidii* on *Rhodamnia rubescens*: Olney State Forest disease exclusion trial

Trial design

A disease exclusion trial was established in Olney State Forest (SF) (33° 07' 53" S, 151° 15" 30" E) on the Central Coast of NSW to quantify the effect of repeated damage from *A. psidii* on *R. rubescens* and examine the progress of disease symptoms over time. The site selected was a wet sclerophyll forest in a moist gully and had an abundance of *R. rubescens* ranging in size from newly emerging seedlings to 12+ m trees. Overstorey trees included *Syncarpia glomulifera* and *Eucalyptus* spp.*,* with the understorey dominated by *R. rubescens* and *Allocasuarina* sp*. Austropuccinia psidii* was first detected in Olney SF in October 2010 (Carnegie & Cooper 2011), six months after *A. psidii* was detected in Australia; Olney SF is less than 10 km north of the first known infected location in Australia. It is likely that *A. psidii* had been present for several months prior to being detected.

Twenty trees were selected by walking a line-transect through the forest and every 5 m selecting the nearest *R. rubescens* tree ~0.5 to ~4.0 m in height (trees above this height would be too difficult to spray). Ten trees were then randomly assigned as treated (sprayed) and 10 as untreated (not sprayed). All foliage on treated trees was sprayed to run-off with the fungicide triadimenol (50 ml/100 L)—which is registered in Australia for control of *A. psidii* (http://permits.apvma.gov.au/PER12319.PDF)—with a manual pressurized back-pack spray unit. Fungicide application generally occurred monthly from August 2011 to October 2014. From June 2013, the 10 treated trees were split into two groups with five individuals randomly selected for ongoing fungicide treatment while the other five were no longer treated with fungicide (hereafter termed "partially treated"). The aim here was that once trees had recovered and had been free of disease for some time, we wanted to follow the progression of disease on these un-diseased trees, similar to what would have occurred when *A. psidii* first established in the forest.

Tree assessments

The whole crown of each tree was assessed for crown transparency (Schomaker *et al*. 2007) monthly from August 2011 to October 2014 to provide an indication of the impact on tree health due to repeated damage from *A. psidii*: low transparency (e.g. 25%) indicated many leaves in the crown and limited impact from *A. psidii* infection while a high transparency (e.g. 75%)

indicated few leaves and a high level of impact. No other causal agent of defoliation (e.g. herbivores or drought) was observed during the course of the study. Incidence and severity of *A. psidii* was assessed on leaves to gain an understanding of the relationship between leaf damage and crown transparency and follow the progression of damage through time. Incidence (% infected) and severity (% leaf area affected on diseased leaves) of *A. psidii* was assessed on leaves on individual branches from August 2011 to December 2012 and thereafter on leaves in the whole crown up to October 2014 as follows. Three branches per tree were randomly selected and tagged 30 cm from the tip and each month from August 2011 to December 2012 the number of leaves (immature and mature leaves combined) on each branch counted and the incidence and severity of *A. psidii* on these leaves assessed. "Immature" leaves had recently been produced and were still susceptible; "mature" leaves, representing several leaf cohorts, had previously been susceptible, but had since matured and were no longer susceptible to new infection. From March 2013 to October 2014, individual branches were no longer assessed and the incidence and severity of *A. psidii* on the immature leaves only, across the whole crown, was assessed at monthly intervals. The reason for this change in methodology was that many of the tagged branches on the untreated trees had died by March 2013, and so to continue to obtain data we began assessing *A. psidii* in the whole crown. Only these later assessments were used to conduct comparative analysis with crown transparency and leaf flush. An estimate of the proportion of immature leaves in the whole crown, providing an indication of leaf flush events, was also assessed monthly from March 2013. Development/production of flowers and fruit, and incidence of rust on each, were to be assessed, however neither flower nor fruit production were observed during this study.

Quantification of diseased leaf area and leaf size on Rhodamnia rubescens at Olney SF

Within six months of initiation of the Olney SF disease exclusion trial we observed a difference in disease severity and an apparent difference in the size of newly developed (immature) leaves between treated and untreated trees. We hypothesized that this difference in leaf size was due to repeated severe leaf damage and subsequent defoliation on untreated trees resulting in reduced carbon assimilation, thus affected ongoing leaf development. To further examine this, leaves were collected and the leaf area damaged by *A. psidii* (severity) and the total leaf area (size) of both treated and untreated trees were assessed. Three branches per tree from each of the 20 trees were randomly selected (but not the tagged branches above) and two leaves per leaf category (old, mature and immature) were sampled six months after treatment began (i.e. 2 leaves x 3 leaf categories x 3 branches = 18 leaves/tree). For this experiment we designated three categories of leaf age to try to differentiate the effect of treatment on leaf production (= leaf size): "old" leaves had matured prior to commencement of the trial and so any rust on these was from previous episodes of infection; "mature" leaves, representing several leaf cohorts, would have been produced after the trial commenced and so would have been susceptible, but had matured and were no longer susceptible at the time they were sampled; "immature" leaves had recently emerged and were susceptible. Whole leaves were removed, placed in paper bags, pressed in a herbarium press while still fresh and leaf area determined by scanning using an HP Color LaserJet CM3530fs MFP. The image processing software QUANT (Vale et al. 2003) was used to quantify leaf area (mm^2) and the percentage of leaf area damaged by *A. psidii* (*A. psidii* severity).

Site 1 Effect of *Austropuccinia psidii* on foliage production and survival of *Rhodamnia rubescens* – *Olney State Forest, NSW*

A fungicide exclusion trial was established in Olney State Forest (37° 07' 53" S, 151° 15" 30" E) on the Central Coast of NSW to quantify the impact of *A. psidii* on *R. rubescens*. The site selected was in a moist gully and had an abundance of *R. rubescens* ranging in size from newly emerging seedlings to 12+ m trees. Over-storey trees included *Syncarpia glomulifera* and *Eucalyptus* spp., with the understory dominated by *R. rubescens* and *Allocasuarina* sp. *Austropuccinia psidii* was first detected in Olney SF in October 2010, six months after *A. psidii* was detected in Australia; Olney SF is less than 10 km north of the initial infected premises. It is likely that *A. psidii* had been present for several months prior to being detected.

Twenty trees were selected by walking a line-transect through the forest and selecting every 5 m selecting the nearest *R. rubescens* tree ~0.5 to ~4.0 m in height (trees above this height would be too difficult to spray). Trees were randomly assigned as treated (sprayed) or untreated (not sprayed), 10 plants per treatment. All foliage on treated trees was sprayed to run-off with the fungicide triadimenol (50 ml/100 L) with a manual pressurised back-pack spray unit, with untreated trees not sprayed. Fungicide application generally occurred monthly from August 2011 to October 2014

From June 2013, the 10 treated trees were split and five of these randomly selected for ongoing fungicide treatment while the other five were no longer treated with fungicide (hereafter termed "partially treated"). The aim here was that once trees had recovered and had been free of disease for some time, we wanted to follow the progression of impact on these un-diseased trees, similar to what would have occurred when *A. psidii* first established in the forest.

Tree assessments

Each month, the whole crown of each tree was assessed for crown transparency (Schomaker *et al*. 2007) where low transparency (e.g. 25%) indicated many leaves in the crown and limited impact from rust infection while a high transparency (e.g. 75%) indicated few leaves and a high level of rust impact. Trees were also assessed for incidence and severity of infection on immature leaves, and number of new leaves produced since last assessment (providing an indication of a "flush" event). These later assessments, and corresponding analysis with weather data, are to be reported elsewhere. Development/production of flowers (generally August-October) and fruit (generally October-December) (Floyd 1989), and incidence of rust on each, were to be assessed, but none were produced during this study.

Diseased leaf area assessments

To further examine the impact on foliage, leaves were collected and a leaf area quantification programme used to quantify total leaf area (size) and leaf area damaged by *A. psidii* of both treated and untreated trees. Two leaves from each leaf-age category (old, mature and immature) from each tree were sampled in February 2012 (six months after treatment began). "Old" leaves had matured prior to commencement of the trial, were thus not susceptible to *A. psidii*, and so were an indication of previous disease; "mature" leaves, representing several leaf cohorts, would have been produced after the trial commenced and so would have been susceptible; "immature" leaves had recently been produced and were still currently susceptible. Three branches per tree were randomly selected and two leaves per leaf category removed and placed in paper envelopes (i.e. 2 leaves x 3 branches = 6 leaves). Leaves were pressed then scanned using an HP Color LaserJet CM3530fs MFP. The image processing software QUANT (Vale *et al*. 2003) was used to quantify leaf size (mm2) and the percentage of area damaged by *A. psidii*.

The impact of *Austropuccinia psidii* on selected species across their native range

To gain an understanding of the impact of *A. psidii* on our selected species, and ascertain whether there was any variation in susceptibility, we assessed native stands of each species across the range of their natural distribution. Stands were selected across their natural distribution ranges through knowledge of local ecologists (e.g. Forestry Corporation of NSW; National Parks & Wildlife Service) and from species location data obtained from the Atlas of Living Australia (www.ala.org.au/). While some stands were already known to have a history of *A. psidii*, such as those listed in the Atlas of NSW Wildlife (www.bionet.nsw.gov.au/), many sites were selected without any prior knowledge of *A. psidii* presence to remove bias from site selection. Stands were selected if they were in native forests and ideally contained at least 20 individuals. At each site (GPS coordinates obtained), a central point was located within the stand and the nearest 20 individuals marked for assessment. Individuals smaller than ~0.5 m in height were not included.

For each tree, assessments were made of *A. psidii* infection and damage: (1) crown transparency (Schomaker *et al.* 2007) (Figure 2), (2) incidence of *A. psidii* (% infected) on (a) immature leaves, (b) mature leaves and (c) flowers and fruits (if present), and (3) a disease rating score (Pegg et al. 2012). Dead trees were classed as 100% crown transparency; results from the Olney SF exclusion trial, and our extensive field observations, indicated that such trees might produce epicormics growth or re-shoot, but that this foliage subsequently became infected and died.

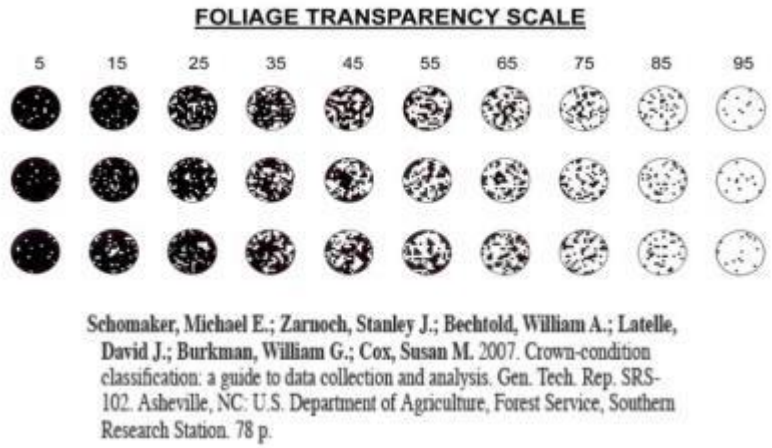

Figure 2. Crown transparency scale used to assess impact of *Austropuccinia psidii* on species of Myrtaceae (Schomaker *et al*. 2007)

No other causal agent of defoliation (e.g. herbivores or drought) was observed during our assessments. For disease incidence assessments, immature leaves were those that had not fully expanded and were thus still susceptible to *A. psidii;* mature

leaves were no longer susceptible, but may have previously been infected when immature and were still retained on trees. Disease rating (1–4 scale) was based on the scale developed by Pegg et al. (2012), where:

- 1 = minor leaf spots with rust pustules on <10% of immature leaves, only a few pustules per infected leaf;
- 2 = rust pustules present on 10–50% of immature leaves and shoots, moderate numbers of pustules per infected leaf;
- 3 = rust pustules present on 50–80% of immature leaves and shoots, multiple pustules per leaf/stem, leaf and stem blighting and distortion, evidence of rust on juvenile stems and older leaves;
- 4 = rust pustules present on majority of immature leaves, shoots and juvenile stems, multiple pustules per leaf/stem, foliage dieback, evidence of stem and shoot dieback.

Based on results from the Olney SF disease exclusion trial, and *a posteriori* knowledge of our study species (e.g. Pegg *et al.* 2014), we were confident that if severe damage from *A. psidii* was observed in trees at our sites then *A. psidii* was the main cause of any crown loss. For example, Pegg *et al*. (2014) followed the progression of disease on individual *R. angustifolia* trees from initial infection by *A. psidii* to severe defoliation and dieback within 15 months. Both our indicator species are non-deciduous, and there is no evidence of seasonal effects during the 10 month period of our assessments, which covers all seasons, that could account for any changes in crown transparency.

Binoculars were used to assess tall trees where necessary. Tree height (m) was measured with either a height pole or laser rangefinder/height meter. Sites were assessed between January and October 2014, roughly 3—3.5 years after *A. psidii* had established in natural ecosystems across the natural range of these two species.

At each site we examined trees for typical symptoms of *A. psidii* infection and damage (Carnegie & Lidbetter 2012; Pegg *et al*. 2014) to confirm presence of the disease. This included yellow pustules on immature leaves and stems, old greyed pustules on mature leaves which had been infected when immature, and stem dieback. No other disease established in Australia presents similar symptoms (Walker 1983). At a selection of sites, samples were collected for further examination in the laboratory and molecular confirmation of *A. psidii* (results presented in Pegg *et al*. 2014; Machado *et al*. 2015).

Statistical analyses

Effect of repeated damage by Austropuccinia psidii on Rhodamnia rubescens: Olney State Forest disease exclusion trial

The values for the crown transparency data were measured at regular intervals so the data are a time series and the observations over time on the same experimental unit (tree) cannot be assumed to be independent. A mixed effect model was used to model the auto-correlation structure. An auto-correlation between the residuals of different time points was modeled by introducing a stationary auto-correlation function of order 1 (Chatfield 2003; Diggle 1990). This error structure models the residuals at time t (ut) as a function of residuals at time t-1 (ut-1) along with the noise (ε_t):

$$u_t = \rho u_{t-1} + \varepsilon_t \qquad (2)$$

The parameter ρ is unknown, and needs to be estimated from the data. This error structure results in the following correlation structure:

$$cor(u_t, u_s) = \begin{cases} 1, \ if \ t = s \\ \rho^{|s-t|}, \ else \end{cases} \qquad (3)$$

Treatment and time were used as fixed effects. Initial plotting indicated a non-linear trend with time, so a smoothing spline was fitted with time. The fitted model is:

$$Response\ variable = intercept + Treatment + f_1(time) : Treatment + \varepsilon \qquad (4)$$

where crown transparency is the response variable, treatment (= treated, untreated and partially treated), and time is the number of days since the start. A separate spline function (f_1) is fitted for each treatment over time rather than assuming a linear relationship. The model was fitted as before using likelihood ratio tests for significance testing.

Only data from March 2013-October 2014 were used for comparative analysis of incidence and severity, crown transparency and leaf flush.

Quantification of diseased leaf area and leaf size at Olney SF

Observations were made within trees for the leaf area data, so the observations are not independent and hence a mixed effects model was also fitted to these data. Fixed effects that were included in the model were treatment, crown transparency and leaf class (old, mature, immature). Crown transparency was included in the model as we had hypothesized that high crown transparency would result in a reduction in photosynthetic area and thus a reduction in leaf size due to depletion of reserves to produce foliage. For the analysis, we included crown transparency assessment dates that we believed would have had some effect on foliage production for each leaf class (i.e. crown transparency prior to or at the time of foliage production): for immature leaves, we used mean crown transparency from the two preceding assessments (February 2012 and December 2011); for mature leaves we used mean crown transparency for the December 2011 and November 2011 assessments (these leaves had matured by the February 2012 assessment); for old leaves we used crown transparency from August 2011.

As the design is nested, a random effect for tree is included in the model. The full model fitted is:

$$Response\ variable = intercept + Treat + LC + Trans + Treat{:}\,LC + \alpha + \varepsilon \qquad (5)$$

where Response variable is the leaf area or the severity of *A. psidii* on leaves (*A. psidii* severity), Treat, LC, Trans, Treat:LC, are the terms for fixed effects for treatment, leaf class, crown transparency and the interaction of treatment and leaf class, and α, ε are the random effects for the tree and the error terms. The variances for the treated and the untreated for percentage damaged were different. The heteroscedasticity structure was specified by weights argument in the model. The model was fitted using likelihood ratio tests for significance testing.

The impact of Austropuccinia psidii on selected species across their native range

The crown transparency data has an inherent nested structure as the trees are nested within locations and cannot be assumed to be independent as is required for linear regression. The data were therefore analyzed using mixed models (Pinheiro & Bates 2000). Restricted maximum likelihood (Zuur *et al*. 2009) was used to compare nested models in which only the random effects differed. Following the final random effect structure the model was tested for fixed effects. Likelihood ratio tests and t statistics were used to identify the significant fixed effect terms in the model.

Previous rust (site with a known history of *A. psidii*), disease rating, disease incidence on immature leaves, disease incidence on mature leaves, and height were used as the fixed variables. Location was used as the random variable. The full model that was fitted was:

$$Response\ variable = intercept + DR + PR + ML + Ht + IL + \alpha + \varepsilon \qquad (1)$$

where crown transparency is the response variable, DR, PR, ML, IL, Ht, are the fixed effect terms for disease rating, previous rust, disease incidence on mature leaves, disease incidence on immature leaves and height, and α, ε are the random effects for the location and the error terms. We included previous rust in the analysis to determine whether there was any bias in our selection of sites we already knew had disease compared to those with an unknown disease history.

We also tested whether region—using Köppen climate classification and seasonal rainfall data (www.bom.gov.au)—had an effect on disease of *R. rubescens* and *R. psidioides* across the survey sites. However, there was no effect so we did not report on this further.

All analyses were conducted using R (R Core Team, 2014), nlme (Pinheiro *et al*. 2014) and plotting was done using ggplot2 (Wickham 2009) and lattice (Sarkar 2008).

Impact on other selected species

Additional surveys have also been conducted on the remaining populations of *Lenwebbia* sp. Blackall Range. The genus *Lenwebbia* consists formally of two species, *L. lasioclada* and *L. prominens*, and occurs in mesic forests along or near the east coast of northern New South Wales to north-eastern Queensland. However, the latter species is now recognised as comprising two additional species: *Lenwebbia* sp. Blackall Range and *Lenwebbia* sp. Main Range. *Lenwebbia* sp. Blackall Range is currently listed as Endangered in Queensland and *L. prominens* as Near Threatened (Queensland; Nature Conservation Act 1992 & Amendment Regulations (1) 2010). Populations of *Lenwebbia* sp. Blackall Range across areas of south-east Queensland, (Doonan, Eudlo, Maleny) were assessed for impact of myrtle rust following reports of decline from local council groups. Two populations of *L. prominens* from northern NSW were also assessed.

Other species assessed, but only on a small number of populations within their natural range distribution, include:

- *Decaspermum humile*
- *Gossia myrsinocarpa*
- *Rhodamnia maideniana*
- *Rhodamnia sessiliflora*
- *Tristaniopsis exiliflora*

Predicting impact of myrtle rust on species of Myrtaceae

Ex-situ plantings of Myrtaceae within Mt Coo-tha and Lismore Botanical Gardens were assessed to determine susceptibility levels and potential impact of myrtle rust on a range of species from a broad geographic range in Australia. The gardens are now an important resource to help identify potential impact on these species and associated plant communities over time. Mt Coo-tha has a large collection of Australian subtropical and tropical Myrtaceae, whereas collections in the Lismore Gardens is focussed on rainforest species of northern New South Wales. Both sites were assessed using the methods mentioned previously to determine impact of repeated *A. psidii* infection. Results of this study will help prioritise species and plant communities at risk in regions of Australia where myrtle rust has not as yet reached and focus research and conservation programs in areas affected by rust.

Impact of myrtle rust on tree species was measured as detailed previously. However, it was identified that in addition to transparency, which can be difficult to assess in dense forest ecosystem, data assessing overall tree health or impact would be valuable. Tree health was scored based on:

- **Branch Death –** as a percentage of the total branches. This recording method can be applied to regeneration, under-, mid- and over-story species and individuals and adds to the value of assessing transparency levels.
- **Branches with evidence of dieback** – as a percentage of branches showing evidence of foliage loss and apical tip dieback but have not been killed.
- **Healthy branches** – as a percentage of the branches with no evidence of defoliation or dieback

When assessing disease levels on new growth flush the following data was also recorded including:

- **Proportion of susceptible foliage** – new growth as a percentage of the total foliage present. This may be more relevant when looking at patterns of disease development in relation to flush cycles, changes in host growth patterns in relation to repeated infection
- **Disease incidence on new growth**
- **Disease severity –** either recorded as a percentage of leaf area affected (average across the infected foliage) or as rating as previously mentioned

Impact of *Austropuccinia psidii* on *Rhodamnia rubescens* fruit development

Our initial aim was to assess the impact of *A. psidii* on fruit development in Olney SF, but no flowers set during 2011–2014. A fortuitist observation of flower development in *R. rubescens* in Tucki Tucki Nature Reserve in north-coastal NSW allowed us to conduct such a trial. A single, mature, *R. rubescens* was selected for the trial when the presence of flower buds was first noted. Unfortunately inconsistent flowering across the site, most likely due to previous myrtle rust impact, prevented us from conducted the experiment on a larger sample size. Two low branches were used for the trial, with 10 branchlets selected and tagged on each branch; each branchlet had approximately 60 fruit. The two branches were randomly assigned the nil treatment (control) and the fungicide application treatment. The fungicide treatment was Amistar 250SC (250g/L Asoxystrobin) at 0.4 ml/L of solution, with spray applied to the point of runoff. The fungicide was applied at 14 day intervals until the surviving fruit had matured. For each branchlet, the total number of flowers/fruit and the number with *A. psidii* infection was recorded fortnightly. Mature fruit was captured for further analysis by placing shade-cloth under the two branches. Fruit was then dried and seed extracted and weighed.

The response variables for the fruit data were disease incidence percentage (% infested) and the total number of fruits. This is a before, after, control impact (BACI) design where before and after is defined by period and control impact is the treatment. We are interested in the interaction between treatment and period. As the data was collected weekly from the same branch the observations cannot be assumed to be independent. An auto-correlation between the residuals of different time points is modelled by introducing a stationary auto-correlation function of order 1 (Chatfield, 2003 & Diggle, 1990). This error structure models the residuals at time t (u_t) as a function of residuals at time t-1 (u_{t-1}) along with the noise (ε_t):

$$u_t = \rho u_{t-1} + \varepsilon_t$$

The parameter ρ is unknown, and needs to be estimated from the data. This error structure results in the following correlation structure:

$$cor(u_t, u_s) = \begin{cases} 1, & if\ t = s \\ \rho^{|s-t|}, & else \end{cases}$$

Also the non- linear trend over time was modelled using smoothers. The model that was fitted for both the response variables is:

$$\%\ Infested\ or\ no.of\ fruits = intercept + Treat + Period + Treat{:}Period + f_1(week){:}Treat + \varepsilon$$

where $\%\ Infested\ or\ no.of\ fruits$ are the response variables, *Treat* is the treatment (treated or untreated), *Period* is the before and after time period, and *Treat:Period* is the period and treatment interaction effect, *week* is the week when the measurements were taken, and f_1 is smooth functions estimated by the model using maximum likelihood estimation. $f_1(week){:}Treat$ indicates that a separate spline function was fitted to the treated and untreated observations. All analysis was done using R (R Core Team, 2014), nlme (Pinheiro *et al*. 2014) and plotting was done using ggplot2 (Wickham 2009) and lattice (Sarkar 2008).

Impact of *A. psidii* on regeneration of Myrtaceae in coastal heath following wildfire

The impact of *A. psidii* on regeneration of a range of Myrtaceae following a wildfire event was assessed. The fire occurred in December 2013 within a dry and wet heathland environment north of Lennox Head, a coastal community of northern New South Wales. The fire was initiated following a lightning strike and burnt more than 500 ha of coastal heath north from Lennox Heads to Broken Head. Fire had been absent from the region for more than 40 years (Pers. comm. Jali Land Council). The site has been described as a "pristine" (Erskine *et al*. 2002) coastal ecosystem and is controlled by the traditional land owners from the Jali Land Council, Ballina, New South Wales. Permission to access land was sought before studies commenced.

Study sites

The vegetation at the site can generally be described as coastal sclerophyll (heath), dominated by *Banksia* spp. with low-lying areas inhabited by *Melaleuca* wetland. Two distinct vegetation types exist within the area burnt; dry and wet heathland environments (Figure 3)

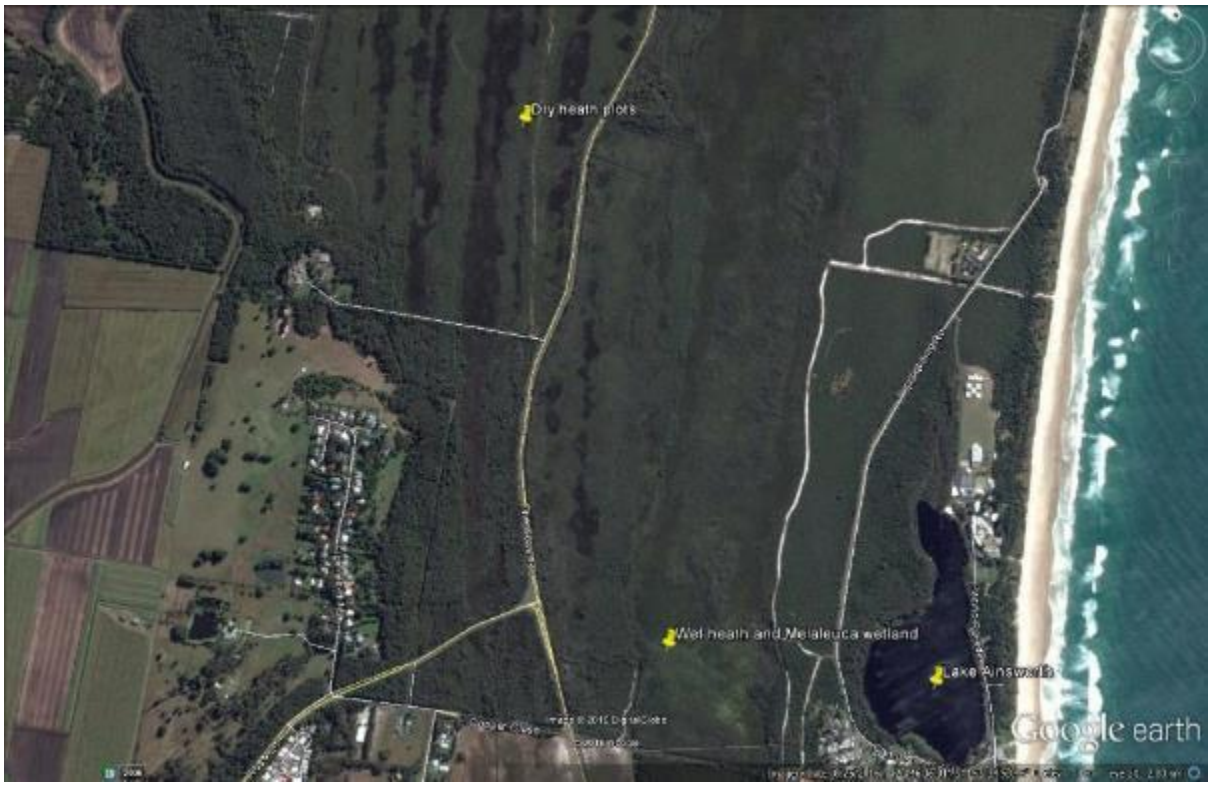

Figure 3 Map showing study sites within coastal heath near Lennox head in northern New South Wales affected by wildfire following a lightning strike in December 2013.

Impact of repeated A. psidii infection on Myrtaceae

To monitor the development of myrtle rust and determine the impact of *A. psidii* on regeneration of Myrtaceae following wildfire plots were established in areas consisting of different vegetation types and were focussed on areas where Myrtaceae were present and dominant within the landscape rather than being randomly situated. Plots were established after the presence of regeneration (coppice/epicormic/seedling) was detected (Figure 4). Plots were 4m x 4m in size, further divided into 16 subplots to enable easier assessment of plant species and to allow for assessment of symptoms progression and subsequent dieback levels. The corner of each subplot was marked using metal pegs and "builders" twine used to help demarcate these. Four plots were established in total, with two in the dry heath and two in the wet heath environments to capture impact on the following species of Myrtaceae:

- *Lophostemon suaveolens*
- *Melaleuca quinquenervia*
- *Melaleuca rigidus*
- *Leptospermum polygalifolium*
- *Leptospermum whitei*
- *Leptospermum laevigatum*
- *Baeckea frutescens*
- *Melaleuca nodosa*
- *Austromyrtus dulcis*

Ideally control plots using fungicide control to provide disease free comparisons would have been applied but due to close proximity to waterways this was not possible.

Austropuccinia psidii infection assessments were made monthly to:

- Monitor the progress of disease development
- Determine species susceptibility and impact of infection over time

Disease assessments commenced in April 2014 when coppice regeneration was first identified and completed in July 2015. Plots were initially assessed fortnightly to capture early stages of regeneration and initial stages of disease development but extending to monthly as growth rates slowed.

Figure 4 Coppice regeneration following wildfire in a coastal heath ecosystem and plots established on sites dominated by Myrtaceae

The following data was captured at each assessment:

- Species of Myrtaceae - identified by botanists through photographic evidence or where necessary confirmed from pressed specimens
- Number of each species present – based on coppice regeneration (seedling regeneration was limited)

Disease levels were assessed as:

- Incidence of infection as a percentage of the total number of individuals for species within the plots
- *Austropuccinia psidii* infection levels were assessed using a 0-5 rating scale where:
    - 0 = no infection (no pustules present) on new shoots, stems of young leaves
    - 1 = Low incidence (<5%) of lesions throughout tree/shrub on new shoots and young leaves; lesions small in size – 1-2 pustules per leaf; no evidence of stem infection
    - 2 = Moderate incidence (5-25%) of lesions throughout tree/shrub on new shoots and young leaves; lesions small in size 2-5 per leaf
    - 3 = Moderate to high incidence (26-50%) of lesions throughout tree/shrub occurring on new shoots and young leaves; low incidence (<10%) of infection on juvenile stems; lesion size moderate with evidence of blighting
    - 4 = High incidence (51-80%) of lesions throughout tree/shrub occurring on new shoots and young leaves; large lesions, blighting and evidence of distorted growth on leaves and shoots; moderate incidence (up to 75%) of infection on juvenile stems. Some shoot distortion and death evident
    - 5 = Infection on all (100%) new shoots and young leaves and juvenile stems. Evidence of stem and shoot dieback on at least 50% of growth; shrub like growth appearance with loss of apical dominance; some shoots still alive

The effect of repeat infection was assessed by determining incidence of branch dieback and branch death per tree. This assessment was done 18 months after assessments commenced. When present, the number of flowers/fruit on each shrub/tree was also recorded along with the percentage showing *A. psidii* infection.

Additional surveys outside of the established plots were conducted to determine if the impact observed within the plots was representative of larger populations. This data was also used to examine distribution and frequency of species within the ecosystem and location of individuals showing higher levels of resistance/tolerance to myrtle rust. A multi-species transect was also established to examine susceptibility of species that were not initially captured within the plots.

Weather data for the assessment period, including rainfall and temperature were collated from SILO DATA DRILL (https://www.longpaddock.qld.gov.au/silo/). Patterns of disease incidence and severity were compared to climate factors including monthly rainfall, days of rainfall per month and average maximum and minimum temperatures.

Symptom development and impact of repeat infection by Austropuccinia psidii on coppice regeneration of Melaleuca quinquenervia following wildfire in a swamp ecosystem

Three 50m transects were established within a *M. quinquenervia* swamp which can be inundated with water for extended periods following rainfall. Aside from understory fern and reed species, no other tree species was present.

Following the fire event and prior to the development of coppice regeneration, trees along each transect were marked and numbered with a total of 140 assessed for severity of myrtle rust infection and impact over time. Trees that didn't produce coppice regrowth were excluded from the final assessment. Assessments for susceptibility to *A. psidii* were conducted as previously described using the 0-5 rating scale. To examine the interaction between A. psidii and native insect "pests", the presence and severity of mirid bug (*Eucerocoris suspectus*) damage was also assessed using the following severity scale:

- 0 = No evidence of leaf blotching mirid damage present on new growth flush
- 1 = Mirid bug damage present on >25% of new growth flush
- 2 = Mirid bug damage present on 25-50% of new growth flush
- 3 = Mirid bug damage present on 51-75% of new growth flush

- 4 = Mirid bug damage present on >75% of new growth flush

Presence of flowers/seed was recorded and levels of dieback assessed 18 months after myrtle rust symptoms were initially detected to demonstrate the effects of repeated infection on coppice regeneration. Dieback was recorded as a percentage of the total number of main branches showing dieback.

Analysis
Normality of data and equality of variance were assessed using an F test. All proportion data were Arcsine square root transformed prior to analysis using ANOVA and compared using Fishers PLD post hoc test (Statview®). Back-converted data were used to present data graphically.

Site 2 Impact of myrtle rust on regeneration of *Melaleuca quinquenervia* and interaction with insect populations

To examine the impact of *A. psidii* on *Melaleuca quinquenervia* regeneration, growth and reproduction, a fungicide exclusion trial was established on coppiced trees at Bungawalbin near Woodburn in NSW. Additionally, the interaction between *A. psidii* and native insect pests was studied. The experiment was established using a randomised block design (Fig. 5) with the following treatments:

1. Untreated control
2. Fungicide only (Bayfidan® = Triademenol 250g/L + Zaleton® = Tebuconazole 200g/L, Trifloxystrobin 100g/L)
3. Fungicide + insecticide (Bayfidan® +Zaleton®; Confidor® = Imidacloprid 200g/L+ Maverick® = Tau-fluvalinate 7.5g/L)
4. Insecticide only (Confidor® + Maverick®)

Treatments were applied monthly once coppice regeneration commenced and applied as foliar applications at label recommended rates. Herbicide was applied when necessary to control weed growth within the site. Plots were 3 m x 3 m with a 1 m buffer around the outside of each plot. A marker was placed in the centre of each plot and stump location marked based on a "clock-face" direction and distance measured from the central point to ensure each stump could be identified.

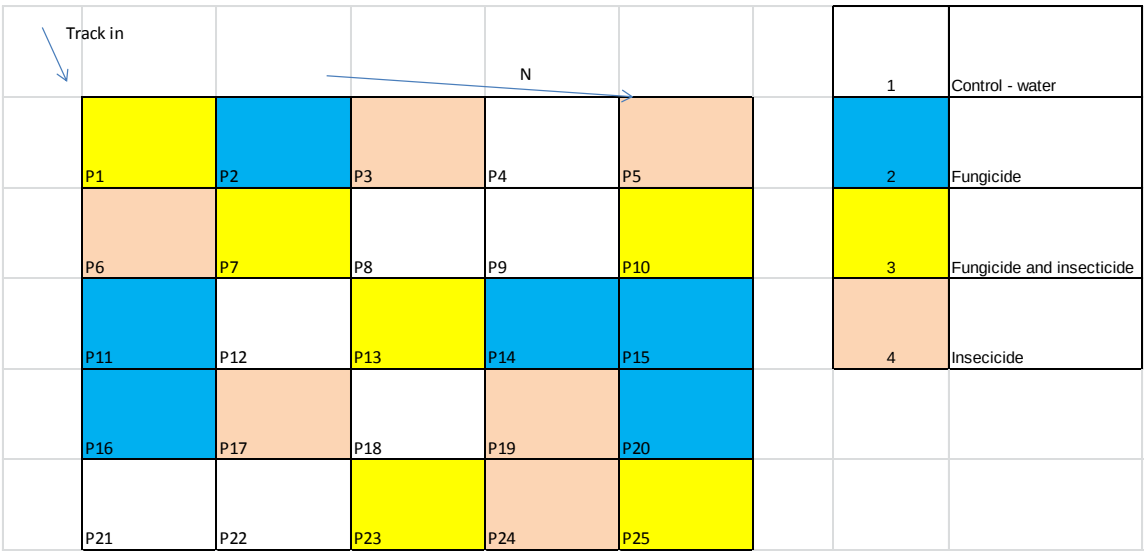

Figure 5 *Melaleuca quinquenervia* myrtle rust interaction and exclusion trial plot layout, northern NSW

Assessments commenced once coppice regeneration was uniform across the site. Initial measurements included:

- Diameter of stump
- Number of coppice shoots emerging from stumps

Coppice heights were measured every six months using the tallest coppice from each stump. Additional measurements were:

- Diameter at Breast Height (DBH) – recorded 18 months from the time of establishment of coppice regeneration on the tallest coppice shoot on each stumps. DBH was not recorded on trees below 1m in height.

- Foliage density - assessed for each tree as a measure for estimating foliage loss due to insect and myrtle rust attack and was done using a density rating method (Frampton *et al*. 2000) similar to the transparency scale mentioned previously
- Leaf area and internode length - The number of leaves present was also recorded to determine internode length as a possible indicator of growth. Leaf area was determined by removing branches from each tree and then taking leaves from a 10cm section of branch. Only fully expanded leaves were measured to remove the influence of variability in growth stages between trees. Leaf area was measured as previously mentioned.

Insect and disease assessments were conducted monthly. Assessments were conducted on new growth flush for both insect and myrtle rust. The percentage of new growth flush in relation to total foliage present was recorded for each tree. *Austropuccinia psidii* infection levels were recorded as incidence (% new shoots and expanding foliage infected) and severity on trees with rust rated as:

- 1 = lesions small in size – average <3 pustules per leaf; no evidence of stem infection (Fig. 6)
- 2 = lesions small in size average 3-5 pustules per leaf; no evidence of stem infection (Fig. 7)
- 3 = multiple lesions per leaf; lesion size moderate with some evidence of blighting; infection may also be present on juvenile stems – <3 per stem (Fig. 8)
- 4 = multiple lesions per leaf; lesion size moderate - large, blighting and evidence of distorted growth on leaves and shoots; multiple lesions on juvenile stems. Some evidence of shoot distortion (Fig. 9)
- 5 = multiple lesions per leaf; lesion size large, in some cases covering the entire leaf; Severe leaf blighting and distorted growth; multiple lesions on juvenile stems causing shoot distortion and dieback (Fig. 10 & 11)

Figure 6 Severity rating 1 for *Austropuccinia psidii* infection on *Melaleuca quinquenervia* - lesions small in size – >5 pustules per leaf; no evidence of stem infection

Figure 7 Severity rating 2 for *Austropuccinia psidii* infection on *Melaleuca quinquenervia* - lesions small in size 5 or more per leaf; no evidence of stem infection

Figure 8 Severity rating 3 for *Austropuccinia psidii* infection on *Melaleuca quinquenervia* –multiple lesions per leaf; lesion size moderate with some evidence of blighting; infection may also be present on juvenile stems

Figure 9 Severity rating 4 for *Austropuccinia psidii* infection on *Melaleuca quinquenervia* –multiple lesions per leaf; lesion size moderate - large, blighting and evidence of distorted growth on leaves and shoots; moderate sized lesions on juvenile stems. Some evidence of shoot distortion

Figure 10 Severity rating 5 for *Austropuccinia psidii* infection on *Melaleuca quinquenervia* –multiple lesions per leaf; lesion size large, in some cases covering the entire leaf; Severe leaf blighting and distorted growth; large lesion on young green stems, shoot distortion and dieback evident

Figure 11 Severity rating 5 for *Austropuccinia psidii* infection on *Melaleuca quinquenervia* - multiple lesions per leaf; lesion size large, in some cases covering the entire leaf; Severe leaf blighting and distorted growth; large lesion on young green stems, shoot distortion and dieback evident

Insect impact was also scored as incidence (% of new shoots and expanding foliage with evidence of insect damage). The type of insect attack (chewing, tip death, leaf necrosis caused by mirid bugs, leaf etching) was also recorded and samples collected or photographed for identification where possible. Insect impact was assessed using a 1-4 scale:

- 0 = No evidence of insect damage present on new growth flush
- 1 = >25% of leaf area damaged on foliage attacked by insects
- 2 = 25%-50% of leaf area damaged on foliage attacked by insects
- 3 = 51-75% of leaf area damaged on foliage attacked by insects
- 4 = >76% of leaf area damaged on foliage attacked by insects

Climate data

Rainfall and temperature data was collated using Silo data drill (https://www.longpaddock.qld.gov.au/silo/). Leaf wetness levels were recorded over time using a Leaf wetness data logger (OM-CP-LF101A-KIT). Disease and insect impact levels were compared in relation to different climatic factors.

Analysis

Normality of data and equality of variance were assessed using an F test. All proportion data were Arcsine square root transformed prior to analysis using ANOVA and compared using Fishers PLD post hoc test (Statview®). Back-converted data were used to present data graphically. For the purpose of this report average of trees from each treatment were used

Impact of repeated infection by *Austropuccinia psidii* on species survival and composition within in subtropical wet sclerophyll/rainforest ecosystems with high density of Myrtaceae - Tallebudgera Valley, Queensland

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Myrtle rust impact was first observed in 2014 in a wet sclerophyll site with a rainforest understory in the Tallebudgera Valley, Queensland. The site has a history of forest logging activities and clearing for cattle grazing but has more recently been allowed to regenerate naturally. The vegetation is dominated by Myrtaceae (Table 1) with over-story currently made up of *Eucalyptus grandis* and *Lophostemon suaveolans and occasional Syzygium oleosum; Decaspermum humile, Archirhodomyrtus beckleri, and Gossia hilli dominating the mid and under story*. *Rhodamnia maideniana* is common in the understory. *Acmena smithii* is also common in the area, particularly in the open forest edges as is *Rhodamnia rubescens* and *Rhodomyrtus psidioides*. Large rainforest trees also present in the open areas include *Syzygium hodgkinsonia* and *S. corynanthum* with some tree exceeding 25 meters in height. In the absence of any further disturbance it is likely that this ecosystem would transition to rainforest.

Table 1 Myrtaceae species in Tallebudgera Valley assessment site and original host species *Austropuccinia psidii* susceptibility rating as per Pegg et al. 2014

Host species	Canopy Position	Rust susceptibility rating
Eucalyptus grandis	Canopy	Relatively Tolerant-Moderate Susceptibility
Lophostemon confertus	Canopy	Resistant
Syzygium oleosum	Canopy/Forest edge	Highly Susceptible
Syzygium corynanthum	Canopy/Forest edge	Relatively Tolerant
Syzygium hodgkinsoniae	Canopy/edge	Not Rated prior to 2014
Rhodomyrtus psidioides	Forest edge	Extremely Susceptible
Rhodamnia rubescens	Forest edge	Highly - Extremely Susceptible
Rhodamnia maideniana	Understory/Forest edge	Extremely Susceptible
Decaspermum humile	Mid-story	Extremely Susceptible
Archirhodomyrtus beckleri	Mid-story	Not Rated prior to 2014
Gossia hillii	Mid-story	Highly - Extremely Susceptible
Acmena smithii	Understory/forest edge	Relatively Tolerant-Moderate Susceptibility

In 2014, myrtle rust impact levels were assessed for *Rhodomyrtus psidioides*, *Rhodamnia rubescens* (reported as per Carnegie et al. 2015) and *Rhodamnia maideniana* (unpublished) but observations were also captured photographically. Despite indications of some decline on other species (e.g. *Decaspermum humile*) these were not included in any impact assessments in 2014. The site was revisited in 2016 with further assessments made on these before mentioned species. Additionally severe decline of a range of other Myrtaceae was noted.

To determine impact levels on the different Myrtaceae species and potential changes in species composition within the site a series of transect plots were established. Four plots were established along 50 m long x 2m wide line transects. Placement of plots was based on site aspect and potential differences in species composition. The start and finish of each plot was marked and GPS recorded. Trees one metre each side of the centre line were marked with flagging tape and numbered. Only species of Myrtaceae were marked. Samples and photos were taken of plants where necessary to confirm identification with a botanist. The diameter of each tree (DBH) and position within the canopy described as per the following:

- Overstorey (canopy)
- Midstorey
- Understorey
- Regeneration

Myrtle rust disease and impact levels were assessed as described previously including:

- Presence of new flush and proportion in relation to total foliage (% of total foliage present)
- Presence or absence of *A. psidii* symptoms on new shoots, green stems and foliage
- Incidence and severity of myrtle rust on new foliage – (1-5 rating scale)
    - 1 = lesions small in size – average <3 pustules per leaf; no evidence of stem infection
    - 2 = lesions small in size average 3-5 pustules per leaf; no evidence of stem infection
    - 3 = multiple lesions per leaf; lesion size moderate with some evidence of blighting; infection may also be present on juvenile stems – <3 per stem
    - 4 = multiple lesions per leaf; lesion size moderate - large, blighting and evidence of distorted growth on leaves and shoots; multiple lesions on juvenile stems. Some evidence of shoot distortion
    - 5 = multiple lesions per leaf; lesion size large, in some cases covering the entire leaf; Severe leaf blighting and distorted growth; multiple lesions on juvenile stems causing shoot distortion and dieback
- Tree health assessed as:
    - Percentage of dead branches
    - Percentage of branches with dieback (including defoliation)
    - Percentage of tree healthy – no myrtle rust damage
- Transparency score as an indicator of overall tree health and to enable monitoring of change over time

To determine if the level of disease and associated dieback was particular to this site or more widespread, other plant communities in the area were examined for presence of myrtle rust related dieback but to date have not been assessed.

Glasshouse screening – examining populations for resistance

The following procedures have been adopted for conducting glasshouse resistance screening programs:

Spore production for inoculation

Maintaining *Syzygium jambos* plants is useful for the production of spores used in controlled inoculation assessments. Unlike other plant species (e.g. *Rhodamnia rubescens*), *S. jambos* appears more robust and continues to thrive and produce new growth flushes despite repeat *A. psidii* infection. It also produces large quantities of urediniospores.

Spore storage methods

Once collected spores are placed through a sieve to remove other extraneous matter and then placed onto an open petri dish and into a desiccator. Spores remain in the desiccator for 5-7 days to ensure moisture levels are at a minimum. Spores are then transferred into vials and weighed before being placed into a -80^{o}C freezer. Alternatively, and where facilities are available, spores can be stored under liquid nitrogen.

Seedling/plant preparation

Plants must be actively flushing to optimise the disease screening process. Stressed (nutrient deficient, water stressed) seedlings/plants will not flush at a rate that will give an accurate disease rating. Plants should be fertilised 2-3 weeks prior to inoculation. In our studies we have used a combination of Seasol™ and Nitrosol™. Screening should be restricted to young plant material where possible or plants coppiced and allowed to regenerate before inoculation.

Inoculation procedure

Urediniospores are removed from -80°C storage and allowed to warm to room temperature before to being added to sterile distilled water (SDW). The surfactant Tween 20 (white oil can also be used) is added at a rate of two drops per 100 mL SDW and the spore suspension stirred to reduce clumping. Spore counts are conducted using a haemocytometer and the suspension adjusted to a concentration of 1 x 10^5 spores/mL.

In our studies we inoculated plants using a fine mist spray (29 kPa pressure), generated by a compressor driven spray gun (Iwata Studio series 1/6 hp; Gravity spray gun RG3), applied to the upper and lower leaf surfaces of the seedlings, ensuring all leaves were coated with a fine mist but run-off of the spore suspension was avoided. The use of highly susceptible plant material should be included as controls.

Once inoculated seedlings are placed onto a metal bench lined with plastic sheeting. Immediately after inoculation, seedlings are covered with another plastic sheet creating a sealed system. Plants are then placed into a Controlled Environment Chamber (CER) set at 18°C in the dark for 24 hours ensuring high humidity levels and leaf wetness is achieved. Hot tap water (60°C) can be applied to the lower plastic sheet immediately before plants are placed into the CER to ensure high humidity levels and leaf wetness is achieved rapidly.

Twenty-four hours after inoculation the plastic coverings are removed and plants grown on in a shade- or glass-house and hand watered as required.

Disease assessment

Disease symptom progression should be monitored regularly as host species present symptoms at different rates. Symptoms on more resistant individuals within a species can appear first and present as “flecks”. Symptom assessment should be conducted on the primary and secondary foliage. As the foliage ages it becomes less susceptible to infection. The following disease assessment ranking system has been modified from that developed by Junghans et al. (2003). We adopted a similar rating system to that used in Brazil so that we could compare our findings to those conducted using different biotypes of *A. psidii* on similar host species.

The following disease rating scale is:

- 1 = no symptoms evident or presence of flecking (yellow/clear);
- 2 = presence of a hypersensitive reaction (HR) with fleck or necrosis;
- 3 = small pustules, <0.8mm diameter, with one or two uredinia;
- 4 = medium-sized pustules, 0.8–1.6 mm diameter with about 12 uredinia;
- 5 = large pustules, >1.6 mm diameter, with 20 or more uredinia on leaves, petioles and/or shoots (Junghans et al., 2003b; Fig. 12, 13, 14).

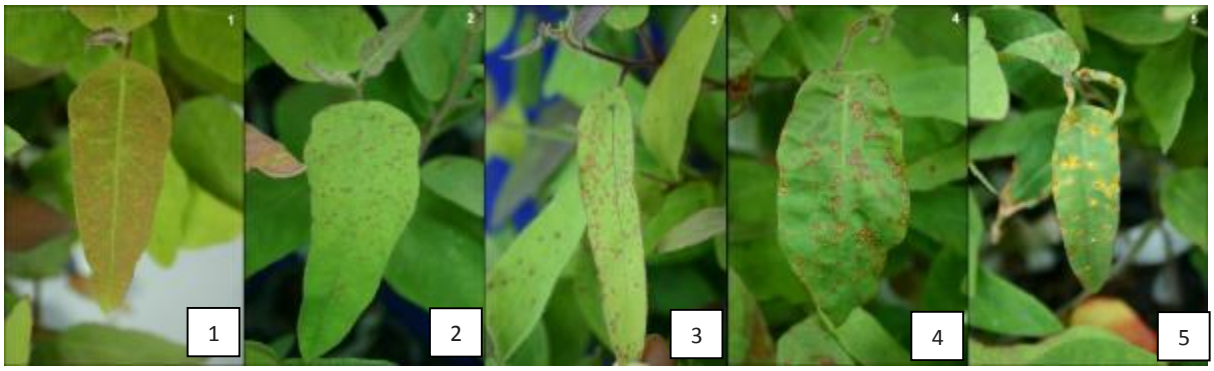


Figure 12 Susceptibility ratings on spotted gum (Published in Pegg et al. 2013)

Inoculations should be repeated on seedlings rated 1 to ensure stage of flush development hasn't influenced the result and produced a "false" resistant score. Plants that have are not actively flushing at the time of inoculation are excluded from the rating and also re-inoculated at a later stage.

Figure 13 *Eucalyptus globulus* disease susceptibility ratings Potts/Pegg Unpublished data

Figure 14 Susceptibility levels in *Eucalyptus ovata* – Potts, Pegg Unpublished data

Species assessed

Eucalyptus *and* Corymbia *spp.*
Screening under controlled conditions is an ideal way to examine resistance across and within species, eliminating physiological and climatic issues that may influence disease incidence and severity under field conditions. Disease screening processes have been developed to great effectiveness for *Austropuccinia psidii* in Brazil where there has been a need to select resistant eucalypts for commercial plantation development. A range of plant species, predominantly those of commercial significance, have been examined for resistance/suscepibility. These include:

- *Corymbia spp.* – collaborative studies with DAF Queensland and Sunshine Coast University
    - *C. citriodora subsp. variegata*
    - *C. citriodora subsp. citriodora*
    - *C. henryi*
    - *C. torelliana and associated hybrids*
- *Eucalyptus spp.* collaborative studies with DAF Queensland and Sunshine Coast University, University of Tasmania, CSIRO and FABI (University of Pretoria)
    - *E. cloeziana*
    - *E. grandis*
    - *E. camaldulensis*
    - *E. globulus*
    - *E. pauciflora*
    - *E. ovata*
    - *E. pellita*
    - *E. urophylla*
    - *E. argophloia*

Studies on these species were conducted to examine the risk *A. psidii* poses both from an environmental and commercial production perspective. Resistance/susceptibility patterns were examined at a provenance and family level and for spotted gum species and *E. globulus*. Resistance patterns within species was then compared with those for endemic pathogens. In the case of spotted gum, resistance patterns found when inoculated with *A. psidii* were compared to provenance and family resistance data for *Quambalaria pitereka* based on both glasshouse screeing studies and historical field trial data. For *E. globulus*, *A. psidii* resistance/susceptibility provenance and family pattern were compared with historical field trial data examing susceptibility to the foliage pathogens *Mycosphaerella* spp. This work is currently being prepared for publication.

Other studies have also been conducted using material screened as part of this project to gain an understanding of the genetics controlling resistance (Bala et al. 2013) and genetics of the different modes of resistance (Butler et al. 2015).

Backhousia citriodora

DAF Queensland currently holds the largest collection of *Backhousia citriodora* (lemon myrtle) with material collected from across its natural range maintained in trial plantings. In collaboration with Sunshine Coast University (A/Prof David Lee), and funding from RIRDC, cuttings were assessed using controlled inoculations to determine resistance levels between and within provenances. Additionally cuttings from the same sources have been established in field trials in northern New South Wales and south east Queensland and will be monitored for *A. psidii* impact under field conditions. This work will be conducted by Emily Lancaster (PB CRC PhD Candidate) and will also serve to validate the results from the screening trials assessing.

Broad-leaved Melaleuca *spp*.

As part of this project, *Melaleuca* was identified as a species of risk from *A. psidii* with any changes in the wetland ecosystem likely to have significant long term ramifications. Field trials have identified variations in resistance/susceptibility levels within a few populations. However, it is not known if these populations are representative of *M. quinquenervia* across it natural range or indeed an indicator of susceptibility of closely related *M. leucadendra* and *M. viridiflora*. Therefore a study was established to examine variability in susceptibility to *A. psidii* within populations of *Melaleuca quinquenervia* and related broad-leaved paperbark species *M. leucadendra* and *M. viridiflora.* This findings from this study have been submitted for review prior to publication (Feb 2018).

Seed material used in this study was sourced from the Australian Seed Bank collection comprised of seed collected prior to *A. psidii* being detected in Australia (Table 2). For *M. quinquenervia* an additional seed-lot (Boggy Creek) was collected from a study site where trees were assessed for rust impact over time and seed collected from trees rated as resistant (G. Pegg). Provenances within each species were made up of bulked seed-lots from at least five parent trees where possible. There were however, exceptions and these were included to ensure that there was a good representation from across the species native range.

Table 2 Melaleuca species and provenances tested for susceptibility to *Austropuccinia psidii* under controlled glasshouse screening

Species	State	Provenance	No. parent trees
Melaleuca quinquenervia	New South Wales	Boggy Creek	9
	New South Wales	Hawks Nest	8
	New South Wales	Long Jetty	10
	New South Wales	Port Macquarie	5
	New South Wales	Tuggerah Lake	4
	New South Wales	Worrel Creek	11
	Queensland	Bribie Island	10
	Queensland	Caloundra	20
	Queensland	Dohles Rocks	10
	Queensland	Gympie	6
	Queensland	Julatten	5
	Queensland	Kuranda	5
	Queensland	Moreton Island	20
	Queensland	Mt Molloy	25
	Queensland	Rokeby National Park	5
	Queensland	Teddington	12
	Queensland	Tozers Gap	8
Melaleuca leucadendra	Northern Territory	King River	7
	Northern Territory	Wangi, Litchfield National Park	5
	Northern Territory	Buffalo Creek	5
	Queensland	Mareeba	3
	Queensland	Iron Range	1
	Queensland	St Lawrence	20

	Western Australia	Cambridge Gulf	5
	Western Australia	Kalumburu Mission	5
	Western Australia	Nimalaica Claypan	6
Melaleuca viridiflora	Northern Territory	Wangi, Litchfield National Park	5
	Northern Territory	east Baines River	5
	Queensland	Rockhampton	10
	Queensland	Round Hill Head	17
	Queensland	Chillagoe	5
	Queensland	Prosperpine	12
	Queensland	north Kennedy River	5
	Queensland	Lakeland	8
	Queensland	Laura	5
	Queensland	Weipa	10
	Western Australia	Theda Station Kalumbura	5
	Western Australia	Ningbing Range Road	5

5. Results

Host range and susceptibility

Host range

The host range of *A. psidii*, both within Australia and internationally, continues to be expand with an extensive list now compiled for publication on the CABI website Invasive Species Compendium;

Giblin FR, Carnegie AJ 2014 *Austropuccinia psidii*. CABI Invasive Species Compendium Datasheet and the Australian Network for Plant Conservation website:

Giblin FR, Carnegie AJ 2014. *Austropuccinia psidii* (Myrtle Rust): Australian and Global Host Lists, http://www.anbg.gov.au/anpc/resources/Myrtle_Rust.html

New host species have been identified through data captured from surveys of natural ecosystems and through public reporting (Biosecurity Queensland). New susceptible species identified since Pegg *et al*. (2014) includes *Syzygium hodgkinsonia* (MS), *Neofabraciae myrtifolia* (RT-MS), *Leptospermum barneyense* (RT), A*rchirhodomyrtus beckleri* (RT-HS), *Syzygium maraca* (RT), *Leptospermum madidum* subsp. *sativum* (RT), *Melaleuca comboyensis* (RT-MS), *Pilidiostigma rhytispermum* (RT-MS), *Xanthostemon fruticosus* (HS) and *Osbornia octodonta* (RT). *Osbornia octodonta* is a Myrtaceous mangrove species with a native range along the Queensland coast, extending into the Northern Territory and northern coastal regions of Western Australia.

The current (2016) host numbers for Australia is 347 species from 57 different genera. Of these, 242 have been identified from infections under field conditions, with the remainder identified from glasshouse screening studies.

Susceptibility of host species

Susceptibility of species under field conditions was primarily assessed in Queensland (Table 3). A total of 180 species have been rated for susceptibility to *A. psidii* with 30 (16.66%) assessed as highly (HS) or extremely susceptible (ES) with no evidence of variability in susceptibility. An additional 11 (6.11%) species susceptibility levels variable and ranging from moderately (MS) to HS or ES, 14 (7.77%) species with individual susceptibility levels ranging from relatively tolerant (RT) to HS or ES, 41 (22.77%) species rated as MS, 16 (8.88%) rated with susceptibility ranges from RT to MS and 68 (37.77%) species rated as RT.

When examining patterns across the different Myrtaceae Tribes, HR and ES species come from Myrteae, Melaleucaeae, Syzygieae, Backhousieae, Eucalypteae, Xanthostemoneae, Kanieae, Chamelaucieae and Lophostemoneae.

Susceptibility data collated to date has identified that of the 23 species listed as threatened (Endangered, Vulnerable, Near Threatened) in Queensland, 11 species are considered highly or extremely susceptible (Table 4). Of the five endangered species, all are considered highly or extremely susceptible. However, the natural range of *Backhousia oligantha* is considered less favourable for disease development based on climatic conditions. The impact of myrtle rust across the natural range of most of these species is still unknown.

Table 3 Current known host list of *Austropuccinia psidii* in Queensland rated for susceptibility levels: Relatively tolerant (RT) = restricted leaf spot or spots only; Moderate susceptibility (MS) = blight symptoms on new shoots and expanding foliage; High susceptibility (HS) = blight symptoms on new shoots and expanding foliage and juvenile stems; Extreme susceptibility (ES) = death of new shoots and severe blighting on all foliage types, shoot and stem dieback. Susceptibility ratings are based on observations to date.

Host name	Tribe*	Disease susceptibility rating	Flower/fruit infection
Acmena hemilampra	Syzygieae	RT	
Acmena ingens	Syzygieae	RT	
Acmena smithii	Syzygieae	RT-MS	X
Acmenosperma claviflorum	Syzygieae	MS	
Agonis flexuosa	Leptospermeae	ES	
Anetholea (Backhousia) anisata	Backhousieae	RT-HS	
Archirhodomyrtus beckleri	Myrteae	RT-HS	
Asteromyrtus brassii	Leptospermeae	RT	
Austromyrtus dulcis	Myrteae	RT-HS	X
Austromyrtus sp. (Lockerbie Scrub)	Myrteae	RT	
Austromyrtus tenuifolia	Myrteae	RT	
Backhousia angustifolia	Backhousieae	RT	
Backhousia bancroftii	Backhousieae	RT	
Backhousia enata	Backhousieae	RT-MS	
Backhousia citriodora	Backhousieae	MS-HS	X
Backhousia gundarara (Prince Regent)	Backhousieae	RT	
Backhousia hughesii	Backhousieae	MS	
Backhousia leptopetala	Backhousieae	RT-HS	
Backhousia myrtifolia	Backhousieae	RT-MS	
Backhousia oligantha	Backhousieae	MS-HS	
Backhousia sciadophora	Backhousieae	RT	
Backhousia subargentea	Backhousieae	RT	
Baeckea frutescens	Chamelaucieae	RT-MS	X
Chamelaucium uncinatum	Chamelaucieae	ES	X
Corymbia citriodora subsp. *variegata**	Eucalypteae	RT	
*Corymbia ficifolia × C. ptychocarpa**	Eucalypteae	RT	
*Corymbia henryi**	Eucalypteae	RT	
*Corymbia torelliana**	Eucalypteae	RT	
Darwinia citriodora	Chamelaucieae	MS	
Decaspermum humile	Myrteae	ES	
Decaspermum humile (North Qld form)	Myrteae	RT	
Eucalyptus carnea	Eucalypteae	RT-HS	
*Eucalyptus cloeziana**	Eucalypteae	RT	
Eucalyptus curtisii	Eucalypteae	RT-HS	
Eucalyptus grandis	Eucalypteae	RT-MS	
*Eucalyptus planchoniana**	Eucalypteae	RT-MS	
*Eucalyptus tereticornis**	Eucalypteae	RT	
*Eucalyptus tindaliae**	Eucalypteae	MS	
Eugenia natalitia	Myrteae	MS	

Eugenia reinwardtiana	Myrteae	ES	X
Eugenia uniflora	Myrteae	MS	X
*Eugenia zeyheri**	Myrteae	MS	
Gossia acmenoides	Myrteae	HS	
Gossia bamagensis	Myrteae	RT	
Gossia bidwillii	Myrteae	RT	
Gossia floribunda	Myrteae	RT	
Gossia fragrantissima	Myrteae	MS	
Gossia gonoclada	Myrteae	HS	
Gossia hillii	Myrteae	HS-ES	
Gossia inophloia	Myrteae	ES	
Gossia lewisensis	Myrteae	MS-HS	
Gossia macilwraithensis	Myrteae	MS	
Gossia myrsinocarpa	Myrteae	MS-HS	X
Gossia punctata	Myrteae	MS	
Homoranthus melanostictus	Chamelaucieae	MS	
Homoranthus papillatus	Chamelaucieae	MS	
Homoranthus virgatus	Chamelaucieae	MS	X
Hypocalymma angustifolium	Chamelaucieae	RT	
Lenwebbia lasioclada	Myrteae	RT	
Lenwebbia prominens	Myrteae	HS	X
Lenwebbia sp. Blackall Range	Myrteae	RT-ES	
Leptospermum barneyense	Leptospermeae	RT	
Leptospermum liversidgei	Leptospermeae	MS	
Leptospermum luehmannii	Leptospermeae	RT	
Leptospermum madidum	Leptospermeae	MS	
Leptospermum madidum subsp. sativum			
Leptospermum petersonii	Leptospermeae	RT	
*Leptospermum semibaccatum**	Leptospermeae	RT-MS	
Leptospermum trinervium	Leptospermeae	MS	
Lindsayomyrtus racemoides	Lindsayomyrteae	RT	
Lithomyrtus obtusa	Myrtaea	RT	
Lophostemon suaveolens	Lophostemoneae	RT	
Melaleuca cheelii	Melaleucaeae	RT	
Melaleuca comboyensis	Melaleucaeae	RT-MS	
Melaleuca fluviatilis	Melaleucaeae	HS	
Melaleuca formosa	Melaleucaeae	RT	
Melaleuca leucadendra	Melaleucaeae	RT-HS	X
Melaleuca linariifolia	Melaleucaeae	RT	
Melaleuca nervosa	Melaleucaeae	HS	
Melaleuca nesophila	Melaleucaeae	RT	
Melaleuca nodosa	Melaleucaeae	HS-ES	
Melaleuca pachyphylla	Melaleucaeae	RT	
Melaleuca paludicola	Melaleucaeae	HS	
Melaleuca polandii	Melaleucaeae	HS	
Melaleuca quinquenervia	Melaleucaeae	RT-ES	X
Melaleuca salicina	Melaleucaeae	RT	
Melaleuca saligna	Melaleucaeae	MS	
Melaleuca viminalis	Melaleucaeae	MS-HS	
Melaleuca viridiflora	Melaleucaeae	HS	
Metrosideros collina	Metrosidereae	RT	
Metrosideros collina × villosa	Metrosidereae	RT	
Metrosideros kermadecensis	Metrosidereae	RT	
Metrosideros thomasii	Metrosidereae	RT	
Mitrantia bilocularis	Kanieae	MS	

Myrciaria cauliflora	Myrteae	RT	
Myrtus communis	Myrteae	MS-HS	X
Neofabraciae myrtifolia	Leptospermeae	RT-MS	
Osbornia octodonta	Osbornieae	RT	
Pilidiostigma glabrum	Myrteae	RT-MS	X
Pilidiostigma rhytispermum	Myrteae	RT-MS	
Pilidiostigma tetramerum	Myrteae	MS	
Rhodamnia acuminata	Myrteae	RT	
Rhodamnia angustifolia	Myrteae	ES	X
Rhodamnia arenaria	Myrteae	MS	X
Rhodamnia argentea	Myrteae	MS-HS	
Rhodamnia australis	Myrteae	HS	X
Rhodamnia blairiana	Myrteae	RT-MS	
Rhodamnia costata	Myrteae	RT-HS	
Rhodamnia dumicola	Myrteae	HS	
Rhodamnia glabrescens	Myrteae	MS	
Rhodamnia maideniana	Myrteae	ES	X
Rhodamnia pauciovulata	Myrteae	MS	
Rhodamnia rubescens	Myrteae	HS-ES	X
Rhodamnia sessiliflora	Myrteae	MS-ES	X
Rhodamnia spongiosa	Myrteae	HS	X
Rhodomyrtus canescens	Myrteae	HS	X
Rhodomyrtus effusa	Myrteae	MS	
Rhodomyrtus macrocarpa	Myrteae	MS	
Rhodomyrtus pervagata	Myrteae	MS-HS	X
Rhodomyrtus psidioides	Myrteae	ES	X
Rhodomyrtus sericea	Myrteae	MS	
Rhodomyrtus tomentosa	Myrteae	MS-HS	X
Rhodomyrtus trineura subsp. *capensis*	Myrteae	MS	
Ristantia pachysperma	Kanieae	MS-HS	
Ristantia waterhousei	Kanieae	RT	
Sphaerantia discolor	Kanieae	MS	
Stockwellia quadrifida	Eucalypteae	HS	
Syzygium angophoroides	Syzygieae	MS	
Syzygium apodophyllum	Syzygieae	RT	
Syzygium aqueum	Syzygieae	RT	
Syzygium argyropedicum	Syzygieae	RT	
Syzygium armstrongii	Syzygieae	RT	
Syzygium australe	Syzygieae	RT-MS	X
Syzygium bamagense	Syzygieae	MS	
Syzygium banksii	Syzygieae	MS	
Syzygium boonjee	Syzygieae	RT	
Syzygium canicortex	Syzygieae	RT	
Syzygium cormiflorum	Syzygieae	RT	
Syzygium corynanthum	Syzygieae	RT-HS	
Syzygium cryptophlebium	Syzygieae	MS	
Syzygium cumini	Syzygieae	MS	
Syzygium dansiei	Syzygieae	RT	
Syzygium endophloium	Syzygieae	RT	
Syzygium erythrocalyx	Syzygieae	RT	
Syzygium eucalyptoides	Syzygieae	HS	
Syzygium eucalyptoides subsp. *eucalyptoides*	Syzygieae	MS	
Syzygium forte subsp. *forte*	Syzygieae	RT	
Syzygium forte subsp. *potamophilum*	Syzygieae	RT	

Syzygium jambos	Syzygieae	ES	X
Syzygium hodgkinsoniae	Syzygieae	RT-HS	
Syzygium kuranda	Syzygieae	MS	
Syzygium luehmannii	Syzygieae	MS	
Syzygium luehmannii × S. wilsonii	Syzygieae	RT	
Syzygium macilwraithianum	Syzygieae	RT-HS	
Syzygium maraca	Syzygieae	RT	
Syzygium minutiflorum	Syzygieae	RT	
Syzygium moorei	Syzygieae	RT	
Syzygium nervosum	Syzygieae	HS	X
Syzygium oleosum	Syzygieae	RT-HS	
Syzygium paniculatum	Syzygieae	RT	
Syzygium pseudofastigiatum	Syzygieae	RT	
Syzygium puberulum	Syzygieae	MS	
Syzygium rubrimolle	Syzygieae	RT	
Syzygium suborbiculare	Syzygieae	MS	
Syzygium tierneyanum	Syzygieae	RT	X
Syzygium wilsonii	Syzygieae	RT	
Syzygium xerampelinum	Syzygieae	MS	
Thryptomene saxicola	Chamelaucieae	RT-MS	X
Tristania neriifolia	Tristanieae	MS	
Tristaniopsis exiliflora	Kanieae	HS	X
Tristaniopsis laurina	Kanieae	RT	
Uromyrtus metrosideros	Myrteae	MS	
Uromyrtus tenella	Myrteae	RT	
Waterhousea floribunda	Syzygieae	RT	
Waterhousea hedraiophylla	Syzygieae	RT	
Waterhousea mulgraveana	Syzygieae	RT	
Waterhousea unipunctata	Syzygieae	RT-MS	
Xanthostemon chrysanthus	Xanthostemoneae	RT-MS	
Xanthostemon oppositifolius	Xanthostemoneae	HS	
Xanthostemon youngii	Xanthostemoneae	MS	X
Xanthostemon fruticosus	Xanthostemoneae	HS	

* Tribes according to Wilson *et al*. 2005

Table 4 List of threatened Myrtaceae in Queensland and their myrtle rust susceptibility ratings

Queensland Conservation Status	Myrtle rust susceptibility rating
Endangered species	
Backhousia oligantha	Highly/Extremely susceptible
Gossia fragrantissima	Highly/Extremely susceptible
Gossia gonoclada	Highly/Extremely susceptible
Lenwebbia sp. Blackall Range	Highly/Extremely susceptible
Rhodamnia angustifolia	Highly/Extremely susceptible
Vulnerable	
Eucalyptus argophloia	Highly/Extremely susceptible
Homoranthus papillatus	Moderately susceptible
Leptospermum luehmannii	Relatively tolerant
Mitrantia bilocularis	Moderately susceptible
Ristantia waterhousei	Relatively tolerant
Sphaerantia discolor	Moderately susceptible

Syzygium moorei	Relatively tolerant
Xanthostemon oppositifolius	Highly/Extremely susceptible
Near Threatened	
Eucalyptus curtisii	RT-Highly/Extremely susceptible (Epicormic)
Gossia inophloia (Austromyrtus)	Highly/Extremely susceptible
Lenwebbia prominens	Highly/Extremely susceptible
Melaleuca formosa (Callistemon)	Unknown
Rhodamnia glabrescens	Unknown
Rhodamnia pauciovulata	Unknown
Stockwellia quadrifida	Highly/Extremely susceptible
Syzygium aqueum	Relatively tolerant
Syzygium macilwraithianum	Relatively tolerant

Distribution of Austropuccinia psidii in Australia

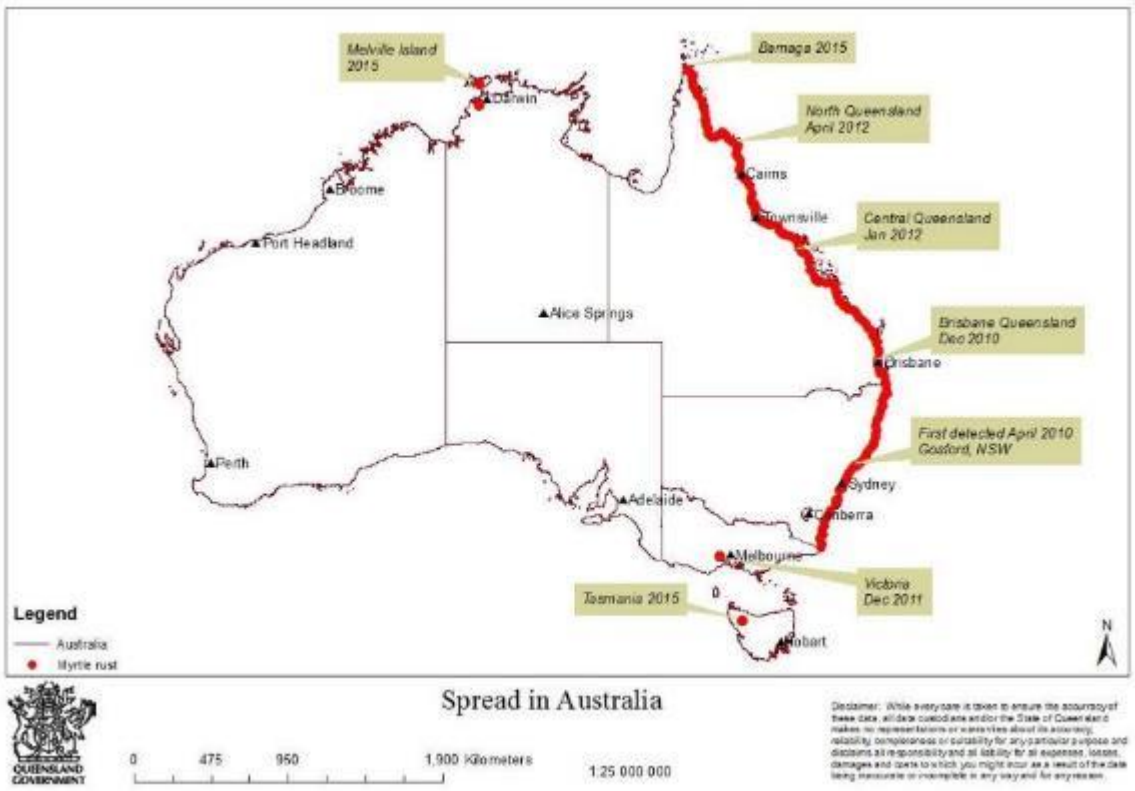


Figure 15 Map showing the dates of original detection of *Austropuccinia psidii* and general distribution across New South Wales and Queensland

The distribution of *A. psidii* (Fig. 15, 16) continues to expand with detections now extending from Tasmania, along the entire east coast of Australia as far north as Bamaga at the tip of Cape York Peninsula and then most recently in the Tiwi Islands and Darwin in the Northern Territory. Reports from west of the Great Dividing Range still remain low with reports generally following periods of wet weather. Detection in these lower rainfall areas have either been in residential gardens (e.g. Geraldton Wax, Warwick, Qld) or along creek/river systems (*Melaleuca* sp., Chillagoe, Qld) where conditions are likely to be more favourable for both host growth and disease development. However, no detailed surveys have been conducted in these regions. The impact that *A. psidii* will have in Darwin and surrounding areas, such as Kakadu National Park, remain unknown.

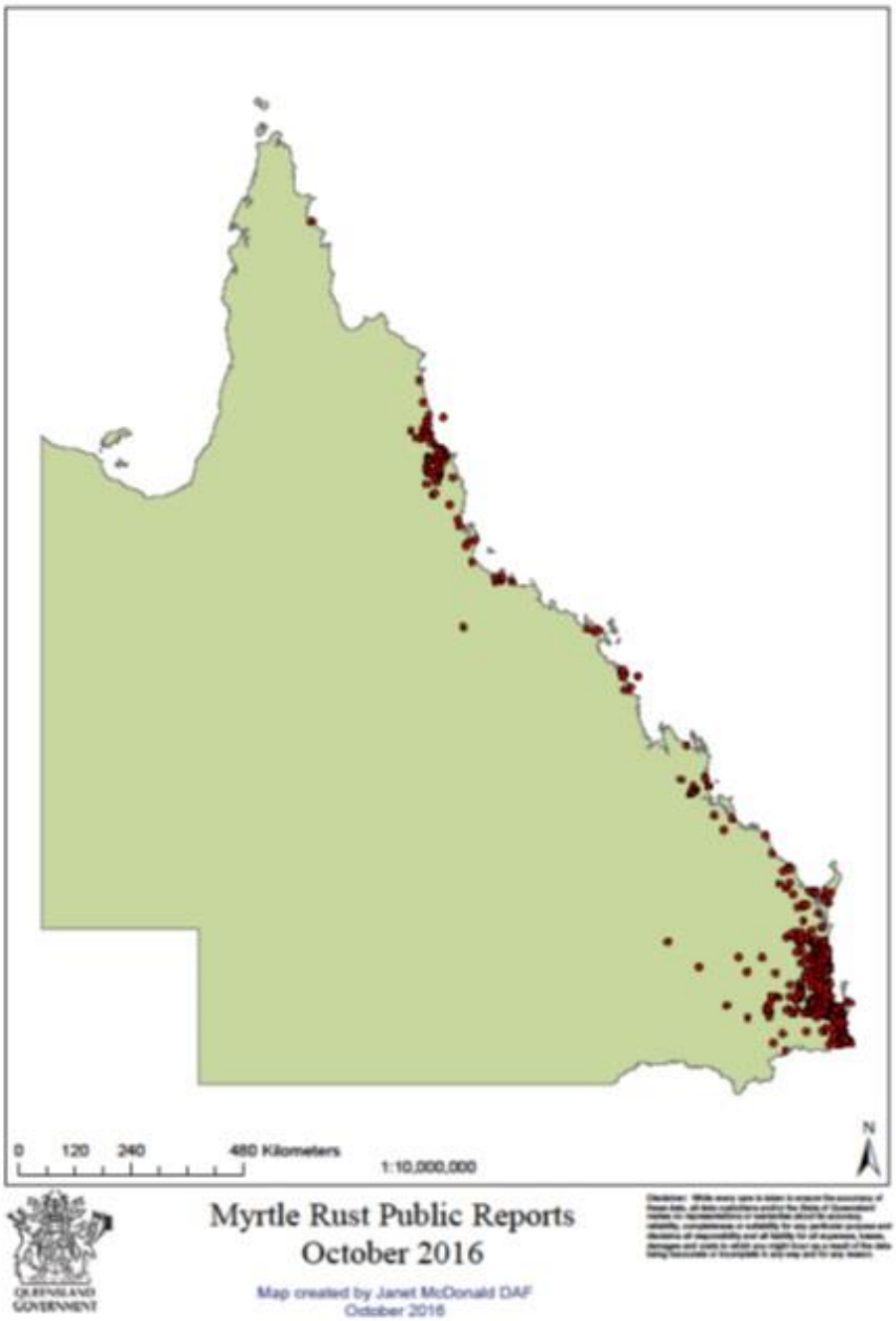


Figure 16 –Public reports of *Austropuccinia psidii* in Queensland between January 2011 and October 2016

Impact of *Austropuccinia psidii* on species of Myrtaceae

Effect of repeated damage by Austropuccinia psidii on Rhodamnia rubescens: Olney State Forest disease exclusion trial

Monthly application of the fungicide triadimenol was effective in controlling *A. psidii* on *R. rubescens* in the native environment (Figs 17–18). If fungicide application extended beyond this time-frame, control was not effective (Supplementary Fig. 1, arrow). Active *A. psidii* infection (sori producing yellow urediniospores) was observed at every assessment date on untreated trees. There was a significant difference ($p < 0.001$) in crown transparency between treated, untreated and partially treated trees (Fig. 17). There was a significant autocorrelation (ρ) between values over time for each tree ($\rho = 0.2$, $p = 0.01$). The smoother terms were all significant ($p < 0.001$) and had 5, 3 and 5 degrees freedom for untreated, treated and partially treated trees, respectively. Based on data from March 2013 to October 2014, crown transparency was moderately correlated with incidence ($r = 0.36$, $p < 0.001$) and severity ($r = 0.38$, $p < 0.001$) of disease on immature leaves and with percentage new flush ($r = 0.51$, $p < 0.001$); incidence and severity were highly correlated ($r = 0.86$, $p < 0.001$); and percentage new flush was moderately correlated with incidence ($r = 0.34$, $p < 0.001$) and severity ($r = 0.31$, $p < 0.001$) of disease on immature leaves.

This trial allowed observations of disease progression, and the subsequent impact of this on trees, over time. At the beginning of the trial, all trees had similar crown transparency (Fig. 17) as well as incidence and severity on mature and immature leaves (data not shown). As the trial progressed, incidence and severity of *A. psidii* infection on treated trees effectively became zero while disease on untreated trees fluctuated, but was significantly greater than on treated trees (Fig. 19). This corresponded with an increase in crown transparency on untreated trees and a decrease on treated trees (Fig. 17). Leaf production (leaf flush) generally followed a trend of increasing during warm wet periods of the year (i.e. spring to summer), but this was not always consistent (Fig. 19). Incidence and severity of *A. psidii* generally followed a trend of increasing during periods of high rainfall and reducing during dry periods over winter (Fig. 19), but again this was not always consistent. A similar trend, with a slight time lag, was observed for crown transparency (Fig. 17). Generally, peaks in incidence and severity occurred a month or so following peaks in leaf flush (Fig. 19). A more detailed epidemiological study will be carried out on this data.

Time-series observations of untreated trees revealed that immature leaves became infected and often distorted and died. This resulted in a proliferation of new shoots and immature leaves that subsequently became infected and distorted with many dying. Within six months of the trial commencing, any new (immature) leaves on untreated trees were noticeably smaller than those on treated trees (see section below). Over time, mature leaves that had been retained on untreated trees prior to the trial beginning were shed, with little replacement (thus increasing crown transparency). Occasionally, a new flush of leaves did not coincide with conditions optimal for disease, resulting in little infection and a cohort of leaves surviving to maturity (and a subsequent decrease in crown transparency). In contrast, on treated trees, immature leaves were able to fully expand and were retained on trees, thus resulting in a decrease in crown transparency.

When we divided the treated trees into two groups in June 2013, we saw no noticeable change in disease incidence and severity or crown transparency in the now untreated (partially treated) trees for six months, then a sharp increase in incidence and severity in early 2014 (Fig. 19) followed by an increase in crown transparency (Fig. 17), significantly different ($p < 0.001$) from the treated trees.

In December 2013 we began to observe some untreated trees almost completely defoliated and with any retained immature leaves distorted and dead (e.g., Fig. 18). These trees subsequently produced a small amount of new flush, which was again severely infected, and by August 2014 these trees ceased to produce new flush and had died. A separate assessment of 100 trees in this stand (see section below), from 1.0 to 15.0 m tall, revealed that 53% of trees (all 1.0 to 4.0 m tall) had died by October 2014. Thus, tree mortality had occurred in this native ecosystem less than four years after *A. psidii* had established in this forest.

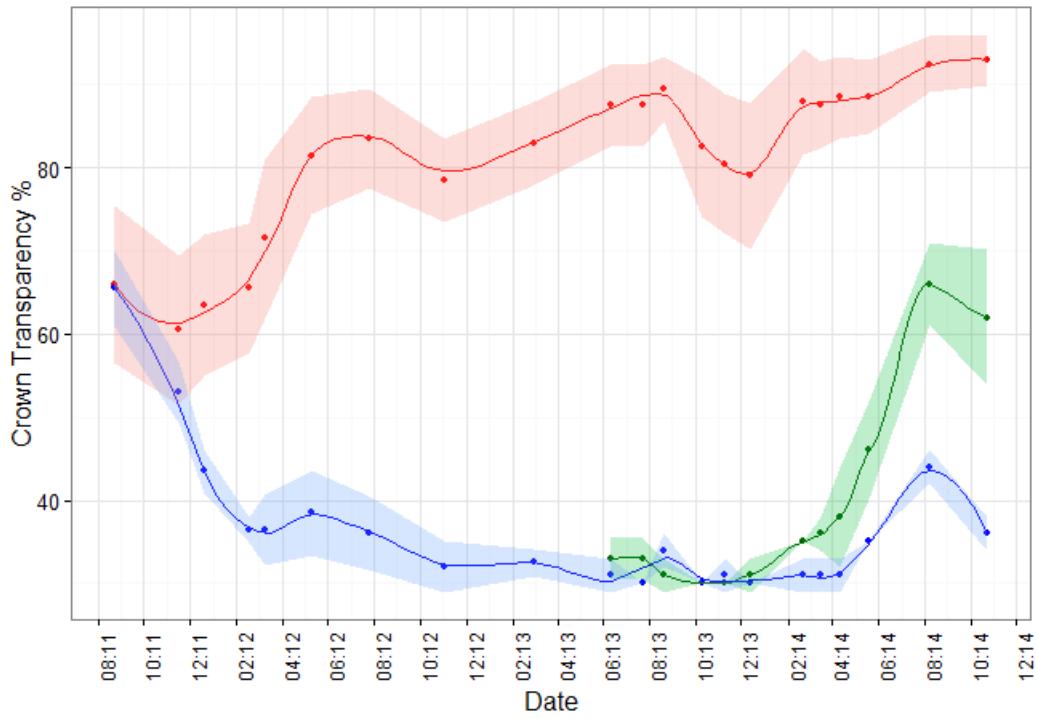


Figure 17 Time series plot of mean crown transparency of all *Rhodamnia rubescens* trees for the disease exclusion trial at Olney State Forest. The lines are locally weighted scatterplot smoothing curves (loess) and the shaded areas are the 95% confidence interval. Red = untreated, Blue = treated, Green = partially treated (treatment ceased in June 2013).

Figure 18 Comparison of untreated tree (**a**) and treated tree (**b**) of *Rhodamnia rubescens* in the disease exclusion trial at Olney State Forest 24 months after commencement of the trial and approx. three years after *Austropuccinia psidii* established in the forest.

Quantification of diseased leaf area and leaf size

Severity of *A. psidii* on leaves collected from the disease exclusion trial at Olney SF was significantly ($p < 0.001$) higher on the untreated compared to the treated trees for all three leaf classes, but more so for the mature and immature leaves (Table 5). The size of leaves (leaf area) was not significantly different between treated and untreated trees for the old and mature leaf class, but was significantly different between treatments for the immature leaf class ($p = 0.004$) (Table 5). Immature leaves were produced generally 4–5 months after initiation of the trial, and so we expected some influence of reducing crown transparency of untreated trees on leaf production. However, previous crown transparency on trees from which leaves were collected was not a significant factor in determining leaf area or disease severity.

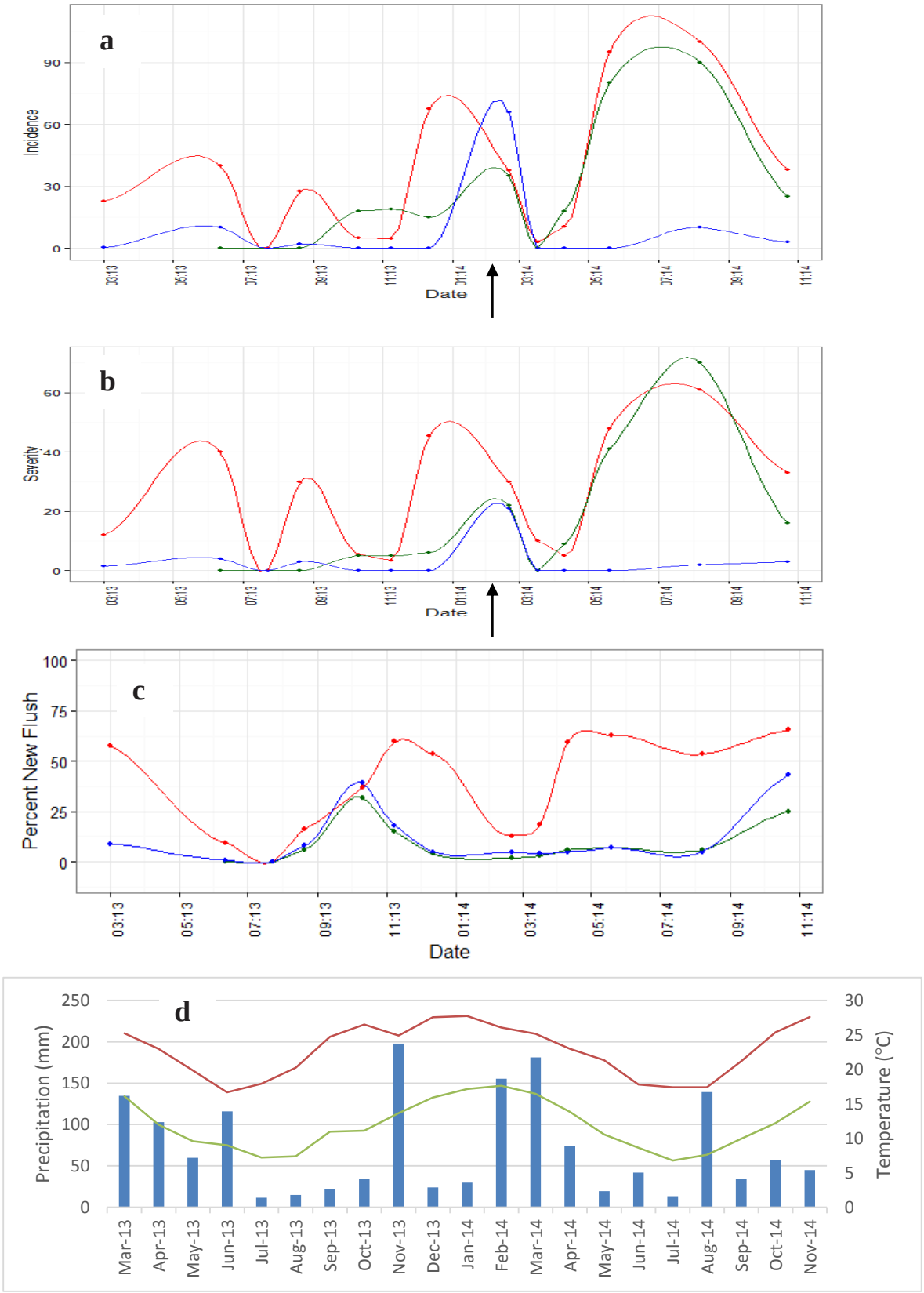


Figure 19 Time series plots for **(a)** incidence and **(b)** severity of *Austropuccinia psidii* on immature leaves, **(c)** percentage of crown with new flush (indicating growth event), and **(d)** temperature (mean max and mean min) and mean monthly rainfall (www.bom.au). For consistency, only data from March 2013 is shown. Arrows indicate increase in disease incidence and severity following delay in fungicide treatment.

Table 5 Mean and standard error (SE) of percentage severity of *Austropuccinia psidii* and leaf area of old, mature and immature leaves from treated and untreated trees analyzed with the image processing software QUANT from the Olney SF disease exclusion trial.

		***A. psidii* severity (%)**		**Leaf Area (mm^2)**	
Treatment	**Leaf class**	**Mean**	**SE**	**Mean**	**SE**
0	Old	16.81	3.70	71.32	8.51
1	Old	6.40	0.78	68.83	8.27
0	Mature	11.06	2.54	58.03	11.43
1	Mature	0.36	0.08	69.12	8.50
0	Immature	19.13	5.81	14.03	3.21
1	Immature	0.97	0.50	49.44	5.61

0= untreated; 1 = treated

The impact of Austropuccinia psidii on selected species across their native range

For *R. rubescens*, we assessed 43 sites across the native range from Murramarang National Park (35° 40’ 45” S, 150° 16’ 55” E) near Batemans Bay, NSW, to Traveston Crossing (26° 11’ 43 S, 152° 25’ 30” E) near Gympie, Queensland (Fig. 20), with *A. psidii* present at all sites. The mean crown transparency was 76.29% (SE 0.81%), with the majority (70%) of trees having greater than 60% transparency (Fig. 21). Based on the disease exclusion trial, and *a posteriori* knowledge of the species, we surmise the normal crown transparency of *R. rubescens* in an understorey is approx. 30–35%. We observed tree mortality at 18 sites, mostly only a few trees, but five sites with between 25–40% of trees dead, one site with half the trees dead and another with three-quarters of the trees dead (Table 6). Overall, 11.5% of trees surveyed were classed as dead (Fig. 21). There was no evidence of any other primary causal agent that could have been responsible for this tree mortality. Mean disease incidence was greater on immature leaves (56.37% [SE 2.08%]) than on mature leaves (29.76% [SE 1.16%]), with a mean disease rating (score) of 2.40 (SE 0.08). Crown transparency was significantly negatively correlated with tree height, and positively correlated with disease rating and incidence of disease on mature leaves (Table 7), but not with incidence of disease on immature leaves or previous presence of rust at the location. The disease rating score was highly correlated with incidence on immature leaves ($r = 0.89$, $p < 0.001$).

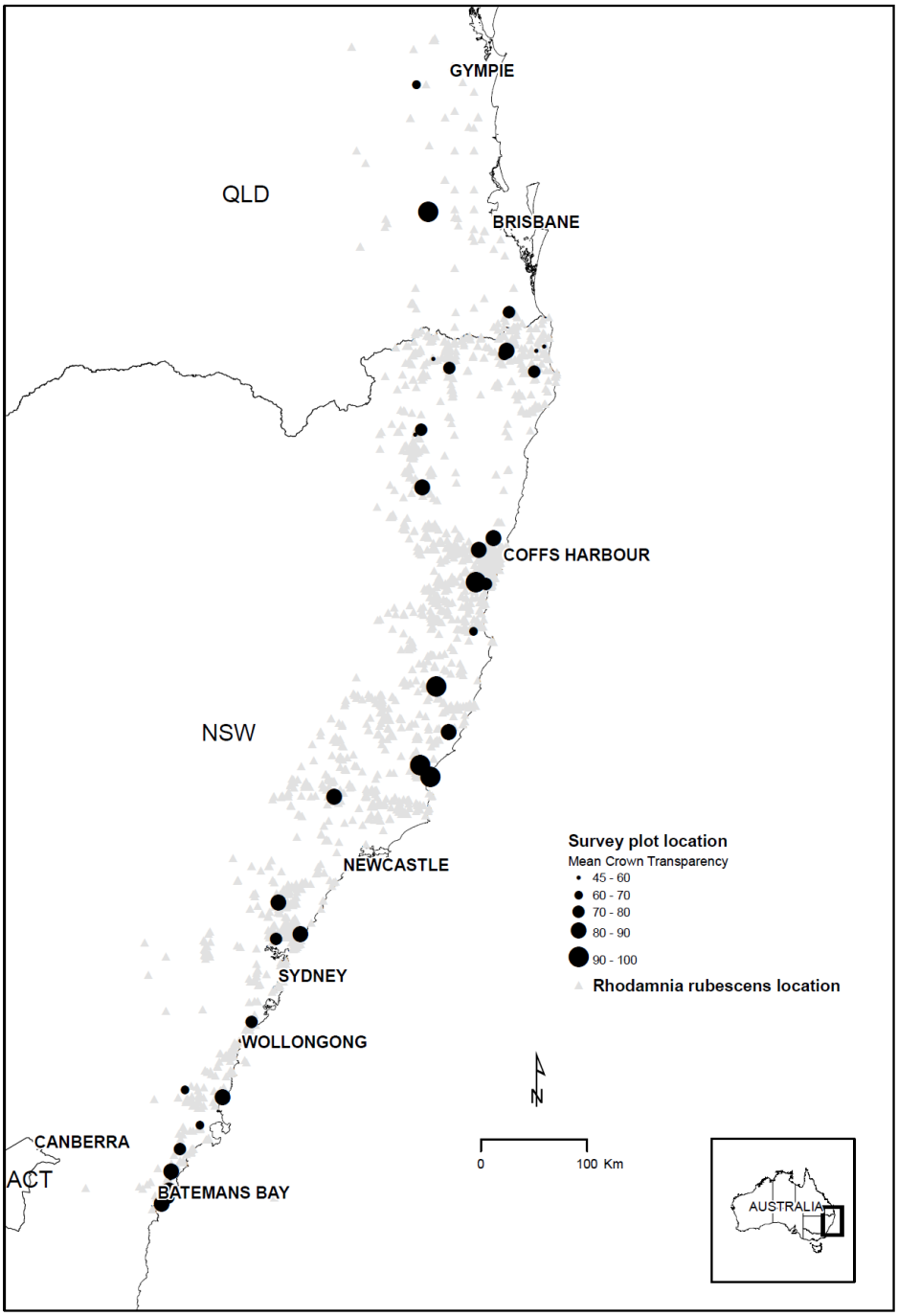


Figure 20. Map of *Rhodamnia rubescens* survey sites. Native distribution of *R. rubescens* (grey triangles) obtained from Atlas of Living Australia (www.ala.org.au) and mean crown transparency of survey plots (graduated circles).

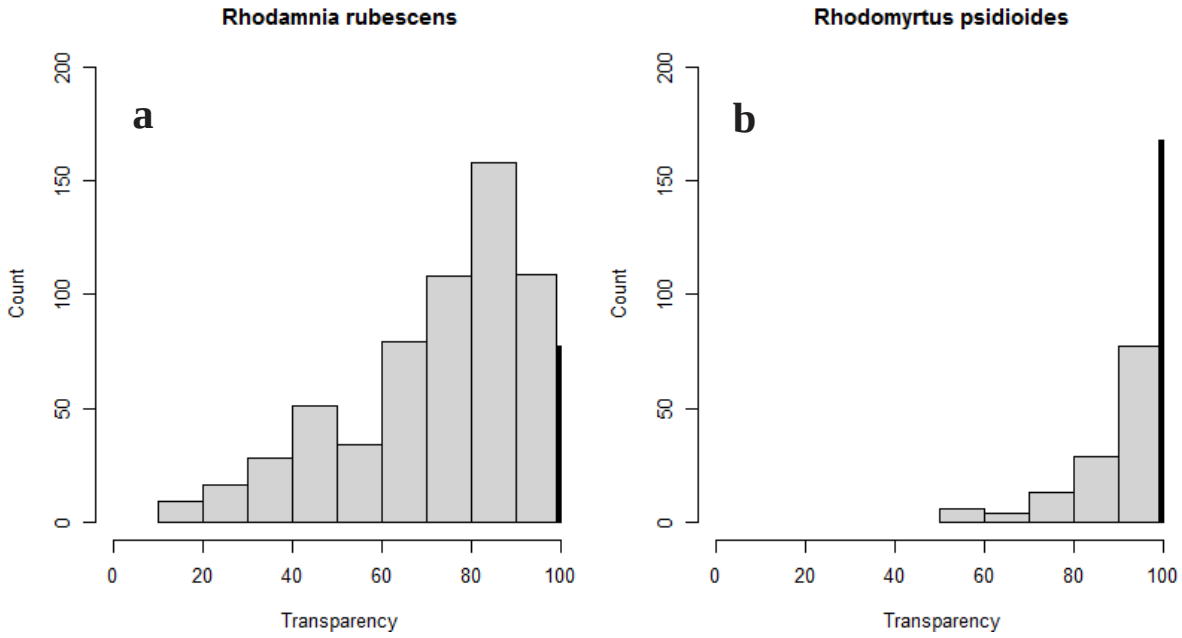


Figure 21: Total tree counts (across all sites) for crown transparency (grey bars)—in 10% classes—and tree mortality (black bar) associated with *Austropuccinia psidii* for (**a**) *Rhodamnia rubescens* and (**b**) *Rhodomyrtus psidioides* from field assessments across the species' native ranges.

Table 6: Percentage of *Rhodamnia rubescens* trees assessed as dead at each survey site.

Location	Percent dead
Austinmer, NSW	0.0
Bagawa SF*, NSW	15.0
Bongil Bongil NP*, NSW	10.0
Brill Brill SF, NSW	30.0
Brisbane Water NP, NSW	0.0
Chichester SF, NSW	8.3
Conglomerate SF, NSW	0.0
Cunninghams Gap, Qld	0.0
Ewingar SF 1, NSW	0.0
Ewingar SF 2, NSW	0.0
Flat Rock SF, NSW	0.0
Gibraltar Range NP, NSW	0.0
Gold Creek Reservoir, Qld	73.3
Goongery, NSW	15.4
Kiwarrak SF, NSW	26.3
McDonald SF, NSW	4.8
Mebbin NP 2, NSW	0.0
Mebbin NP 4, NSW	0.0
Middle Brother SF, NSW	0.0
Morton NP, NSW	0.0
Murramalong NP 1, NSW	4.5
Murramalong NP 2, NSW	8.3
Murramalong NP 3, NSW	16.7
Olney SF 1, NSW	53.3
Olney SF 2, NSW	0.0

Pine Creek SF, NSW	0.0
Red Head, NSW	40.0
Richmond Range NP, NSW	0.0
Royal NP, NSW	23.3
Seven Mile Beach NP, NSW	16.7
Tallebudgera Valley 1, Qld	25.0
Tallebudgera Valley 2, Qld	0.0
Termeil SF 1, NSW	0.0
Termeil SF 2, NSW	0.0
Tomerong SF, NSW	0.0
Tomerong, NSW	0.0
Traveston Crossing 1, Qld	0.0
Traveston Crossing 2, Qld	13.3
Upper Burringbar, NSW	0.0
Upper Sleepy Hollow, NSW	0.0
Wambina NR*, NSW	11.1
Way Way SF, NSW	0.0
Yabbra SF, NSW	0.0

* SF = State Forest; NP = National Park; NR = Nature Reserve

Table 7. ANOVA table for fixed effects of field assessments of *Rhodamnia rubescens.*

Variables	Value	SE	t-value	p-value
Intercept	70.13	3.57	19.66	<0.001
Disease rating	3.17	0.66	4.77	<0.001
Height (m)	-2.15	0.44	-4.90	<0.001
Disease incidence on mature leaves	0.20	0.03	6.04	<0.001

For *R. psidioides,* we assessed 18 sites from Wambina Nature Reserve (33° 24’ 60” S, 151° 20’ 34” E) near Gosford, NSW, to Tallebudgera Valley (28° 7’ 15” S, 153° 12’ 48” E) near Beechmont, Queensland (Fig. 22), with *A. psidii* present at all sites. The mean crown transparency was 94.88% (SE 0.53%), with the majority of trees (82%) having greater than 90% transparency (Fig. 21). Based on *a posteriori* knowledge of the species, we surmise the normal crown transparency of *R. psidioides* in an understorey is approx. 50%. All but 3 sites had exceptional levels of tree mortality (Table 8), with four sites having 50–75% dead trees, two sites with 95% dead trees, and another two sites with all trees (100%) dead. Overall, 56.5% of trees surveyed were dead (Fig. 21). Trees of all sizes were killed, including trees as tall as 12 m in height (Fig. 23), with the stage of decline indicating some had been dead for at least one year (i.e. two years after *A. psidii* established in the region). There was no evidence of any other primary causal agent that could have been responsible for this tree mortality. Mean disease incidence was greater on immature leaves (94.46% [SE 2.12%]) than on mature leaves (38.44% [SE 3.18%]), with a mean disease rating (score) of 3.87 (SE 0.05). Crown transparency was not significantly correlated with any other variable assessed (data not shown). The random location effect was significant ($p < 0.001$).

For both these species, we observed severely damaged trees with epicormic shoots infected and killed by *A. psidii.* Ad hoc observations during surveys revealed few regenerating seedlings or suckers, and all with *A. psidii* damage.

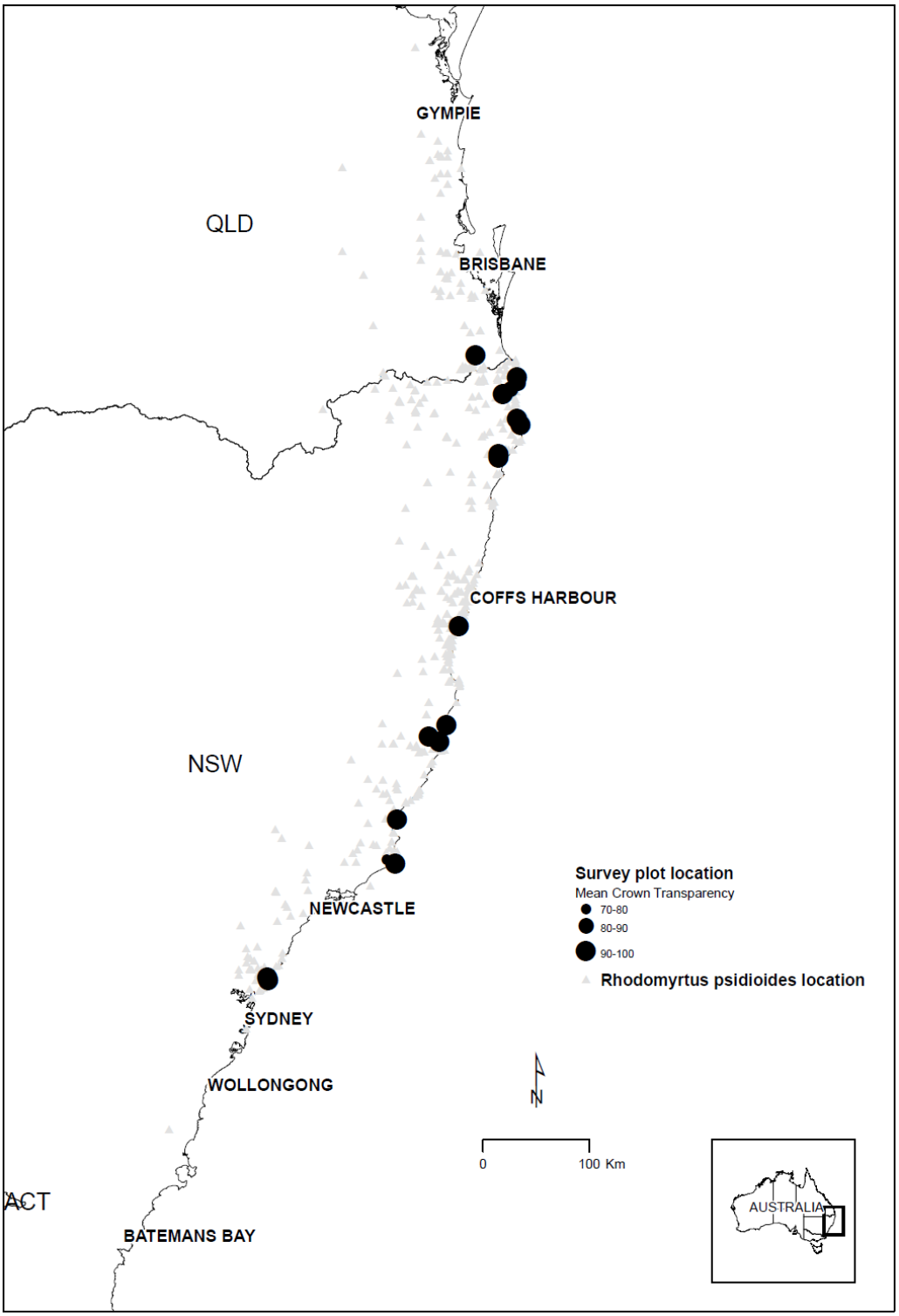


Figure 22 Map of *Rhodomyrtus psidioides* survey sites. Native distribution of *R. psidioides* (grey triangles) obtained from Atlas of Living Australia (www.ala.org.au), and mean crown transparency of survey plots (graduated circles).

Table 8: Percentage of *Rhodomyrtus psidioides* trees assessed as dead at each survey site

Location	Percent dead 2014
Baggotville 1, NSW	69.2
Baggotville 2, NSW	60.0
Bongil Bongil NP*, NSW	72.5
Broken Ridge, NSW	100.0
Cudgen NR*, NSW	0.0
Ewingsdale, NSW	100.0
Goolawah RP*, NSW	24.0
Myall Lakes NP, NSW	23.1
Port Macquarie 1, NSW	11.8
Port Macquarie 2, NSW	0.0
Red Head, NSW	0.0
Seal Rocks RP, NSW	20.0
Tallebudgera Valley, Qld	96.7
Tweed Coast, Qld	15.0
Upper Burringbar, NSW	95.7
Upper Sleepy Hollow, NSW	12.5
Wamberal Lagoon NR, NSW	50.0
Wambina NR, NSW	33.3

* NP = National Park; NR = Nature Reserve; RP = Regional Park

Figure 23 Native stand of mature *Rhodomyrtus psidioides* in north coastal NSW where the majority of trees have been killed within 2–3 years of *Austropuccinia psidii* establishing. Photo P. Entwistle.

Progression of decline in Rhodomyrtus psidioides and Rhodamnia rubescens

Table 9– Progression of decline in selected sites of *Rhodomyrtus psidioides* based on the number of dead trees when assessed in 2014 and then again in 2016.

Location	Percent dead 2014	Percent dead 2016
Bongil Bongil NP*, NSW	72.5	100
Port Macquarie 1, NSW	11.8	69.2
Tallebudgera Valley, Qld	96.7	100

Figure 24 Decline of *Rhodomyrtus psidioides* in Tallebudgera Valley with severe dieback and death in 2014 and absence of regeneration and change in species composition in 2016

Three *R. psidioides* sites originally assessed in 2014 were again assessed in 2016 to determine rates of decline (Table 9). In two of the three sites assessed all trees are now dead with a 57.4% increase in tree mortality recorded at Bongil Bongil NP, NSW. No evidence of root sucker regeneration or seedling germination was evident at Tallebudgera (Fig. 24). Rhodomyrtus psidioides at this site has been replaced by other species including the noxious weed lantana. However, root sucker regeneration has been recorded at a number of sites, some of which were not included in the original surveys. At a single site assessed at Shark Bay, Iluka in NSW 98% of root suckers showed evidence of myrtle rust infection and only 11.6% had low levels of infection at the time of assessment (Fig. 25).

Figure 25 *Rhodomyrtus psidioides* root sucker development (Shark Bay, Iluka, NSW) was identified under dead adult trees with *Austropuccinia psidii* infection on new growth flush (a) and juvenile stems (b) causing dieback (c). A single root sucker was free of symptoms (d) at the time of assessment.

Five *Rhodamnia rubescens* sites were re-assessed in 2016 (two years after the original assessments) to determine rate of decline in the species. Increases in tree mortality was recorded at all sites (Table 10) with decline in tree health observed with dramatic foliage loss, particularly in the lower canopy (Fig. 26, 27). Seedling germination/regeneration was not been observed at any of the sites.

Table 10 Progression of decline in selected sites of *Rhodamnia rubescens* based on the number of dead trees when assessed in 2014 and then again in 2016.

Location	Percent dead 2014	Percent dead 2016
Gold Creek Reservoir, Qld	73.3	91.6
Tallebudgera Valley 1, Qld	25	30
Tallebudgera Valley 2, Qld	0	30.8
Bongil Bongil NP, NSW	10	50
Royal NP, NSW	23.3	50

Figure 26–Comparison of dieback levels on *Rhodamnia rubescens* at Tallebudgera Valley in (a) 2014 and (b) 2016 showing pronounced defoliation in the lower half of the canopy as a result of repeated *Austropuccinia psidii* infection.

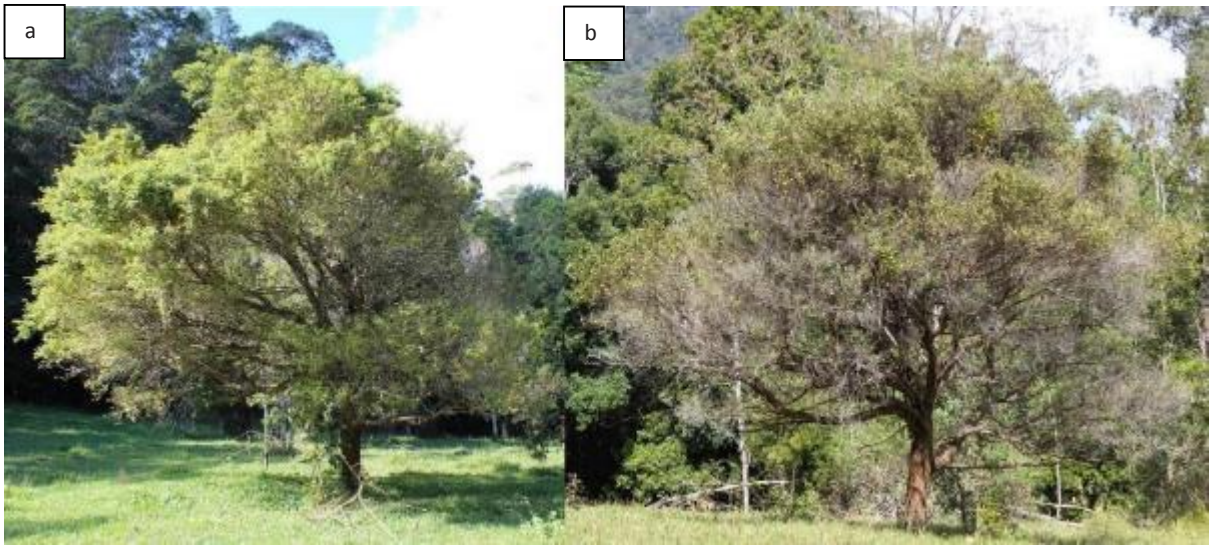


Figure 27– Comparison of dieback levels on *Rhodamnia rubescens* at Tallebudgera Valley in (a) 2014 and (b) 2016 showing pronounced defoliation in the lower half of the canopy as a result of repeated *Austropuccinia psidii* infection

Other Myrtaceae assessed

Table 12 Myrtaceae species assessed for impact of myrtle rust infection within native ecosystems

Myrtaceae	Location	Year assessed	Number trees	Average transparency rating	Dead (%)
Gossia myrsinocarpa	Lake Eacham, Qld	2014	16	62.81	0
	Kuranda Range, Qld	2014	9	53	0
	Barron Falls, Qld	2014	5	73	0
	Clohesy River, Koah, Qld	2014	2	85	0
Rhodamnia sessiliflora	Lake Eacham, Qld	2014	19	58.42	0
	Kuranda Range, Qld	2014	4	47.5	0
	Clohesy River, Koah, Qld	2014	2	50	0
Tristaniopsis exiliflora	Clohesy River, Koah, Qld	2014	7	64.091	0
Lenwebbia prominens	Boomerange Ck, NSW	2015	11	52.27	0
	Minion Falls, NSW	2015	15	55.67	0
	Minion Falls site 2, NSW	2016	9	45.55	0
	Telephone Rd, Ellangowan, NSW	2016	14	57.14	0
Lenwebbia sp. Blackall Range	Doonan Reserve, Doonan, Qld	2014	19	87.89	15.79
	Mary Cairncross Scenic Reserve, Qld	2014	5	53	0
	Eudlo Site 1	2014	1	35	0
	Eudlo Site 2	2014	2	50	0
Rhodamnia maideniana	Tallebudgera Valley	2014	20	68.75	0
Rhodamnia maideniana	Tallebudgera Valley	2016	47	91.34	29.78
Decaspermum humile	Tallebudgera Valley	2016	39	95.61	53.85

Other species of Myrtaceae

The following species were assessed for impact within the native environments but surveys were only conducted at a limited number of sites.

Gossia myrsinocarpa was assessed at four sites in far north Queensland (Table 12) with infection found on all plants assessed with impact at the time of assessment predominantly on new growth flush, resulting in shoot dieback. No tree deaths were recorded at the time but infection on flowering structures was observed.

Rhodamnia sessiliflora was assessed at three different sites with varying levels of impact recorded. Dieback levels were low at all sites, with damage restricted to foliage blighting and some shoot death. Infection on flowers and fruits was also observed.

Tristaniopsis exiliflora is a key species in river ecosystems in tropical regions of Queensland. *Austropuccinia psidii* infection was identified on all life stages with infection found on regenerating seedlings, saplings, juvenile foliage on mature trees, epicomic regrowth and flower buds. However, assessments were only conducted at a single site but reports of infection from other sites across north Queensland have been recorded (K. Kupsch, P. Entwistle pers. Comm).

Stands of *Lenwebbia* sp. Blackall Range were selected for assessment through knowledge of Queensland Government Botanists, Sunshine Coast Council and local ecologists as well as location data obtained from the Atlas of Living Australia (www.ala.org.au/). A total of 27 trees, ranging in height from 0.3 to 7 m, were assessed from across three sites Doonan (Doonan Reserve), Maleny (Mary Cairncross Reserve) and Eudlo (Table 12).

Evidence of *A. psidii* infection was recorded on all but one tree of *Lenwebbia* sp. Blackall Range, with 85% of trees assessed having a transparency rating of greater than 50% and 51% of these trees with >80% transparency. All trees assessed at the Doonan Reserve had transparency rates >75%. Three of these trees were totally defoliated as a result of repeated infection. On trees where foliage remained, branch dieback was evident (Fig. 28).

Figure 28. *Lenwebbia* sp. Blackall Range saplings with dieback and infection (a,b) and trees 100% defoliated as a result of repeat infection by *A. psidii* (c,d).

While no deaths of *Rhodamnia maideniana* were recorded at a Tallebudgera Valley site in 2014, 30% of trees assessed in 2016 were found to be dead (Table 12). Significant levels of dieback were identified on the remaining trees with repeated infection by *A. psidii* causing defoliation, shoot and branch dieback and branch death. While plants were actively producing a new growth flush at the time of the 2016 assessment, all shoots and juvenile foliage were infected (Fig. 29).

Figure 29 Understory rainforest species *Rhodamnia maideniana* in 2014 (a) showing infection on new growth flush but limited levels of defoliation in comparison to the significant decline observed in 2016 where the majority of trees were defoliated with all branches showing evidence of dieback.

Although common in northern New South Wales and Queensland, the impact of *A. psidii* on *Decaspermum humile* has not been well studied. To date only assessments of trees in botanic gardens (Fig. 30) and a single site in Tallebudgera Valley have been conducted. Considering 53% of trees assessed were dead, the species warrants more attention. An average transparency score greater than 95 also suggests that the remaining trees are in severe decline.

Figure 30 *Decaspermum humile* in an ex-situ planting at Lismore Botanic Gardens with significant dieback as a result of repeated *A. psidii* infection. New coppice shoots emerging along branches were all infected.

Predicting Austropuccinia psidii impact on Myrtaceae

Large collections of Myrtaceae from different regions and ecotypes across Australia are present in ex-situ plantings in Botanic Gardens at Mt Coot-tha, Brisbane. Rainforest and some coastal Myrtaceae of significance in northern New South Wales are planted within the Lismore Botanic Gardens. Both sites have been utilised to examine host range when myrtle rust was first detected and to examine potential impact on species as *A. psidii* continues to spread and the effects of repeated infection over time are realised. All species distriubution maps are from The Australasian Virtual Herbarium.

Myrtle rust was first detected in the Mt Coot-tha Botanic Gardens on the 6th of May 2011 on the following species:

Rhodomyrtus psidioides, Rhodomyrtus canescens, Rhodamnia arenaria, Rhodamnia maideniana, Rhodamnia spongiosa, Decaspermum humile, Backhousia sciadophora, Chamelaucium uncinatum

Within three years of rust being reported from the gardens five species died as a result of repeated infection and many others showed evidence of severe decline including defoliation, branch dieback and death.

Species "extinct" from the gardens include:

Rhodomyrtus psidioides – **Native guava**

Original rating – Extremely susceptible

Considered widespread and common in New South Wales and south-east Queensland

Impact of myrtle rust:

- Infection on flowers and fruit
- Fruit reported to be empty of seed
- Death of mature trees, saplings and seedlings

Chamelaucium uncinatum – **Geraldton wax**

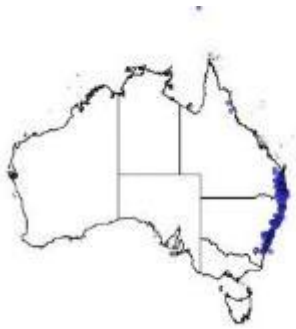
Original rating – Extremely susceptible

Native to south-west Western Australia

Impact of myrtle rust:

- Infection of flower buds and flowers
- Death of trees

Rhodamnia rubescens – **Scrub turpentine**



Original rating – Highly - Extremely susceptible

Widespread and common in rainforests in New South Wales and SE Queensland

Impact of myrtle rust:

- Infection on flowers and fruit
- Decline and death of mature trees
- Death of seedlings

Rhodomyrtus canescens **– Crater ironwood**

Original rating – Highly susceptible

Restricted to rainforests of NE Queensland

Impact of myrtle rust:

- Wild populations have not been assessed
- Impact in the gardens
- Significant and rapid dieback

Lenwebbia lasioclada **– Velvet myrtle**

Original rating – Relatively tolerant

Distribution restricted to rainforests of NE Queensland

Impact of myrtle rust:

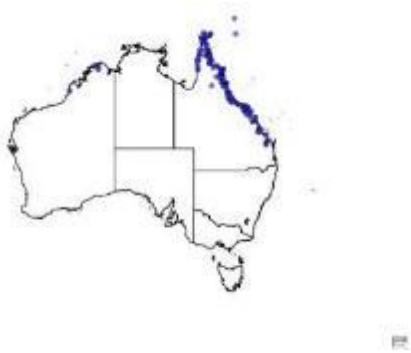
- Wild populations have not been assessed
- Impact in the gardens
- Significant dieback

Species with significant levels of dieback

***Eugenia reinwardtiana* – Beach Cherry**

Original rating – Extremely susceptible

Widespread distribution in coastal rainforests of eastern Queensland. Also New Guinea, SE Asia and Pacific Islands. Used widely in urban plantings.

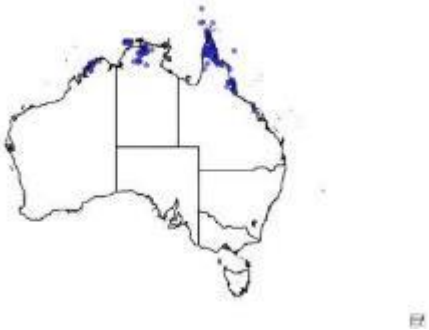


Often the first species reported from new geographic locations as the disease spread

Impact of myrtle rust:

- Repeated infection of new growth flush resulting in foliage loss
- Branch death and dieback
- Infection and premature senescence of flower buds, flowers and fruit

***Syzygium forte* subsp. *forte* – Watergum, Brown Satinash**



Original rating – Relatively tolerant

Restricted to coastal and riverine rainforests of northern Queensland, and northern NT

Severe foliage infection was not identified until 2 years after this species was identified as being a host.

Impact of myrtle rust:

- Repeated infection resulting in loss of new growth
- Branch dieback
- Branch death

***Rhodamnia spongiosa* – Northern malletwood**

Original rating – Highly susceptible

Restricted to lowland rainforests of central and NE Queensland.

Limited impact assessments done within the native range

Impact of myrtle rust:

- Repeated infection of new growth resulting in loss of foliage
- Infection and premature senescence of flower buds, flowers and fruit
- Branch dieback

***Gossia myrsinocarpa* – Malanda ironwood, small flowered lignum**

Original rating – Moderately – Highly susceptible

Restricted to rainforests of central and NE Queensland

Surveys and assessments conducted around Kuranda and Atherton Tablelands

Impact of myrtle rust:

- Repeated infection of new growth resulting in foliage loss
- Infection and premature senescence of flower buds, flowers and fruit
- Significant levels of branch dieback

***Rhodomyrtus pervagata* – Rusty Rhodomyrtus, rusty ironwood**

Original rating – Moderately – Highly susceptible

Restricted to rainforests in Wet Tropics NE Queensland

Limited assessments done across the native range

Impact of myrtle rust:

- Repeated infection of new growth resulting in loss of foliage
- Branch dieback
- Infection of flower buds, flowers and fruit resulting in premature senescence

***Syzygium nervosum* – no common name**

Original rating – Highly susceptible

No assessments conducted within its natural range – restricted to rainforests in Northern Territory and north-west Western Australia

Impact of myrtle rust:

- Repeated infection of new growth resulting in loss of foliage
- Branch dieback

***Syzygium eucalyptoides* – Wild apple**

Original rating – Highly susceptible

Widespread across northern Australia in gallery and riverine forest

No assessments done within its native range

Impact of myrtle rust:

To date impact observed has been restricted to seedlings and coppice regrowth with infection of new growth causing dieback

***Rhodamnia maideniana* – Smooth scrub turpentine**

Original rating – Extremely susceptible

Restricted to rainforests of SE Qld and NE NSW

Some impact assessments conducted across the natural range

Impact of myrtle rust:

- Repeated infection of new growth resulting in loss of foliage
- Significant dieback
- Flower bud, flower and fruit infection resulting in premature senescence

***Lenwebbia prominens* – Southern-velvet myrtle**

Original rating – Highly susceptible

Restricted to rainforests of SE Qld and NE NSW

Conservation status: Near Threatened species (NCA)

Limited assessments across its native range

Impact of myrtle rust:

- Repeated infection of new growth resulting in loss of foliage
- Branch dieback
- Flower bud, flower and fruit infection resulting in premature senescence

Impact assessment

Assessments of 74 species of Myrtaceae within the Mt Coot-tha and Lismore Botanic Gardens were conducted in 2016, five years after myrtle rust was first detected in these plantings. Multiple individuals of a species were assessed where possible with the average tree health score presented (Table 13). All species originally rated as either HS or ES (Pegg et al. 2014) showed high levels of dieback or decline as indicated by the low percentage of healthy crown on trees assessed (Table 13). Species rated MS showed levels of decline apart from *Gossia punctata* and *Leptospermum madidum*. Some species, originally rated as RT, also showed evidence of dieback but the majority were free of myrtle rust related dieback. These species were also compared to Myrtaceae assessed and found to be resistant or free of any symptoms of infection: *Pilidiostigma tropicum*, *Syzygium alliilgneum*, *S. branderhorstii*, *S. jonsonii*, *S. malaccense*, *S. monimioides*, *S. papyraceum*, *S. sayeri*, *S. trachyphloium*, *Xanthostemon* sp. “Mt Tozer” and *X. crenulatus*. All were free of myrtle rust related dieback.

Table 13 – Assessment of Myrtaceae at Mt Coot-tha and Lismore Botanic Gardens examining impact of repeated *Austropuccinia psidii* infection using crown health as an indicator of decline

Myrtaceae	Tree health (% healthy crown)	Original susceptibility rating (2014)
Chamelaucium uncinatum	0	ES
Eugenia reinwardtiana	0	ES
Rhodomyrtus psidioides	0	ES
Rhodomyrtus canescens	0	HS
Melaleuca nodosa	0	HS-ES
Gossia myrsinocarpa	2	MS-HS
Decaspermum humile	3.2	ES
Rhodamnia spongiosa	3.75	HS
Rhodamnia maideniana	5.94	ES
Rhodamnia rubescens	6.25	HS-ES
Rhodamnia argentea	8.33	MS-HS
Gossia hillii	9.37	HS-ES
Rhodamnia australis	22.5	HS
Gossia inophloia	23.75	ES
Rhodomyrtus pervagata	25	MS-HS
Melaleuca leucadendra	27	RT-HS
Rhodamnia dumicola	31.25	HS
Syzygium apodophyllum	37.5	RT
Gossia acmenoides	47.5	HS
Lenwebbia prominens	48.33	HS
Uromyrtus australis	50	NR
Syzygium corynanthum	60	RT-HS
Gossia floribunda	61.6	RT
Waterhousea mulgraveana	65	RT
Syzygium macilwraithianum	66.67	RT-HS
Austromyrtus dulcis	70	RT-HS
Backhousia anisatum	72.66	RT-HS
Syzygium rubrimolle	75	RT
Syzygium forte subsp. potamophilum	78.75	RT-MS
Backhousia citriodora	80	MS-HS
Backhousia oligantha	80	MS-HS
Neofabricia myrtifolia	80	NR
Acmenosperma claviflorum	81.37	MS
Syzygium nervosum	81.6	HS
Gossia fragrantissima	82.5	MS
Backhousia sciadophora	89	RT
Mitrantia bilocularis	90	MS
Gossia bamagensis	90	RT
Syzygium dansiei	90	RT
Syzygium bamagense	91.25	MS
Syzygium xerampelinum	92.5	MS
Backhousia hughesii	95	MS
Acmena hemilampra subsp. hemilampra	95	RT
Lindsayomyrtus racemoides	95	RT

Syzygium minutiflorum	95	RT
Syzygium wilsonii subsp. wilsonii	95	RT
Syzygium oleosum	95	RT-HS
Syzygium australe	96.6	RT-MS
Syzygium tierneyanum	98.33	RT
Gossia punctata	100	MS
Leptospermum madidum	100	MS
Sphaerantia discolor	100	MS
Melaleuca comboyensis	100	NR
Pilidiostigma tropicum	100	NR
Syzygium ingens	100	NR
Syzygium papyraceum	100	NR
Choricarpia subargenea	100	RT
Gossia bidwillii	100	RT
Leptospermum petersonii	100	RT
Melaleuca linarifolia	100	RT
Ristantia waterhousei	100	RT
Syzygium argyropedicum	100	RT
Syzygium canicortex	100	RT
Syzygium cormiflorum	100	RT
Syzygium erythrocalyx	100	RT
Syzygium moorei	100	RT
Tristaniopsis laurina	100	RT
Waterhousea floribunda	100	RT
Waterhousea hedraiophylla	100	RT
Backhousia leptopetala	100	RT-HS
Rhodamnia costata	100	RT-HS
Backhousia myrtifolia	100	RT-MS
Pilidiostigma glabrum	100	RT-MS
Waterhousea unipunctata	100	RT-MS

*NR = Not rated for rust susceptibility pre Pegg et al. (2014)

Impact on flower and fruit development

To date (2016) *A. psidii* infection has been identified having a direct impact on flower and fruit production on 31 species (Table 3). However, this number is based on observations only with limited detailed studies conducted to capture quantitative data documenting both direct and indirect effects on fecundity. *Austropuccinia psidii* infection has been identified on all flower parts and caused senescence of flowers preventing development of fruit/seed. Infection of juvenile fruit has been observed (e.g. *Rhodamnia sessiliflora* - Pegg unpublished) preventing maturation occurring. Infection on mature fruit has also been observed (e.g. *Austromyrtus dulcis*, *Rhodamnia* spp.) but the effects on germination not yet studied. However, reports from seed collecting staff indicate that when fruit of *Rhodomyrtus psidioides* has been found, an increasingly rare event, they are absent of seed. When examining internal structures of *Eugenia reinwardtiana* fruit, *A. psidii* uredinia and urediniospores were identified (Fig. 31). This has also been observed on fruit of the exotic *Myrtus communis*.

Observations have also been made of bees, both European bees (*Apis melifera*) and the native stingless bees (*Tetragonula* sp.) actively collecting *A. psidii* spores (Fig. 32).

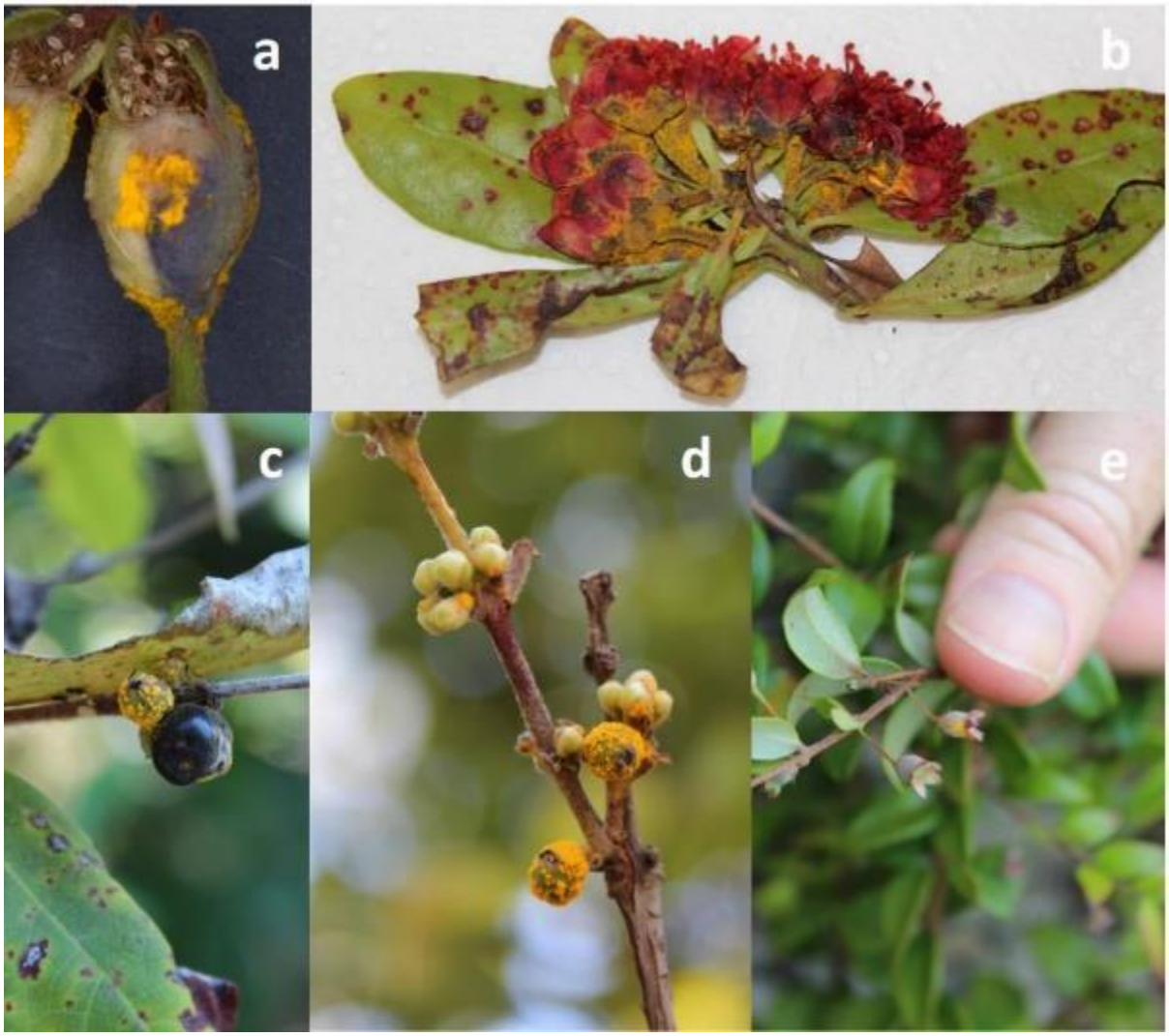


Figure 31 *Austropuccinia psidii* infection on the flowers and fruit has been identified from a range of species having a direct effect on fecundity. *Austropuccinia psidii* urednia and urediniospores were found to occur internally on immature *Eugenia reinwardtiana* fruit (a), *Xanthostemon youngii* flowers, flower buds, immature and mature fruit of *Rhodamnia sessiliflora* and fruit of *Austromyrtus dulcis*.

Figure 32 *Syzygium jambos* foliage covered in *Austropuccinia psidii* uredinia and urediniospores with a European honey bee (*Apis melifera*) foraging rust spores - Photo Vanessa Brake (DAWR)

Impact of *Austropuccinia psidii* on *Rhodamnia rubescens* fruit development

Fortnightly fungicide application protected flowers and fruits of *R. rubescens* from *A. psidii* infection, with a significant difference ($p < 0.05$) in incidence of fruit infected between treatments. The mean % infested at the start of the trial differed between the treated and the untreated trees (1.31%, 16.9% respectively); these values after the treatment was applied were 2.18% and 76.58% (means of three assessments after treatment), indicating a significant increase in the % infested for the untreated branchlets (Fig. 33). For untreated branchlets, incidence of *A. psidii* increased sharply in the first fortnight after the trial began (16.9 to 82.5), plateaued at the 3rd assessment (84.6), and then decreased by the final assessment (62.4) (Fig. 33).

The mean no of fruits at the start of the trial for untreated and treated are similar (60.3 and 65.9, respectively), but after the treatment was applied these values are 31.47 and 51.28 (mean of three assessments) indicating that more fruits are retained by the treated branches than the untreated branches (Fig. 34, 35). At the final assessment, the mean number of fruits per branch for treated trees was 34.7 (4.68 se) and 17.6 (5.78 se) for untreated. The trend over time is non-linear for both the response variables. Number of fruits data showed the presence of high auto-correlation (0.87), so auto-correlation was included in the model. The no. of fruits did not show a significant treatment effect ($p=0.08$) but the treatment by period interaction was highly significant ($p<0.001$). The decline in the number of fruits over time for treated and untreated follows a different trend (Fig. 35). The decline over time is linear for the untreated branches but follows a non-linear pattern for the treated ones. For treated branches the decline is very slow for the first 2 weeks (almost constant) and then the number of fruits drop quickly, whereas for the untreated branches there is a constant drop in the number of fruits over weeks. Qualitative observations indicated that more fruit reached maturity (purple colour) on treated trees compared to untreated trees.

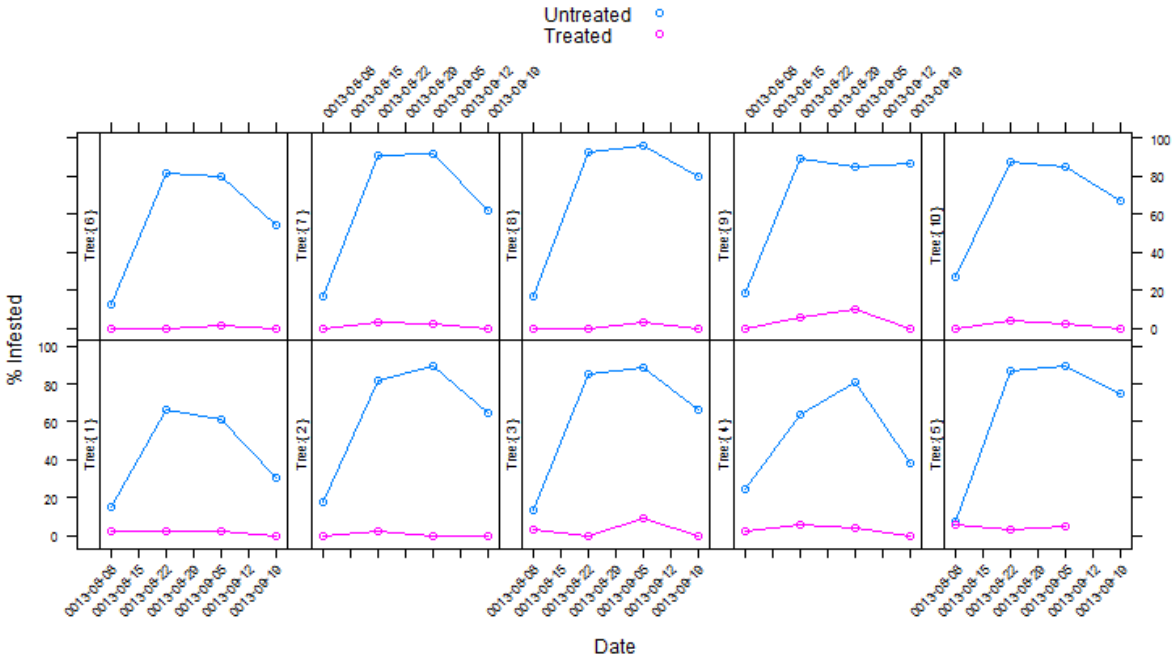


Figure 33 Time series plot of the disease incidence percentage (% infested) of the fruits by branchlet for treated and untreated branches within each branch.

Figure 34 *Rhodamnia rubescens* with fruit at various levels of maturity (a) infected with *Austropuccinia psidii* on immature and mature fruit (b,c) and absence of fruit on untreated branches (d)

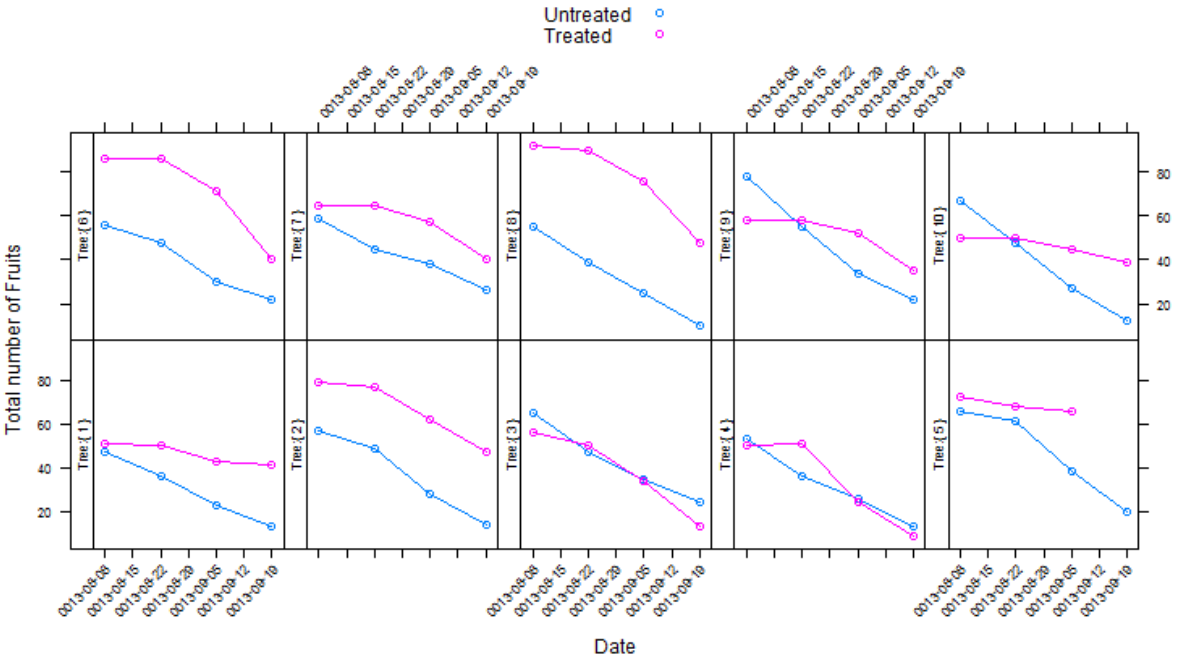


Figure 35 Time series plot of the total number of fruits by branchlet for treated and untreated branches within each branch

Impact of *Austropuccinia psidii* (myrtle rust) on regeneration of Myrtaceae following disturbance within coastal heathland communities of northern New South Wales, Australia

Coppice and epicormic re-growth was first identified in March 2014 following a rainfall (>200mm) event with plots established once species could be identified as being Myrtaceae. Evidence of *A. psidii* infection was first detected in April 2014 with a single tree showing symptoms on new growth flush. Disease incidence (number of trees with symptoms and level of infection per tree) and severity levels rapidly increased in both the *Melaleuca quinquenervia* swamp and on Myrtaceae within the wet and dry coastal heath sites. Disease incidence levels increased and peaked during the autumn to winter months (April-August). Disease incidence was then variable between sites despite the relative close proximity of all sites. No relationship was identified between incidence and severity of *A. psidii* infection and rainfall per month or days of rain per month. Disease incidence (Fig. 36) and severity (Fig. 37) levels in the dry heath site peaked during the cooler winter months (May, June, July) before declining over months of Spring and early Summer before increasing again in late Summer/Autumn. Disease levels on species in the wet heath site followed similar pattern with the exception of a spike in disease incidence in September 2014 (Fig.38).

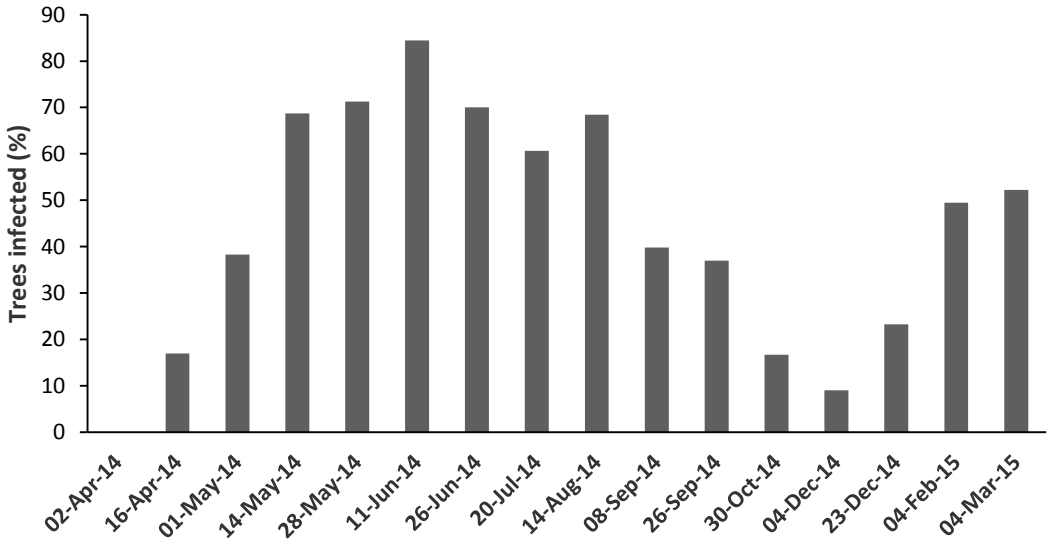


Figure 36 Myrtle rust disease incidence (% trees with infection) on coppice of Myrtaceae species over time in dry heath environment. Species in this environment included *Austromyrtus dulcis*, *Leptospermum whitii*, *L. polygalifolium*, *Melaleuca nodosa* and *M. quinquenervia*.

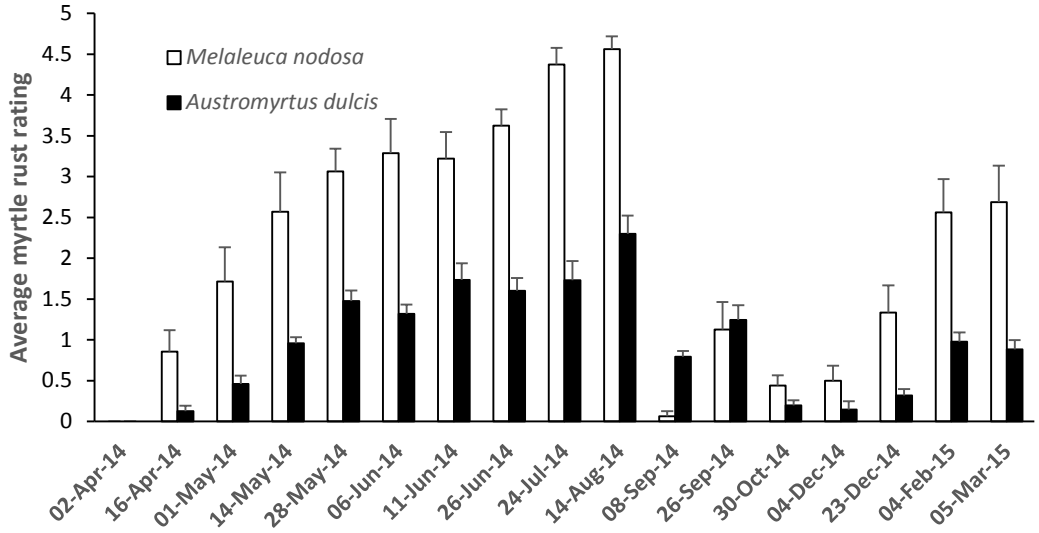


Figure 37 Average disease severity levels over time for two susceptible species in dry coastal heath *Melaleuca nodosa* and *Austromyrtus dulcis*

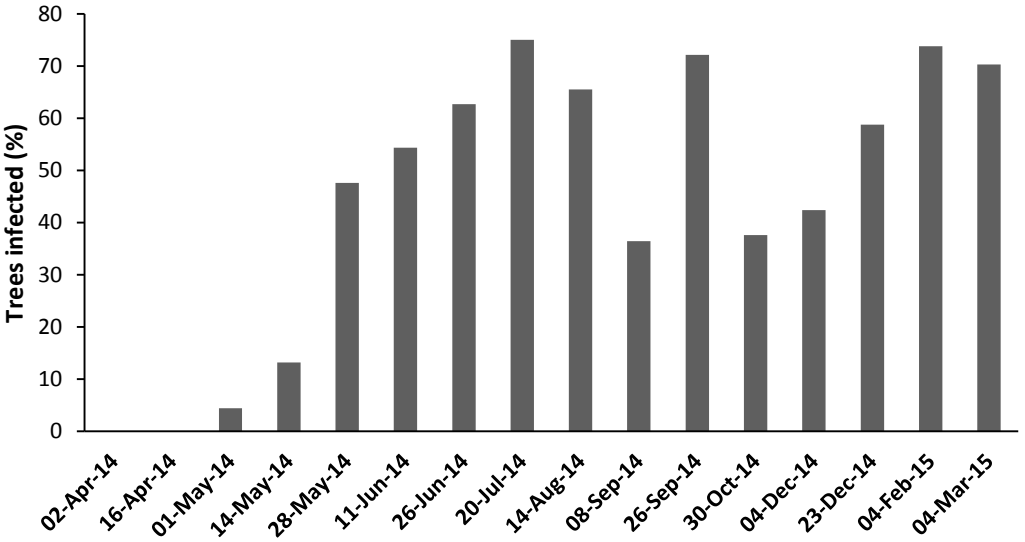


Figure 38 Myrtle rust disease incidence (% trees with infection) on coppice of Myrtaceae over time in wet heath environment. Species in this environment included *Baeckea frutescens*, *Leptospermum levigatum*, *Leptospermum polygalifolium*, *L. whitei*, *Lophostemon suaveolans and Melaleuca quinquenervia*.

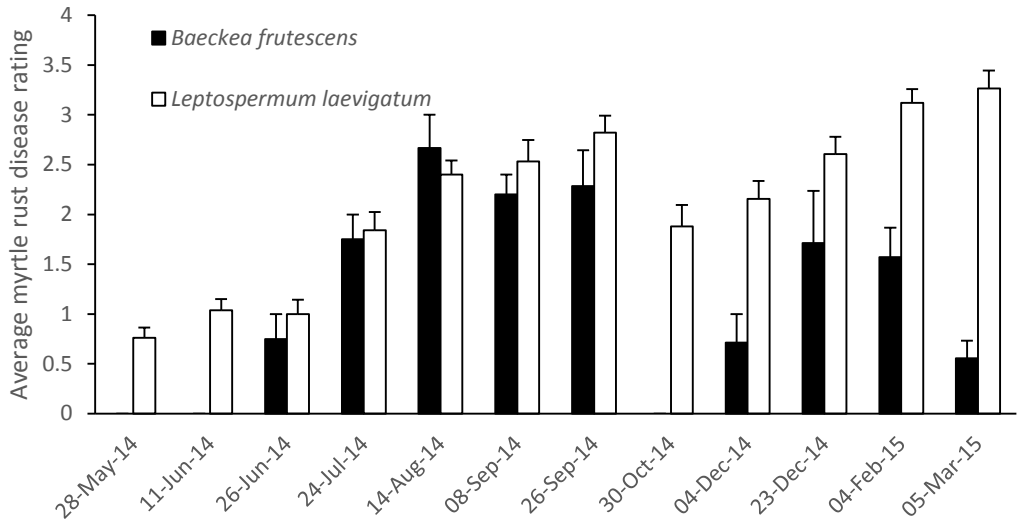


Figure 39 – Average disease severity levels on *Baeckea frutescens* and *Leptospermum laevigatum*, two of the more myrtle rust susceptible species in the wet heath environment

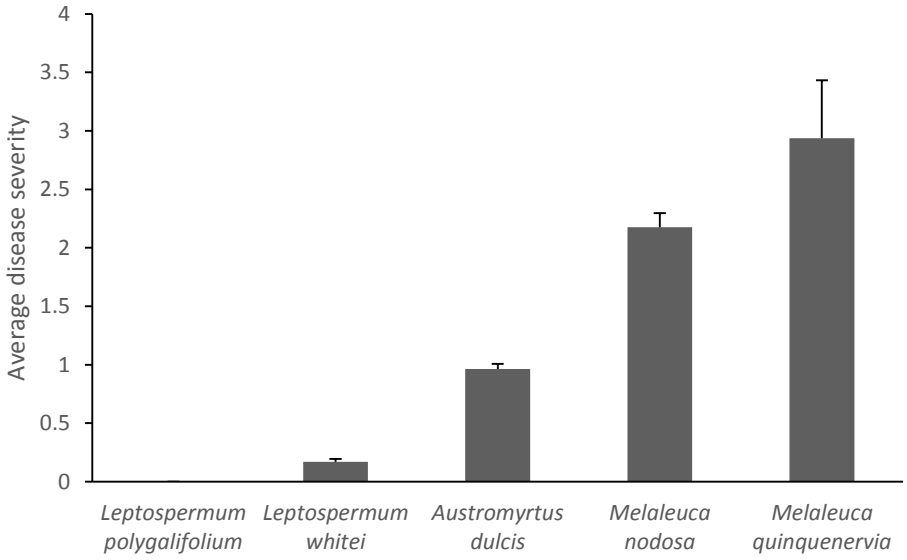


Figure 40 Average disease susceptibility of Myrtaceae within dry coastal heath ecosystem based on a 0-5 rating score of the new growth flush with significant differences in disease susceptibility (P<0.0001).

Melaleuca quinquenervia was the most susceptible species within the dry coastal heath plots (Fig. 40) but only two trees were present within the plots established. The species is scattered in distribution in this environment. Epicormic regeneration on one of the two *M. quinquenervia* was killed as a result of *A. psidii* infection, while coppice regeneration on the second tree remained healthy.

Melaleuca nodosa (Fig. 40, 47) is common within the coastal heath environment and was significantly (P>0.0001) more susceptible to myrtle rust than *Austromyrtus dulcis*.

Only low levels of foliage infection was identified on *Leptospermum whitei* and no disease was detected on *L. polygalophylla* within the plots. As a result of repeated infection branch dieback was identified on *A. dulcis* but significantly less than that recorded on *M. nodosa.* No dieback was recorded for *L. polygalifolium* and *L. whitei* (Fig. 41).

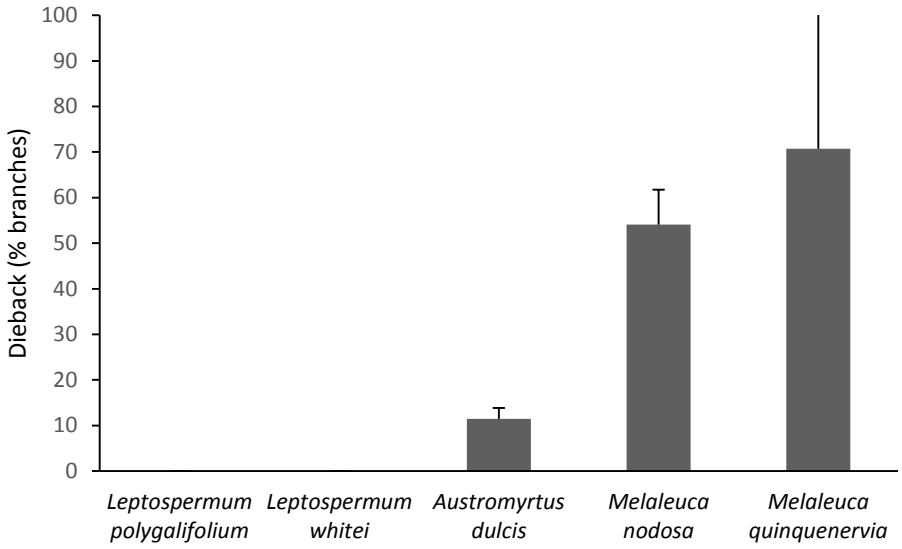


Figure 41 Comparison of dieback levels on Myrtaceae in dry coastal heath plots with significant (P<0.0001) differences identified between species

Austropuccinia psidii severity levels were greatest on epicormic regrowth of *Leptospermum laevigatum* in the wet coastal heath sites. *A. psidii* infection was predominantly identified on juvenile stems rather than foliage for this species, a symptom also observed on *Baeckea frutescens*. Dieback levels on *L. laevigatum* were significantly higher (P>0.0001) than *B. frutescens*.

Similar to the dry heath site, variability in susceptibility of *M. quinquenervia* was observed with repeat *A. psidii* infection on one tree causing death of coppice shoots (Fig. 42-44) while no dieback was recorded on epicormic shoots on a second tree.

Figure 42 – *Melaleuca quinquenervia* coppice regeneration following fire with 30% of trees showing resistance to *Austropuccinia psidii* but were impacted upon by mirid bugs (*Eucerocoris suspectus*) (RHS)

Figure 43 Impact of *Austropuccinia psidii* infection on *Melaleuca quinquenervia* coppice regeneration following fire with infection initially causing blight of new shoots and stems followed by gradual decline and eventual death of shoots.

Figure 44 Impact of *Austropuccinia psidii* on susceptible *Melaleuca quinquenervia* coppice regeneration following a wildfire

Austropuccinia psidii infection was also recorded on *Melaleuca rigidus* and *L. whitei* with repeated infection causing low levels of dieback (Fig. 45, 46). Infection was also detected on *Lophostemon suaveolans* but *A. psidii* pustules were restricted to insect galls present on expanding foliage (Fig. 48). No dieback was recorded for *L. suaveolans*.

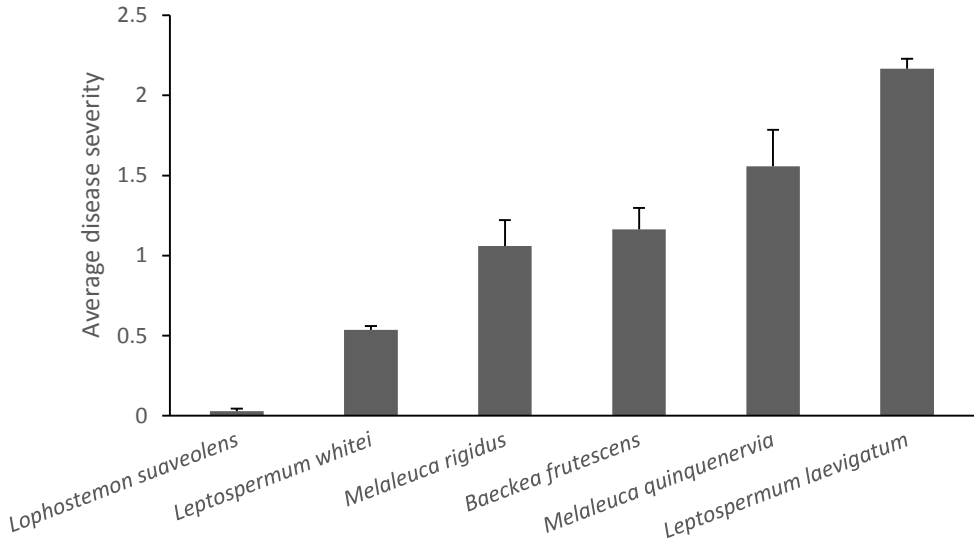


Figure 45 Average disease susceptibility of Myrtaceae within dry coastal heath ecosystem based on a 0-5 rating score of the new growth flush with significant differences in disease susceptibility (P<0.0001).

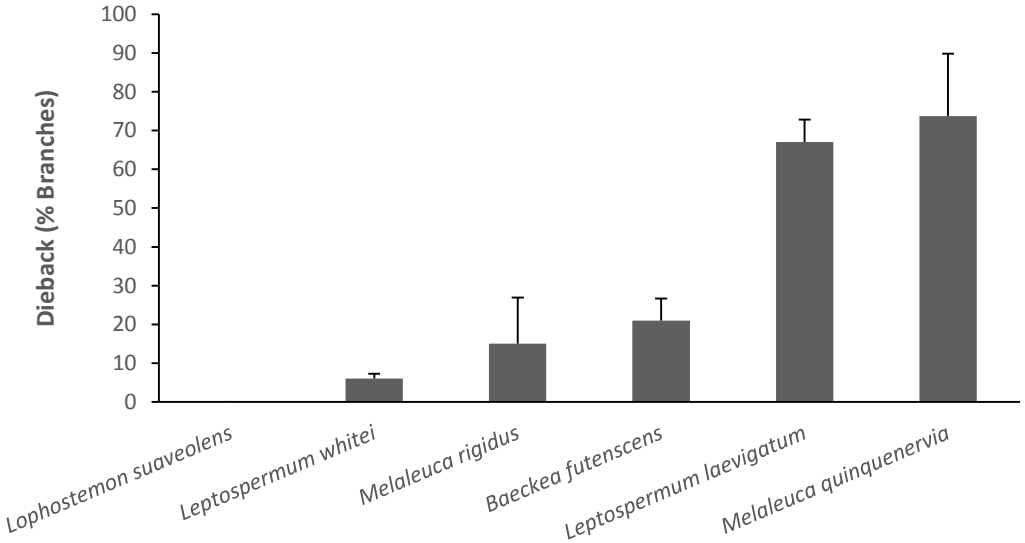


Figure 46 Impact of repeated *Austropuccinia psidii* based on percentage of branches showing evidence of dieback with significant differences between species (P<0.0001)

Figure 47 Impact of *Austropuccinia psidii* on *Melaleuca nodosa* coppice regeneration following wildfires in coastal heath environment near Lennox Head in northern New South Wales. New coppice development (top left, top centre) with juvenile foliage showing symptoms of infection by Austropuccinia psidii (top right). Infection by *A. psidii* causes dieback of growing tips (middle left) with "cankers" found to form on the woody stem with *A. psidii* sori found to be present (middle centre). *A. psidii* infection did not directly impact flowering although number of flowers/seed pods were lower on more susceptible individuals (middle right). Repeated infection of growing tips resulted in branch dieback (bottom)

Figure 48 *Lophostemon suaveolens* with *Austropuccinia psidii* pustules (sori) restricted to insect galls

Seedling regeneration

Information gathered on the impact of rust on seedlings is more difficult to understand. Observations within plots would suggest a change in species composition is occurring with the seedlings, from the resistant *Lophostemon suaveolens* becoming the dominant species, and with *Melaleuca quinquenervia* and *Leptospermum* species becoming less common (Fig. 49, 50). *Austropuccinia psidii* infection was identified on *M. quinquenervia* and *L. polygalifolium* seedling but was not detected on *L. suaveolens*. However, lack of information on what would have occurred in the absence of myrtle rust limits our understanding of this data. More recent observations of *M. quinquenervia* seedlings suggest that seedlings that do survive infection have had repeated events where apical dominance is lost and seedlings are becoming multi-branched (Fig. 51). Again, the lack of information on seedling regeneration in these ecosystems in the absence of rust makes it difficult to draw more specific conclusions. An assessment to determine the pre-existing species composition within the sites may help shed some light on the impact myrtle rust has on species composition.

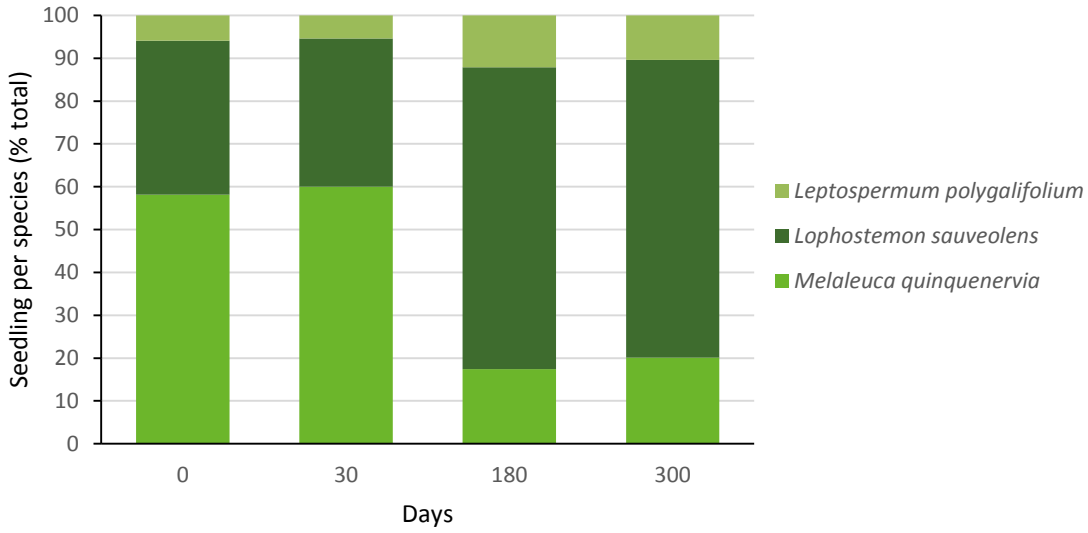


Figure 49 Changes in seedling Myrtaceae species regeneration composition over time within coastal heath following wildfire

Figure 50 Myrtaceae seedlings germinating after the fire were predominantly *Melaleuca quinquenervia*, *Leptospermum polygalifolium* and *Lophostemon suaveolens*. Within plots established (a) *M. quinquenervia* was most affected by *A. psidii* (b,c,d) with only minor infection found on *L. polygalifolium* (c,d) and no infection on *L. suaveolens*.

Figure 51 Impact of *Austropuccinia psidii* on *Melaleuca quinquenervia* seedlings following wildfire in coastal heath environments; (top & bottom left) loss of apical dominance evidence of infection and death of growing tips due to infection by *A. psidii* compared to (bottom right) apically dominant seedlings showing resistance to infection.

Additional surveys

To determine if the *A. psidii* impacts on species within plots was representative of broader impact one-off surveys were conducted and selected species assessed for impact:

Melaleuca nodosa

One hundred and forty four trees were assessed for *A. psidii* impact based on the percentage of branches showing dieback. 31.94% of trees had *A. psidii* infection related dieback on all branches. Of these trees only 8.7% (4 trees) had seed pods present with three having seed on 10% of branches and the fourth on only 5% of the branches. Only 15.27% of trees assessed had dieback on 25% or less of the tree branches. 63.63% of these trees had seed present on an average of 45.36% of branches.

Baeckea frutescens

Twenty trees were selected randomly from within the wet heath site and assessed for *A. psidii* impact. All trees showed some level of branch dieback with more than 50% of the trees having dieback on 50% or more of their branches. At the time of assessment impact of dieback on flowering levels was not assessed. However, observations at a later date suggested that trees with stem and branch dieback had reduced flowering levels in comparison to trees showing no *A. psidii* related dieback (Fig. 52).

Leptospermum laevigatum

All trees showed some level of dieback caused by repeated *A. psidii* infection. However, only 30% had dieback on 50% or more of the branches (Fig. 53). Again observations by the authors identified reduced flowering rates on trees with dieback.

Leptospermum polygalifolium

While 90% of trees assessed showed some levels of branch dieback caused by *A. psidii*, only a single tree was identified with significant levels of impact with dieback on 70% of branches.

Leptospermum trinervium

Leptospermum trinervium was not present in any of the study plots. However, observations by the authors identified significant levels of *A. psidii* infection and dieback on epicormic regeneration of this species. All 20 trees assessed showed some level of branch epicormic regrowth dieback caused by *A. psidii*. Of these, 10 had greater than 50% of branches showing evidence of dieback (Fig. 54).

Eucalyptus robusta

A common coastal eucalypt, and known koala food source, *Eucalyptus robusta* was found sporadically through the wet heath sites with seedling germination observed following the fire event. While impact of *A. psidii* was not assessed for this species, infection was observed on new growth flush and juvenile stems of seedlings (Fig. 55).

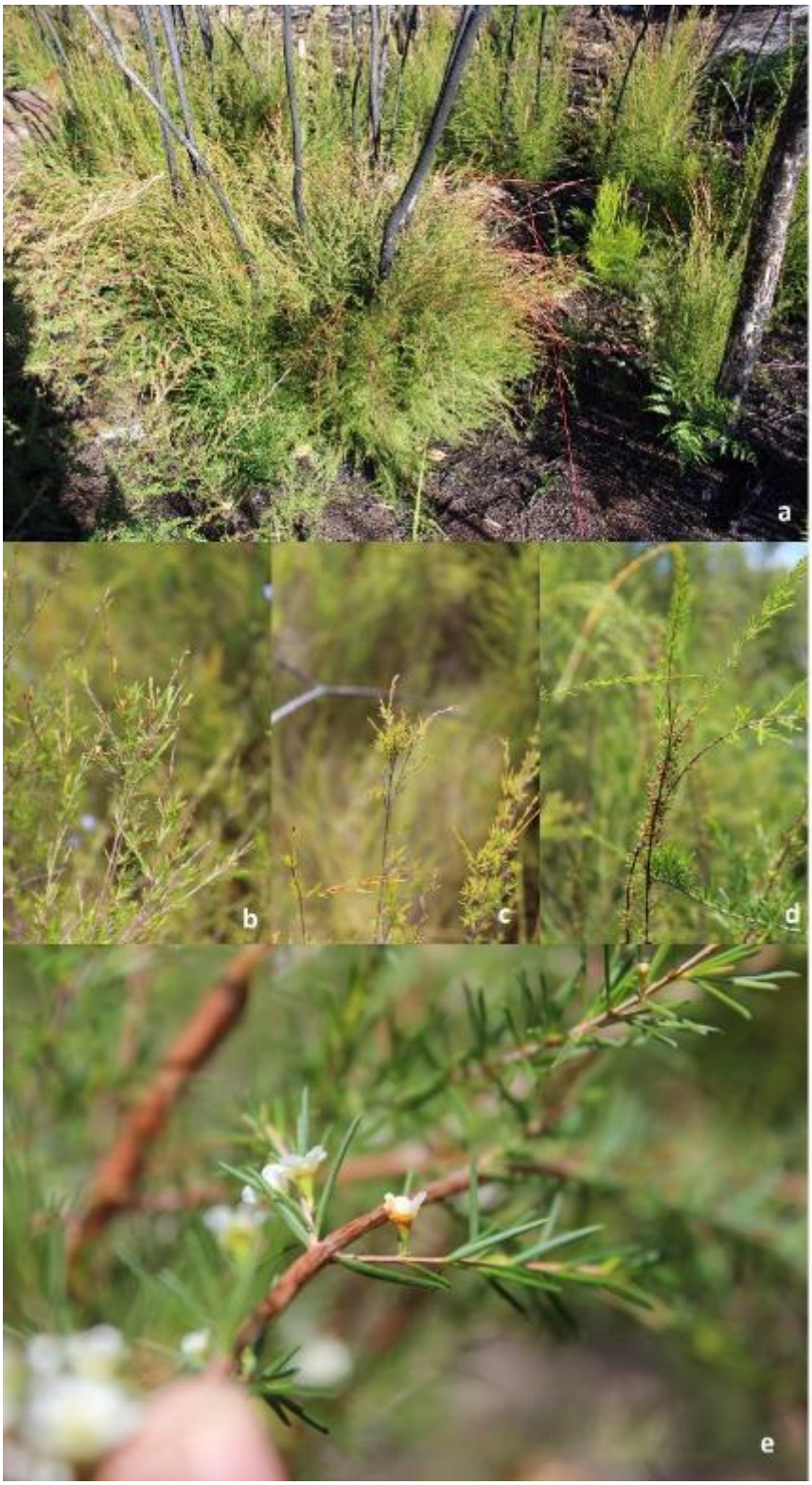


Figure 52 Coppice regeneration of *Baeckea frutescens* following a wildfire (a) with *A. psidii* infections causing stem dieback (b, c). Resistance was identified in the population assessed with flowering occurring on individuals free of dieback symptoms (d). Low levels of infection was identified on flowers.

Figure 53 *Austropuccinia psidii* infection on *Leptospermum laevigatum* was restricted to juvenile stems (a, b) with no evidence of infection on leaves, Infection on stems resulted in distorted growth and dieback (c,d,e). Flower levels on those with dieback were observed to be lower than those where disease symptoms were absent

Figure 54 Coppice regeneration on *Leptospermum trinervium* was found to be highly susceptible to *Austropuccinia psidii* with uredinia and telia identified on juvenile stems and foliage (a, b, c, d) resulting in foliage loss and dieback (e, f)

Figure 55 *Eucalyptus robusta* seedlings were found regenerating following wildfire in wet heath environment with symptoms of *Austropuccinia psidii* infection on juvenile stems and young leaves

Unburnt areas

A 100 meter transect was assessed through an adjacent unburnt site in the dry heath environment and disease levels assessed on Myrtaceae present. An initial survey was conducted in August 2014 recording disease infection levels on new growth flush and again in March 2015 to assess for impact (dieback). *Austromyrtus dulcis*, *Melaleuca quinquenervia* and *M. nodosa* were assessed. For the first assessment only 2 of the 20 *M. nodosa* present had evidence of *A. psidii* infection on new growth flush, both rated 1 using the 0 to 5 rating scale. Similarly only a single *M. quinquenervia* tree was found with infection (rating 2) and three of fifteen *A. dulcis* shrubs had low levels of rust infection (rating 1). At the second assessment there was no evidence of *A. psidii* dieback on *M. quinquenervia*. However, dieback was recorded on 50% of the *M. nodosa* and 22% of *A. dulcis* assessed.

Melaleuca quinquenervia swamp

Austropuccinia psidii had a significant impact on the regeneration of *M. quinquenervia* in a swamp environment following wildfire. Seventy two percent of trees showed some level of susceptibility to *A. psidii*. When disease incidence (number of trees infected) levels were at their highest (September 2014), 70.45% of infected trees rated as highly or extremely susceptible (4 or 5).

In August 2014 mirid bugs (*Eucerocoris suspectus*) impacted on (Fig. 56), in addition to *A. psidii*, new shoots and expanding foliage with feeding causing significant blighting (Fig. 42). Mirid bug levels were highest in December 2014 when *A. psidii* levels were comparatively low. Both insect attack and *A. psidii* levels declined in months when growth flush levels declined in December 2014 and January, May and June 2015. In 2015, the site was inundated for long periods following heavy and persistant rainfall, which appeared to slow tree growth rates, reducing the amount of susceptible flush present and resulting in lower levels of both *A. psidii* infection and mirid bug attack.

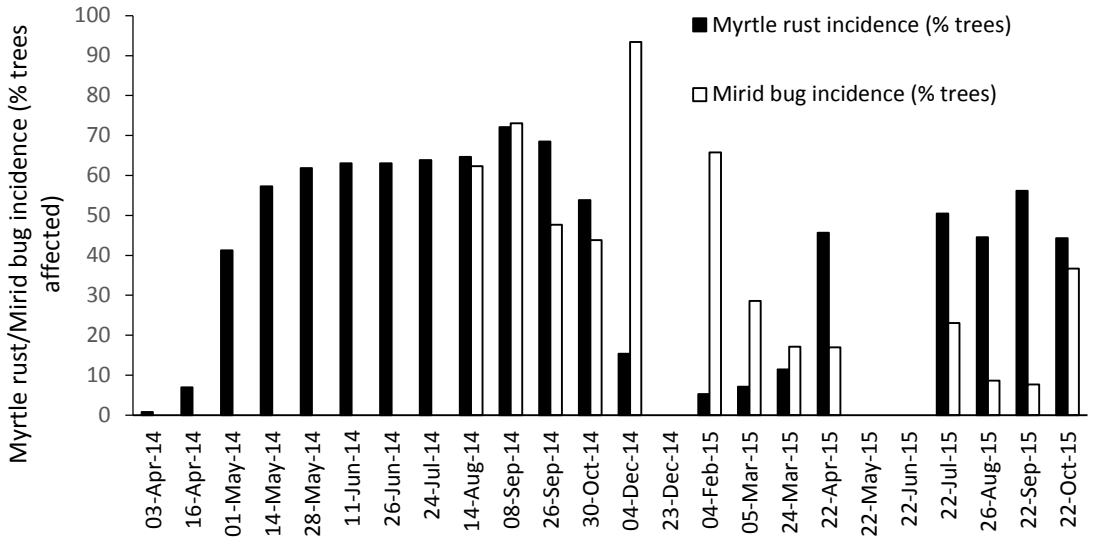


Figure 56 Incidence of *A. psidii* infection and mirid bug (*Eucerocoris suspectus*) attack on coppice regeneration of *Melaleuca quinquenervia* over the assessment period following wildfire in a coastal swamp and heath ecosystem near Lennox Heads in northern New South Wales.

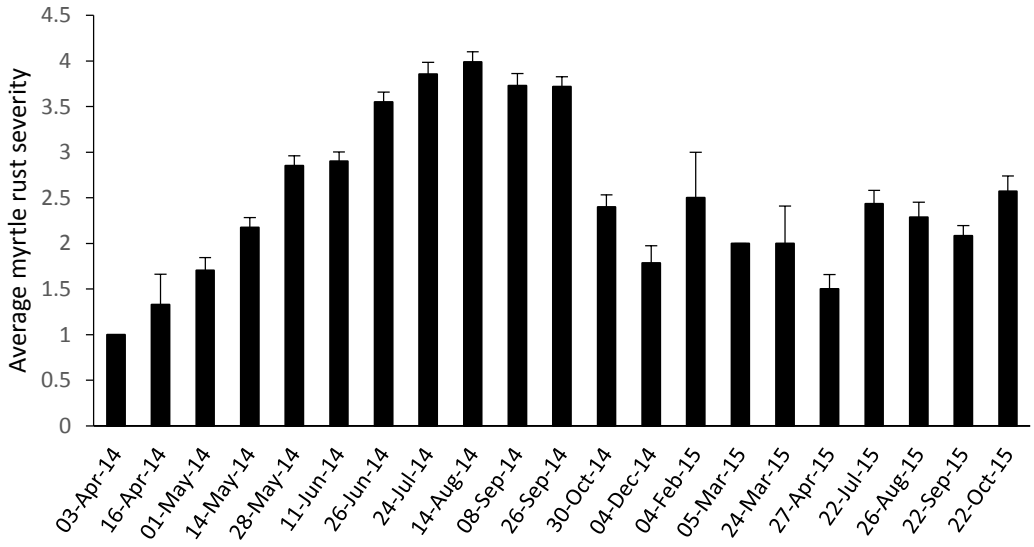


Figure 57 Average disease severity levels on coppice regeneration of *Melaleuca quinquenervia* over the assessment period following wildfire in a coastal swamp and heath ecosystem near Lennox Heads in northern New South Wales.

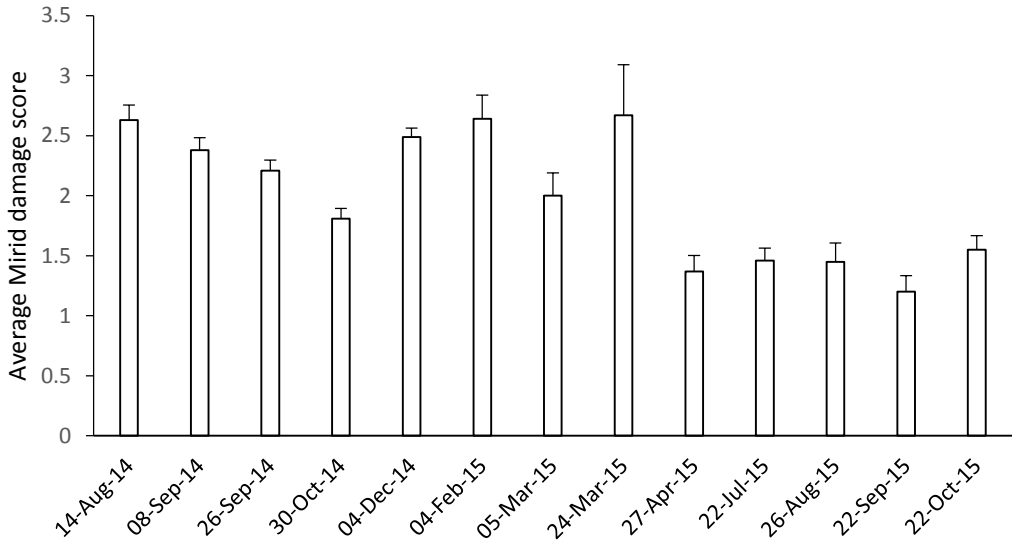


Figure 58 Average severity levels of mirid bug (*Eucerocoris suspectus*) attack on coppice regeneration of *Melaleuca quinquenervia* over the assessment period following wildfire in a coastal swamp and heath ecosystem near Lennox Heads in northern New South Wales.

Dieback as a result of repeated *A. psidii* infection, and potentially additive effects of mirid bugs, was identified on 71.94% of trees assessed. However, only 10% of trees had 50% or more of the coppice regeneration killed.

Impact of *Austropuccinia psidii* on regeneration of *Melaleuca quinquenervia* and interaction with insect populations

Incidence and severity of A. psidii and insect attack

Incidence of myrtle rust infection, based on the number of trees with *A. psidii* symptoms, fluctuated over time with no specific pattern based on season (Fig. 59) or maximum daytime and minimum night-time temperatures (Fig. 60). Disease incidence levels were lowest during late spring/early summer months in 2015/16. In contrast incidence of insect damage was greatest during the spring and summer months followed by very low levels of damage during the winter months of June and July (Fig. 59).

Insect damage incidence levels were strongly correlated with minimum (P = 0.957) and maximum (P = 0.987) temperatures with increasing temperatures linked to an increase in the number of trees showing symptoms of insect damage (Fig. 60). There was no relationship between days of rainfall (Fig. 61), rainfall per month (Fig. 62) or leaf wetness (Fig. 63) and disease incidence or severity levels. This was also the case when examining relationships with incidence and severity of insect damage.

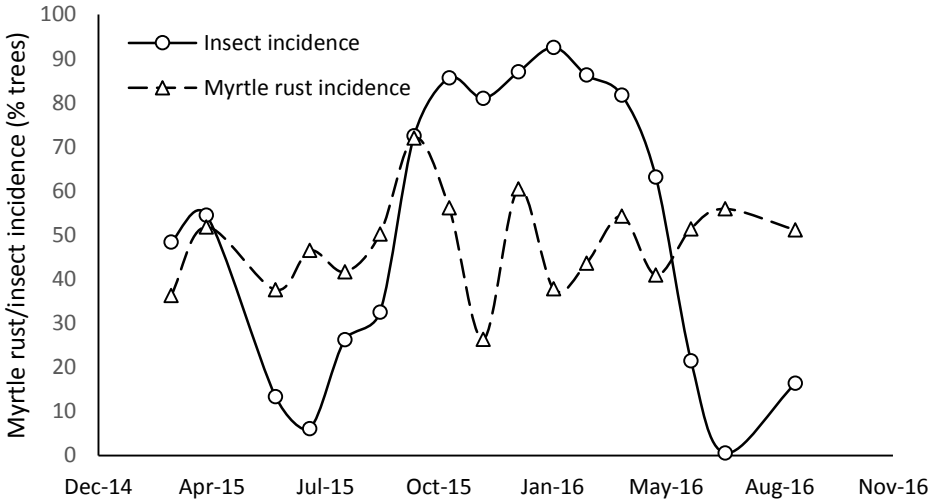


Figure 59 Changes in *Austropuccinia psidii* incidence (% total trees) and incidence of insect damage (% total trees) over the assessment period on new shoots and expanding foliage on coppice regrowth of *Melaleuca quinquenervia*.

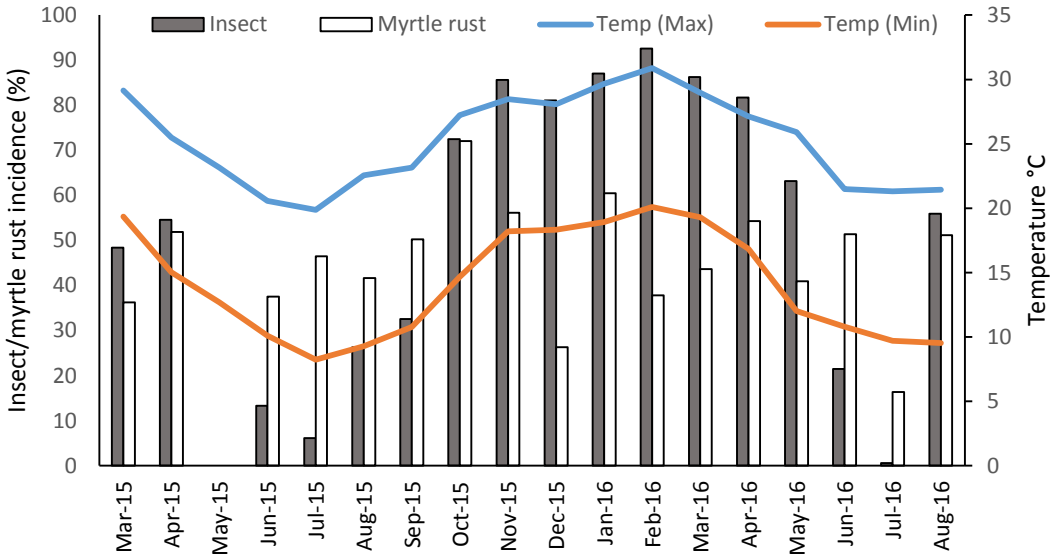


Figure 60 Changes in *Austropuccinia psidii* incidence (% total trees) and incidence of insect damage (% total trees) over the assessment period on new shoots and expanding foliage on coppice regrowth of *Melaleuca quinquenervia* in relation to average monthly temperature (Maximum and Minimum).

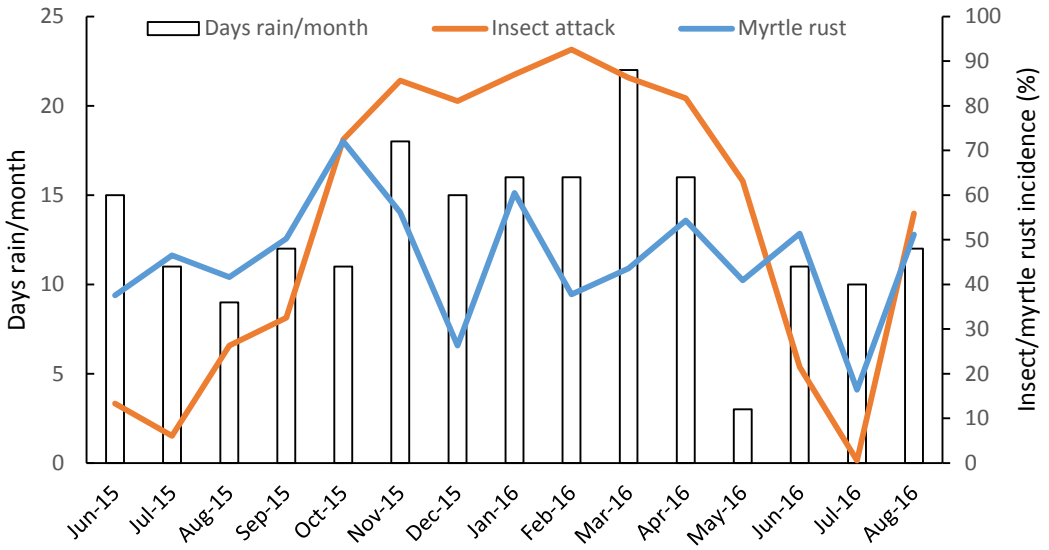


Figure 61 Changes in *Austropuccinia psidii* incidence (% total trees) and incidence of insect damage (% total trees) over the assessment period on new shoots and expanding foliage on coppice regrowth of *Melaleuca quinquenervia* in relation to days of rainfall per month.

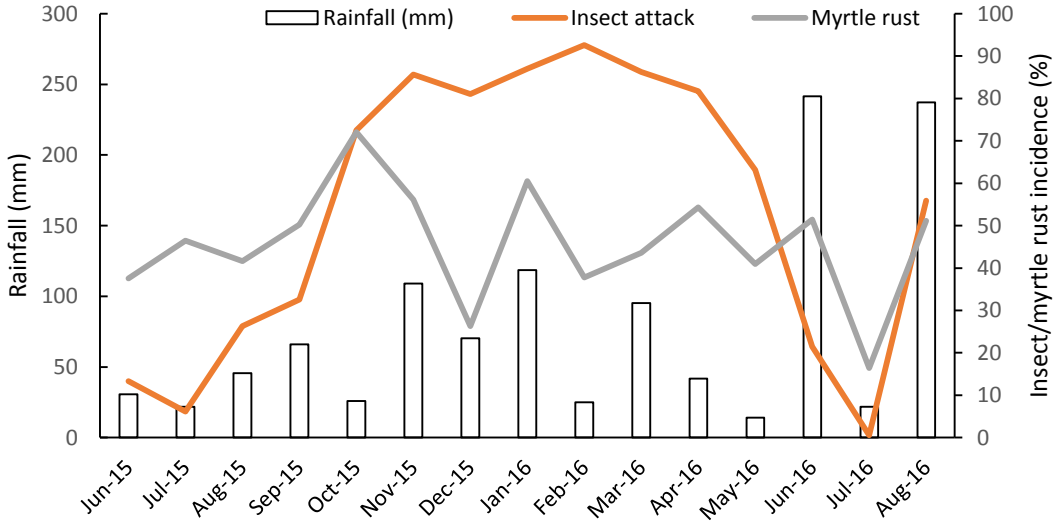


Figure 62 Changes in *Austropuccinia psidii* incidence (% total trees) and incidence of insect damage (% total trees) over the assessment period on new shoots and expanding foliage on coppice regrowth of *Melaleuca quinquenervia* in relation to total rainfall per month.

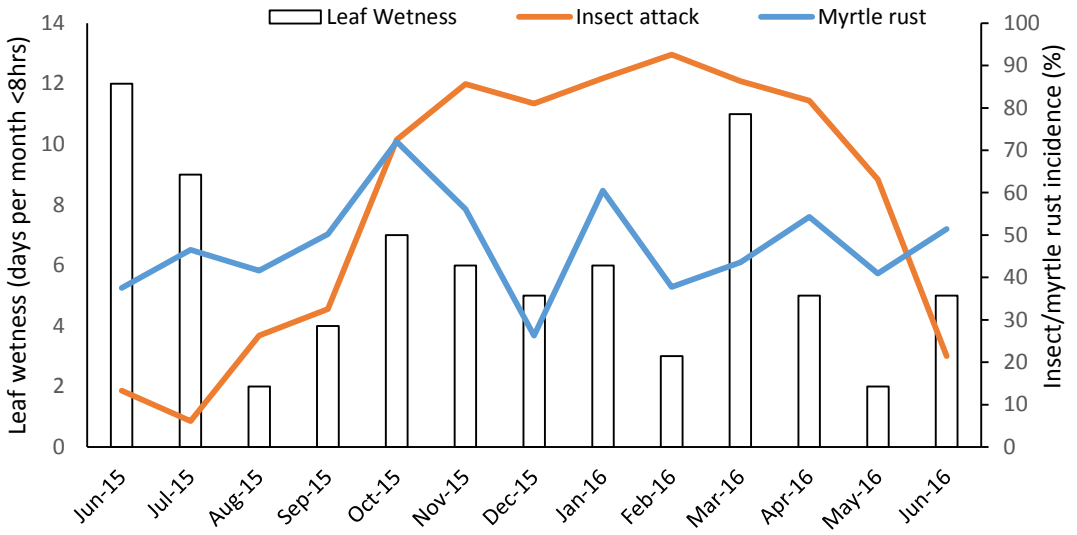


Figure 63 Changes in *Austropuccinia psidii* incidence (% total trees) and incidence of insect damage (% total trees) on *Melaleuca quinquenervia* coppice regeneration over the assessment period in relation to leaf wetness graphed as the number of days leaf wetness exceeded 8 hours overnight.

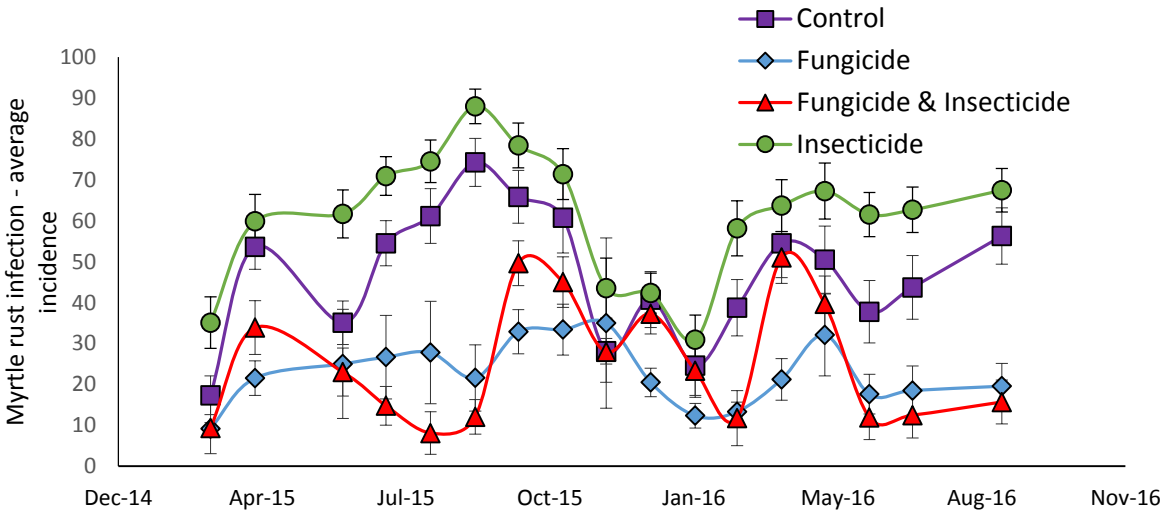


Figure 64 *Austropuccinia psidii* incidence levels (average disease incidence level per tree) on *Melaleuca quinquenervia* coppice regeneration over the assessment period comparing four treatments: untreated control, fungicide, fungicide + insecticide, insecticide

While not completely eliminating *A. psidii*, fungicide application reduced the incidence and severity of infection on susceptible growth flush in comparison to the untreated control and insecticide only treated trees (Fig.64, 65).

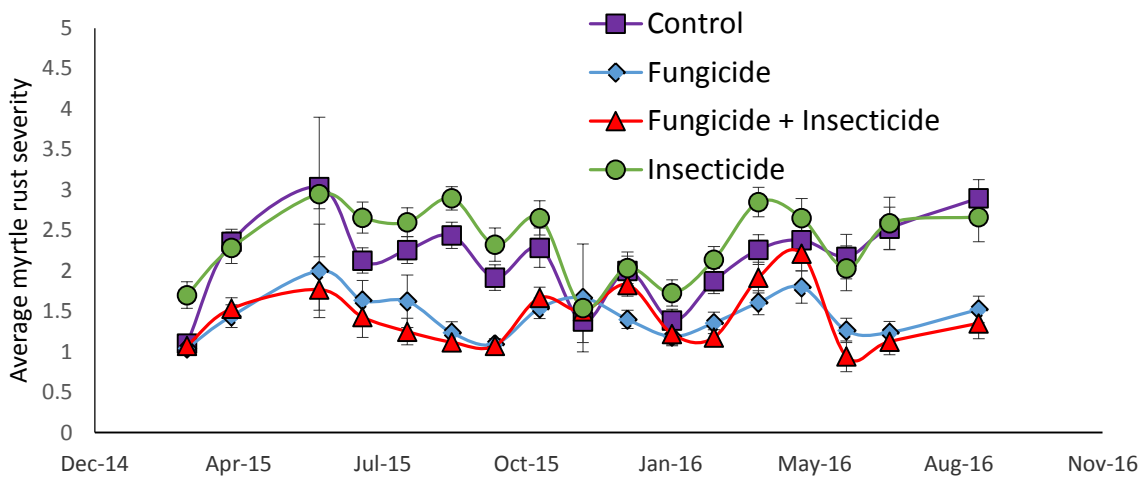


Figure 65 *Austropuccinia psidii* severity levels (average disease severity level per tree using a 1-5 rating scale) on *Melaleuca quinquenervia* coppice regeneration over the assessment period comparing four treatments: untreated control, fungicide, fungicide + insecticide, insecticide

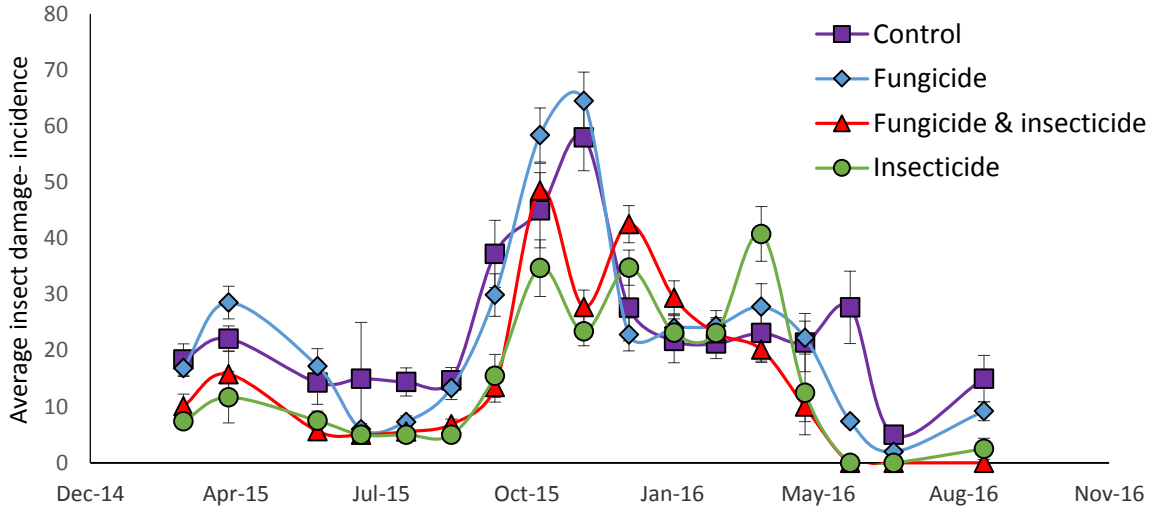


Figure 66 Insect damage incidence levels (average incidence level per tree) on *Melaleuca quinquenervia* coppice regeneration over the assessment period comparing four treatments: untreated control, fungicide, fungicide + insecticide, insecticide

An array of different insects were found causing damage to *M. quinquenervia*, at times making control difficult despite using broad spectrum insecticides (Fig. 66, 67). Insect pests included weevils (*Aterpus griseatus*) (Fig. 70), which stripped the young stems often "ringbarking" branches, leaf tying caterpillars, tip sucking bugs, chrysomellids (including *Geloptera perosa*) and other general leaf chewing insects, many of which could not be identified as they were not present at the time of assessment. Weevil damage was particularly severe at the time of coppice establishment (February-March 2015) in the untreated control and fungicide only treated plots.

Some of the more severe damage was caused by mirid bugs (Fig. 66) which attacked the new growth flush and were particularly severe from November 2015 to January 2016 causing significant levels of defoliation, even on trees treated with insecticide. The fungicide + insecticide and insecticide treated trees, which were tallest and most actively growing at the time (December 2015-March 2016), had greater levels of mirid bug attack than other treatments and this is reflected in insect incidence and severity scores during that period. The faster growth rates in these treatments is likely to influence the efficacy of chemical treatments over time. At times young shoots and expanding foliage was affected by both mirid bug attack and *A. psidii* infection (Fig. 69).

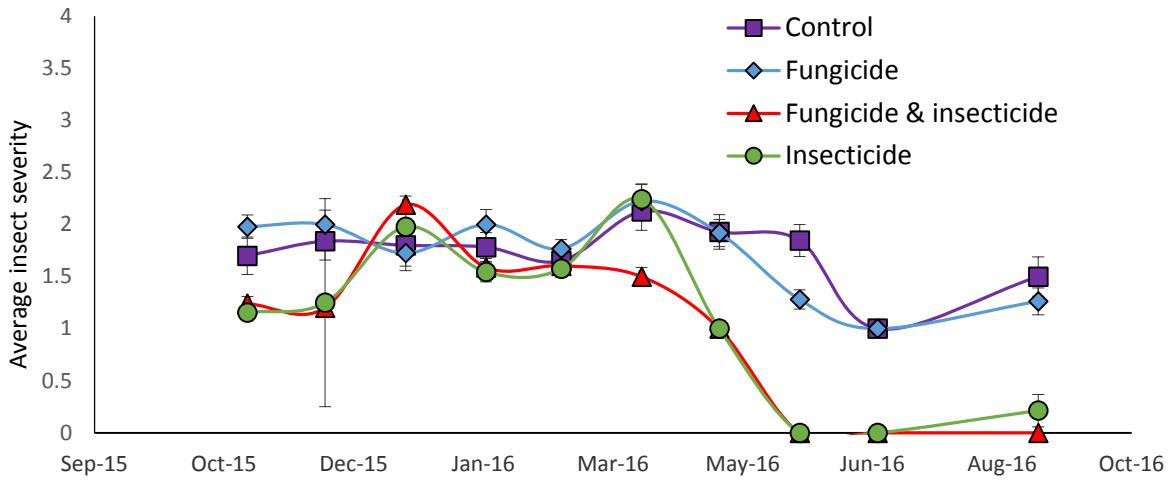


Figure 67 Insect damage severity levels (average disease severity level per tree using a 1-5 rating scale) on *Melaleuca quinquenervia* coppice regeneration over the assessment period comparing four treatments: untreated control, fungicide, fungicide + insecticide, insecticide

Figure 68 Mirid bug (*Eucerocoris suspectus*) damage on expanding foliage and new shoots of *Melaleuca quinquenervia*

Figure 69 *Austropuccinia psidii* and mirid damage occurring in combination on new growth of *Melaleuca quinquenervia*

Figure 70 Weevils (*Aterpus griseatus*) caused damage to bark and cambial layers on young woody stems and branches of *Melaleuca quinquenervia*

Impact of Austropuccinia psidii and insect attack

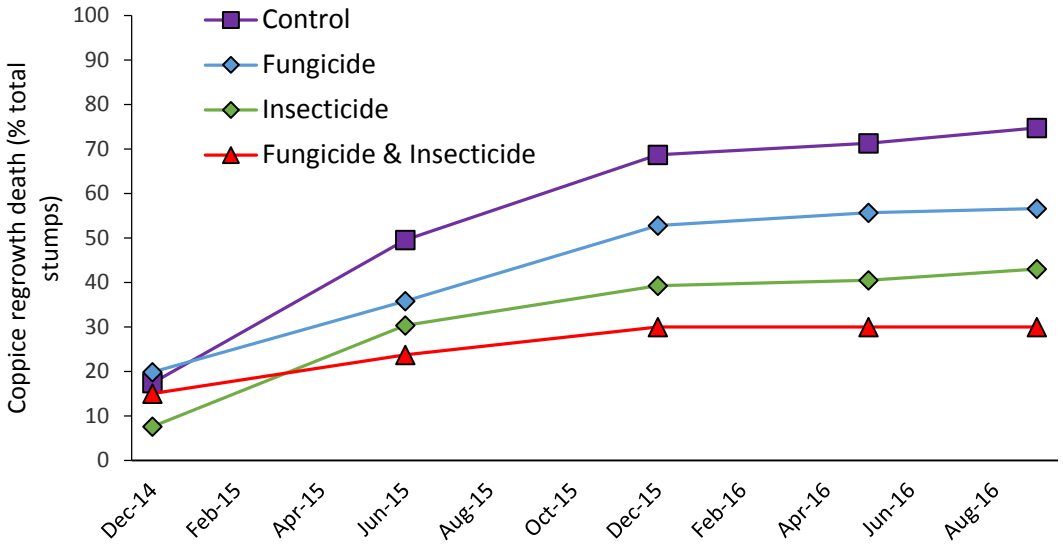


Figure 71 Death of *Melaleuca quinquenervia* coppice over the assessment period comparing four treatments: untreated control, fungicide, fungicide + insecticide, insecticide

Death of *M. quinquenervia* coppice regeneration has been greatest within the untreated control plots with a combination of *A. psidii* infection and insect attack resulting in 74.78% deaths (Fig. 71). Fungicide only treated plots had the second highest levels of dieback (56.6%) followed by insecticide (43.04%). Stumps deaths were least when *A. psidii* and insects were controlled with only 30% of stumps dying back (Fig. 71).

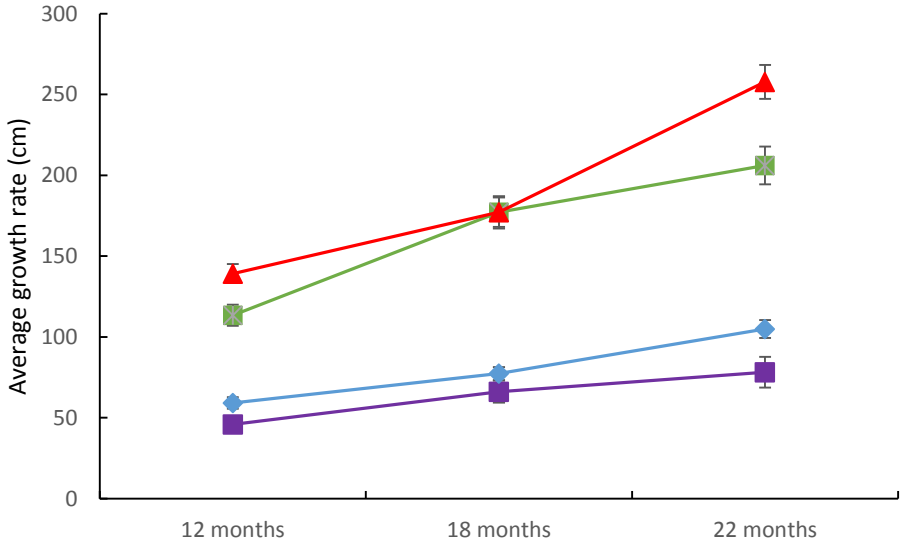


Figure 72 Comparison of growth rates of regenerating *Melaleuca quinquenervia* 12, 18 and 22 months after coppice development commenced comparing four treatments: untreated control, fungicide, fungicide + insecticide, insecticide.

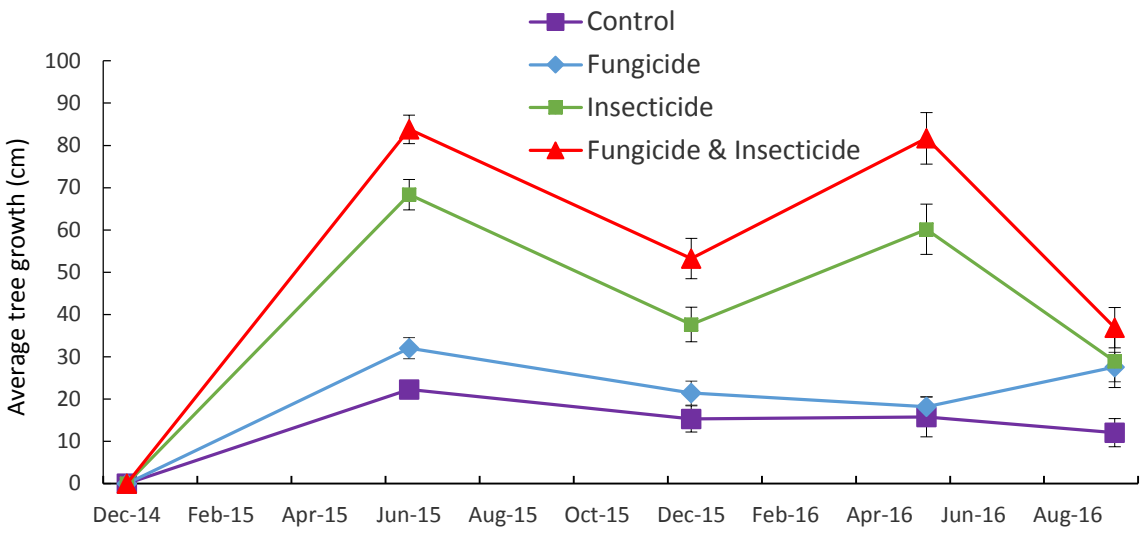


Figure 73 Comparison of seasonal growth rates of regenerating *Melaleuca quinquenervia* after coppice development commenced comparing four treatments: untreated control, fungicide, fungicide + insecticide, insecticide

Tree growth was significantly greater in insecticide and insecticide + fungicide treated trees than fungicide and fungicide and untreated control plots (Fig. 72). Insect attack within the first months significantly slowed growth in trees where insecticide was not applied. To date there is no significant difference between fungicide treated trees and trees in untreated control plots. However, it must be pointed out that approximately 35% of the remaining trees in the untreated control plots have been assessed as being resistant to *A. psidii*. Similarly in the insecticide treated plots, 22% of the remaining trees have been rated as resistant.

When examining tree growth rates based on seasonal differences (growth rates per 6 month period) (Fig. 73), growth is understandably slower during the cooler Autumn and Winter months. Interestingly the rate of growth in fungicide treated trees in the last 6 months has been similar to insecticide and insecticide + fungicide treated trees and is the only treatment showing an increase in growth rate in the last 6 months (Fig. 73). Further assessments are required to determine if this pattern will continue over time. Growth flush levels were also higher in fungicide treated trees in comparison to insecticide and insecticide + fungicide treated trees from March 2016 to September 2016 (Fig. 74)

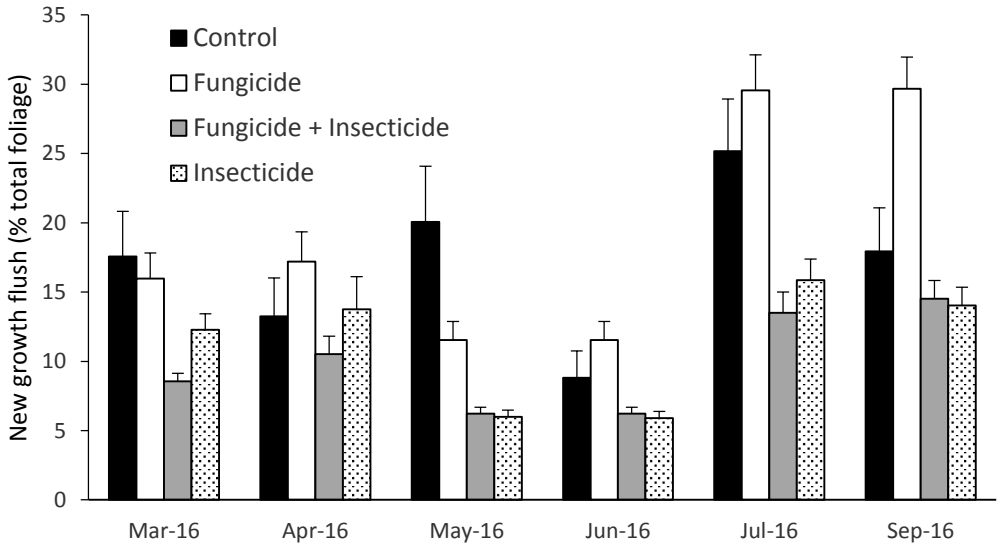


Figure 74 Comparison of growth flush development (new growth as a % of the total foliage present) in *Melaleuca quinquenervia* in coppice regrowth comparing four treatments: untreated control, fungicide, fungicide + insecticide, insecticide

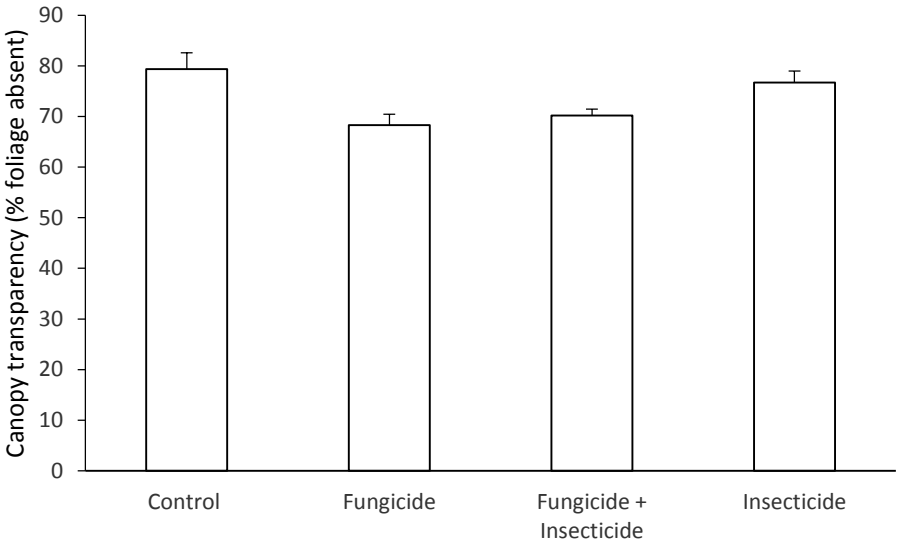


Figure 75 Comparison of canopy transparency levels where increased transparency indicates lower foliage density levels in *Melaleuca quinquenervia* coppice regrowth comparing four treatments: untreated control, fungicide, fungicide + insecticide, insecticide

Foliage transparency levels, indicating lower foliage density, were highest in untreated and insecticide treated trees (Fig. 75) (Fig. 79). Fungicide treated trees and untreated trees lacked apical dominance and are more shrub like in appearance (Fig. 76). Leaf size, based on average leaf area of fully expanded leaves (Fig. 77, 78), was significantly greater in fungicide + insecticide treated trees than untreated ($P<0.0001$), fungicide ($P=0.0002$) and insecticide ($P=0.0001$) treated trees. While there was no significant differences between other treatments, leaf area was higher in fungicide treated trees than insecticide and untreated trees.

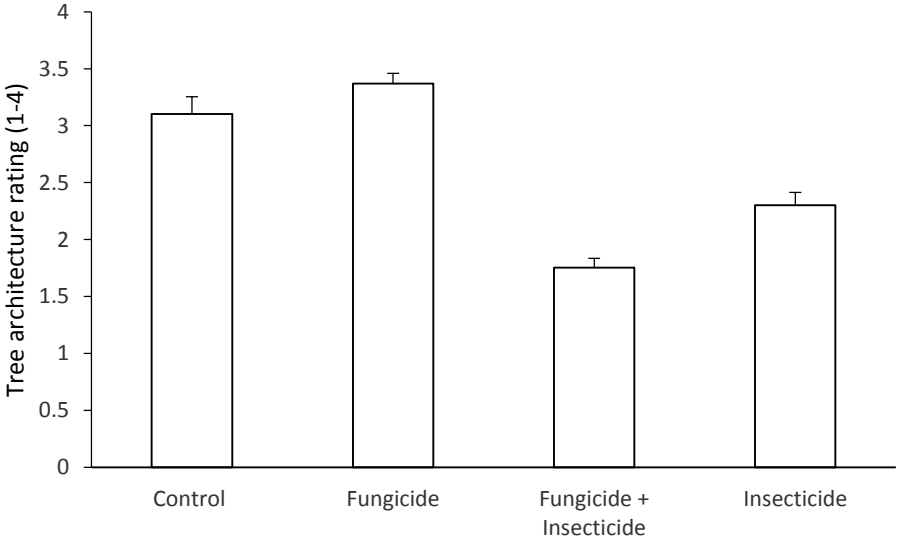


Figure 76 Comparison of tree architecture in *Melaleuca quinquenervia* coppice regrowth comparing four treatments: untreated control, fungicide, fungicide + insecticide and insecticide where a rating of 1 is an apically dominant tree and 4 is shrub like in appearance and lacking obvious apical dominance.

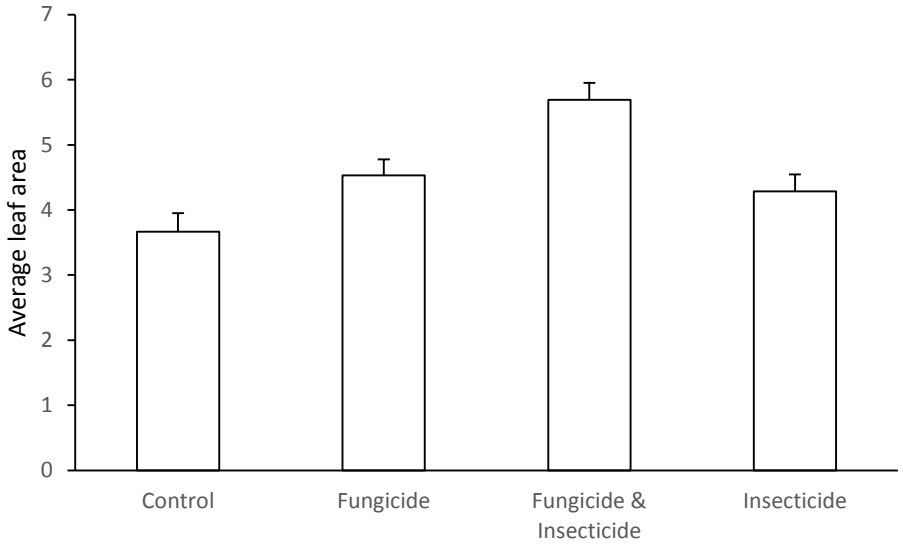


Figure 77 Comparison of average leaf area of fully expanded leaves in *Melaleuca quinquenervia* coppice regrowth comparing four treatments: untreated control, fungicide, fungicide + insecticide and insecticide.

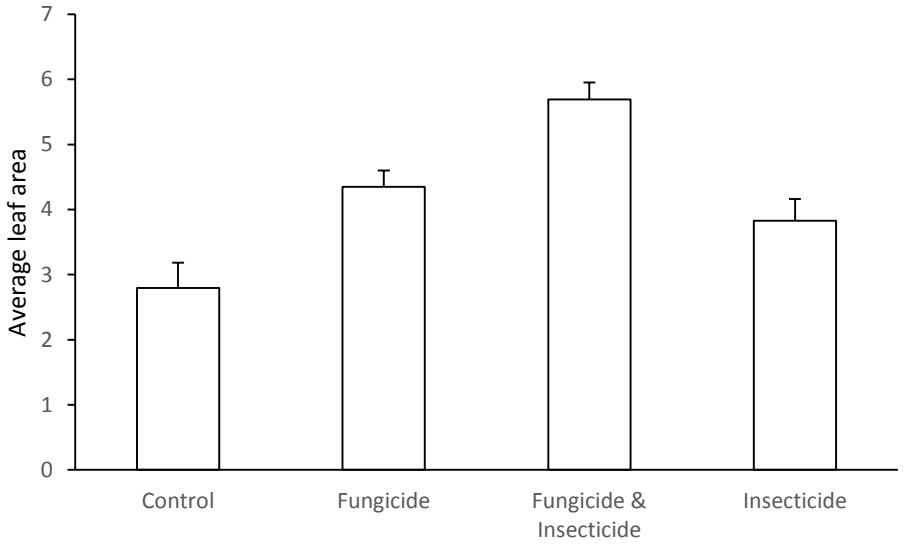


Figure 78 Comparison of average leaf area of fully expanded leaves in *Melaleuca quinquenervia* coppice regrowth comparing four treatments: untreated control, fungicide, fungicide + insecticide and insecticide with rust resistant trees removed from insecticide and control treatments

Figure 79 *Melaleuca quinquenervia* coppice regrowth comparing foliage density and tree form for four treatments: untreated control, fungicide, fungicide + insecticide and insecticide with rust resistant trees removed from insecticide and control treatments

Impact of *Austropuccinia psidii* on regenerating subtropical rainforest/wet sclerophyll ecosystems dominated by Myrtaceae.

Species composition in the understory of the wet sclerophyll/rainforest site in Tallebudgera Valley, Queensland was made up of seven Myrtaceae (Fig. 80) and dominated by *Archrhodomyrtus beckleri*. *Rhodamina maideniana*, *Gossia hilli*, *Acmena smithii* and *Decaspermum humile* were also relatively common with only a few *Pilidiostigma glabrum* and *Syzygium oleosum* occupying this vegetation layer. Impact of *A. psidii* was greatest on *D. humile* with *A. beckleri*, *G. hillii* and *R. maideniana* all had significant levels (P<0.0001) of dieback in comparison to *A. smithii* (Table 14).

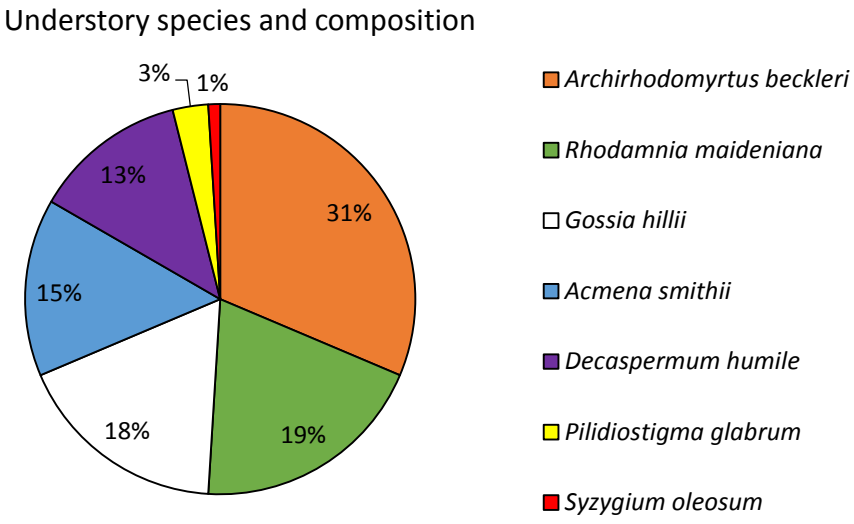


Figure 80 Composition of understory Myrtaceae as of 2016 within a subtropical rainforest/wet sclerophyll site in the Tallebudgera Valley, Queensland.

Table 14 Impact of *A. psidii* infection on the main species making up the understory component of the wet sclerophyll/rainforest ecosystem, Tallebudgera Valley, Queensland.

Tree species	Branch death (%)	Branches dieback (%)	Healthy canopy (%)
Acmena smithii	6.667 $^{\pm 5.156}$ a	1.333 $^{\pm 6.702}$ a	85.417 $^{\pm 6.969}$ a
Archirhodomyrtus beckleri	43.75 $^{\pm 6.611}$ b	97.656 $^{\pm 2.344}$ b	2.344 $^{\pm 2.344}$ b
Decaspermum humile	48.462 $^{\pm 11.027}$ b	100 $^{\pm 0}$ b	0 $^{\pm 0}$ b
Gossia hillii	33.889 $^{\pm 8.22}$ b	97.222 $^{\pm 2.778}$ b	2.778 $^{\pm 2.778}$ b
Rhodamnia maideniana	4.737 $^{\pm 1.687}$ a	93.421 $^{\pm 5.354}$ b	6.579 $^{\pm 5.354}$ b

Archrhodomyrtus beckleri was also the most common species identified in the midstory canopy making up 41% of all the Myrtaceae identified (Fig. 82). *Gossia hillii*, *Decaspermum humile* and *Acmena smithii* were the next most common species. Similar to the understory dieback levels were significantly (P<0.0001) lower on *A. smithii* (Table 15). Epicormic regeneration was found on the main trunk and base of *G. hillii*, *D. humile* and *A. beckleri* trees showing myrtle rust related dieback was (Fig. 81). In many cases, the coppice shoots were also infected by *A. psidii* and dying back.

Figure 81 Coppice regeneration on mid-story (a) *Archirhodomyrtus beckleri* and (b) *Decaspermum humile* trees in severe decline as a result of repeated *Austropuccinia psidii* infection

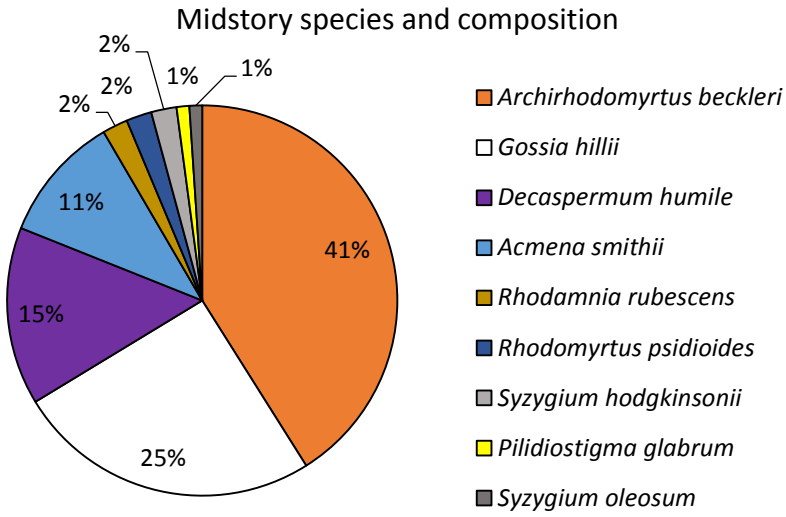


Figure 82 Composition of mid-story Myrtaceae as of 2016 within a subtropical rainforest/wet sclerophyll site in the Tallebudgera Valley, Queensland.

Table 15 Impact of *Austropuccinia psidii* infection on the main species making up the mid-story component of the wet sclerophyll/rainforest ecosystem, Tallebudgera Valley, Queensland.

Tree species	Branch death (%)	Branch dieback (%)	Healthy canopy (%)
Acmena smithii	8 $^{\pm 5.281}$ a	21.0 $^{\pm 13.204}$ a	78.5 $^{\pm 13.124}$ a
Archirhodomyrtus beckleri	22.564 $^{\pm 5.424}$ a	92.308 $^{\pm 3.067}$ b	7.66 $^{\pm 3.063}$ b
Decaspermum humile	86.786 $^{\pm 6.126}$ b	100 $^{\pm 0}$ b	0 $^{\pm 0}$ b
Gossia hillii	42.609 $^{\pm 7.989}$ c	100 $^{\pm 0}$ b	4.348 $^{\pm 4.348}$ b

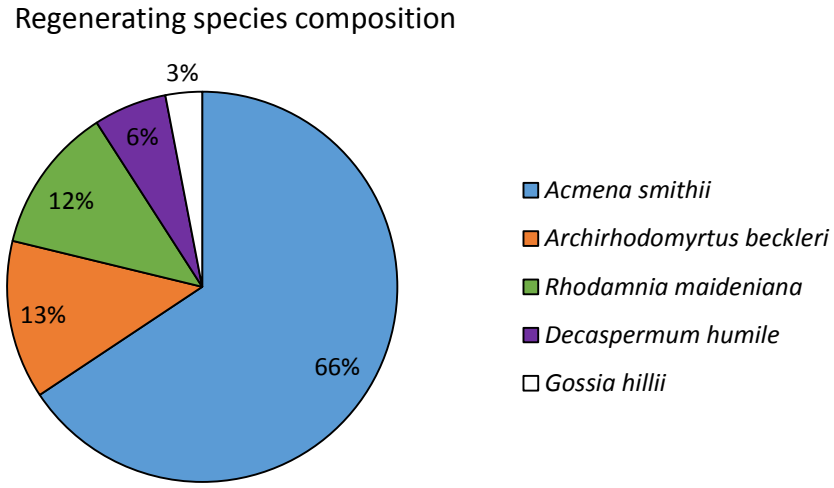


Figure 83 Composition of regenerating Myrtaceae seedlings as of 2016 within a subtropical rainforest/wet sclerophyll site in the Tallebudgera Valley, Queensland.

Acmena smithii is the most common species regenerating, making up 66% of all the Myrtaceae seedlings assessed within the plots (Fig. 83) and showing only low levels of *A. psidii* related dieback (Table 16). *Archirhodomyrtus beckleri, Rhodamnia maideniana* (Fig. 84) and *Gossia hillii* all had significant levels of *A. psidii* related dieback recorded (Table 16). *Decaspermum humile* made up only 6% of the regenerating Myrtaceae but some seedlings at the time of assessment showing only low levels of decline with no branch death but an average of 33% branches showing dieback symptoms.

Table 16 *Austropuccinia psidii* impact levels on regenerating Myrtaceae

Tree species	Branch death (%)	Branch dieback (%)	Healthy canopy
Acmena smithii	0.077 ± 0.077 a	3.231 ±1.444 a	96.75 ±1.459 a
Archirhodomyrtus beckleri	8.846 ±4.535 b	93.846 ±2.839 b	6 ±2.78 b
Decaspermum humile	0 ±0 a	33.333 ±21.082 c	66.667 ±21.082 c
Gossia hillii	0 ±0 a	100 ±0 b	0 ±0 b
Rhodamnia maideniana	2.917 ±2.497 a	100 ± 0 c	0 ±0 c

Other species

Syzygium corynanthum

Three large (25m+ in height) *S. corynanthum* trees are present in the open areas of the study site but outside of the assessment plots. One tree is showing significant levels of decline with >75% defoliation, 20% branch death and the remaining branches showing evidence of dieback (Fig. 85). Epicormic shoots on branches have evidence of older infection causing dieback and fresh infection on new shoots (Fig. 85). On the other two trees examined, foliage loss is restricted to dieback of the very tip of branches and foliage loss is less obvious. However, high levels of *A. psidii* infection of new growth was observed on both trees despite the comparatively low levels of decline.

Syzygium hodgkinsoniae

Juvenile and mature *S. hodgkinsoniae* trees were assessed at the study site (Fig. 86). Juvenile trees were found to have high incidence of rust infection present on new shoots and expanding foliage with shoot and branch dieback evidence of the impact of past infection episodes. The impact on tree health of younger trees was evidenced by their sparse canopies (high transparency level).

The impact of *A. psidii* on mature trees is currently less obvious with foliage density levels high on trees examined at the site (Fig. 87). However, branch dieback and the presence of coppice shoots on stems is evidence of stress. These coppice shoots were found to be infected by *A. psidii* (Fig. 87). Dead growing tips were also found to be present on most branches. Despite this, fruit was found present on one of the trees with no evidence of *A. psidii* infected identified at the time.

Indications of rates of decline of Myrtaceae at this site were also captured through photographic evidence. Photographs taken in 2014 (Fig. 88) show some evidence of decline on *Decaspermum humile* and *Syzygium corynanthum* but little evidence of impact on *Acmena smithii*. In 2016, a similar photo captured the same trees and showed the dramatic change in tree health with considerable dieback on *S. corynanthum* and *D. humile* and a decline in foliage density on a single *A. smithii* tree. When examining other *A. smithii* trees at the site it was found that there was considerable variability in susceptibility to *A. psidii* within the species.

Other sites

To date, two other sites within the Tallebudgera Valley have been examined to determine if the dieback levels identified at the study site are representative of what is happing on a larger scale. At both sites considerable levels of decline in the under- and mid-story were identified with severe impact identified on *A. beckleri*, *G. hillii* and *D. humile*. Dead *Rhodomyrtus psidioides* trees were identified at site 2. Using Google Street View images, changes from 2014 to 2016 were able to be established (Fig. 89-90). Impact assessments are yet to be completed on these sites.

Figure 84 *Rhodamnia maideniana* with *Austropuccinia psidii* sori on new growth flush (a) and expanding foliage (c) and the effects of repeated infection on growing tips (b)

Figure 85 *Syzygium corynanthum* with (a) severe defoliation and branch dieback compared to a relatively healthy tree with a dense canopy. However, on closer examination tip dieback is present on a high percentage of trees. (c) Young myrtle rust infected epicormic regeneration on trees with significant dieback

Figure 86 *Syzygium hodgkinsoniae* showing significant decline in canopy density (a) and severe *Austropuccinia psidii* infection on new shoots and expanding foliage (b, c) resulting in branch dieback (d)

Figure 87 Mature *Syzygium hodgkinsoniae* showing early stage of decline in the lower canopy (a) with evidence of branch dieback and epicormic shoots, all of which are infected by *Austropuccinia psidii* (b). Fruit of *S. hodgkinsonia* were identified on one of the trees.

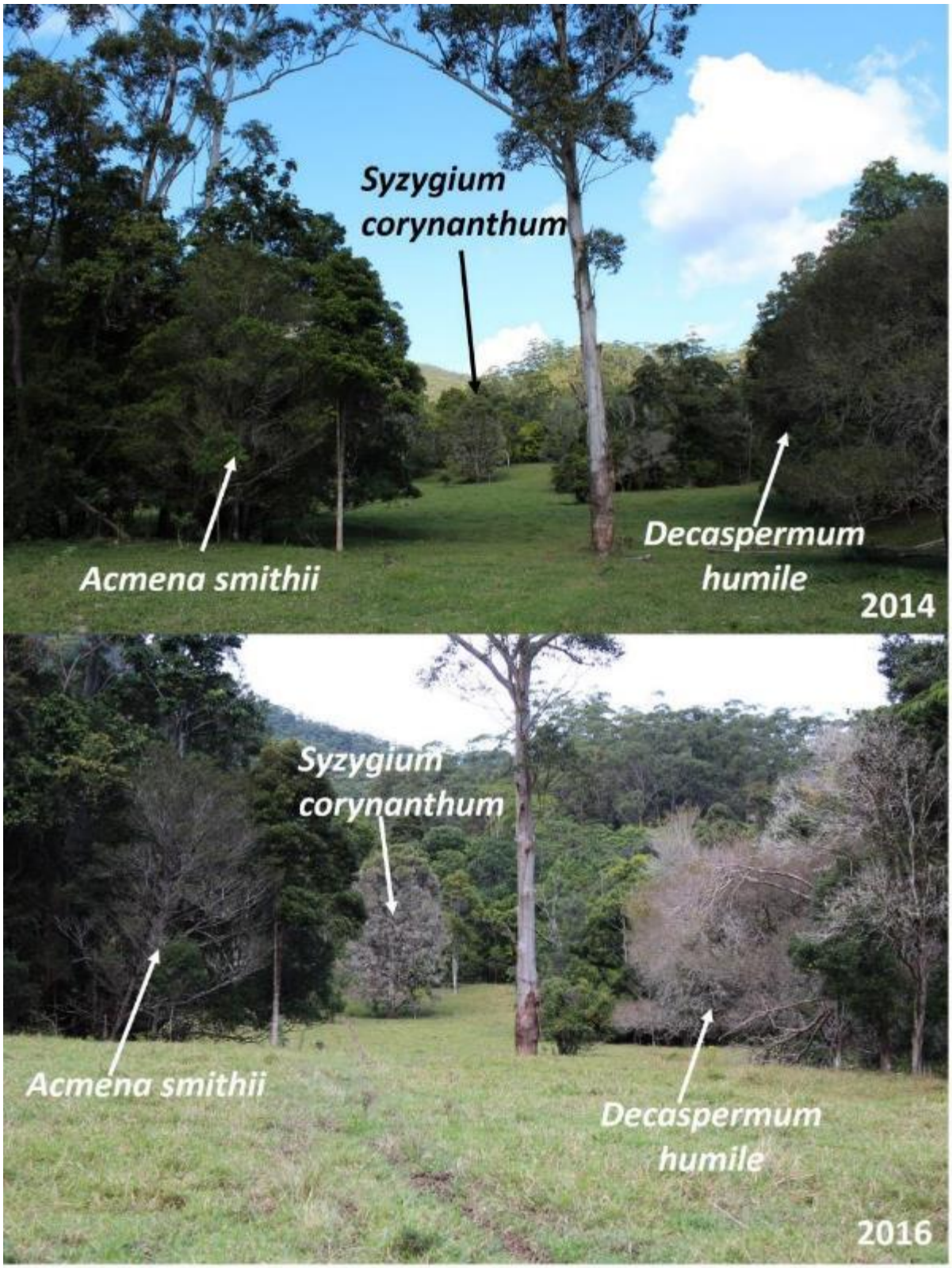


Figure 88 Progression of decline from 2014 (top) to 2016 (bottom) on *Acmena smithii*, *Decaspermum humile* and *Syzygium corynanthum* caused by repeated *Austropuccinia psidii* infection

Figure 89 Photographs showing decline of the mid-story made up of *Archirhodomyrtus beckleri*, *Decaspermum humile* and *Gossia hillii* at a second site in the Tallebudgera Valley. In 2014 (top photo - Google) vegetation density is high with little to no dieback evident in comparison to 2016 (bottom photo) where significant decline is evident in the mid and understory vegetation.

Figure 90 Photographs showing decline of the mid-story made up of *Archirhodomyrtus beckleri*, *Decaspermum humile*, *Gossia hillii* and *Rhodomyrtus psidioides* at a third site in the Tallebudgera Valley. In 2014 (a) vegetation density is high with little to no dieback evident in comparison to 2016 (b) where significant decline is evident in the mid and understory vegetation.

Screening for resistance

As part of this current project, and in conjunction with other research studies, screenings of a range of commercially significant species have been conducted using the methodologies outlined in this report. A summary of the findings and the relevant references are provided below:

Spotted gum

Published as: Pegg GS, Brawner J, Lee DJ, 2014. Screening *Corymbia* populations for resistance to *Austropuccinia psidii*. Plant Pathology. 63, 425-436.

To determine the threat *A. psidii* poses to plantation and native eucalypts, artificial inoculation was used to screen germplasm of spotted gum (*Corymbia* spp.) for resistance to the biotype of *A. psidii* that has become established in Australia. The objective was to characterize resistance to *A. psidii* within the *Corymbia* species complex so that management strategies for the deployment of germplasm from existing breeding programmes of these spotted gum species could be developed.

Plant populations:

To examine the influence of origin on rust resistance patterns, 15 seedlings from between nine and 11 open-pollinated families from a range of provenances were examined for disease levels following inoculation with *A. psidii*. Two provenances from CCC were compared to five CCV and two CH provenances. Of the CCC provenances, one was from an inland location in far north Queensland (Mt Garnet) and a second was from a coastal location at the southern end of the range of CCC (Yeppoon) in Queensland. Presho, a CCV provenance frequently severely damaged by a native fungal foliage pathogen (*Quambalaria pitereka*), originates in the westernmost range of the species. The resistance of Presho to *A. psidii* was compared to the more coastal provenances of Woondum, Brooyar, Mt McEuan and another inland provenance, Ballon. For CH, two provenances were selected from different rainfall zones: Lockyer and Nerang. Resistance levels were also examined at a family level for seven provenances (CCC – Mt Garnet, Yeppoon; CCV – Brooyar, Mt McEuan, Woondum; CH – Lockyer, Nerang) with a family structure. Ballon and Presho provenances were excluded from family level comparison as the seedlings originated from a bulked seed lot. Also included were families from CCV, CH and CT seed orchards. To examine repeatability of results at a family level, a second inoculation was done on seedlings from three CCV provenances, Brooyar, Mt McEuan and Woondum and results compared to the first inoculation. In addition, seedlings from seven full-sib controlled cross *Corymbia* hybrids and eight commercial *Corymbia* clones selected from within control pollinated hybrid families of CT mother trees pollinated with CCV pollen, were included in the study.

Assessment

Seedlings were assessed 12 days after inoculation for incidence of disease (% of seedlings with symptoms) and severity of infection on new shoots and expanding leaves using a disease rating scale: 1 = no symptoms evident or presence of yellow flecking; 2 = presence of a hypersensitive reaction (HR) with fleck or necrosis; 3 = small pustules, <0.8 mm diameter, with one or two uredinia; 4 = medium-sized pustules, 0.8–1.6 mm diameter with about 12 uredinia; 5 = large pustules, >1.6 mm diameter, with 20 or more uredinia on leaves, petioles and/or shoots (Junghans et al., 2003b). Ratings 1–3 are considered as indicating resistance. Disease incidence (I) was also assessed as a percentage of the four youngest inoculated leaves on seedlings showing evidence of pustule development and uredinia. Disease severity (S) was scored as a subjective assessment of the percentage of the total area of infected foliage on diseased leaves only. In total, four assessments were available for analysis as response variables: disease incidence (I) and disease severity (S), disease rating scale (1–5) and the percentage of resistant seedlings based on the disease rating scale.

Outcomes

Inter- and intraspecific variability in rust resistance was observed among spotted gum species. There was no apparent relationship between climatic conditions at the provenance origin and disease resistance. The heritability estimates for all assessments are moderate to high and indicate a significant level of additive genetic variance for rust resistance within the populations. The results of this study clearly identify potential to select for resistance at the family level within the tested populations. While the potential for *A. psidii* to detrimentally impact upon *Corymbia* in the nursery and in young plantations was demonstrated, estimations of the heritability of resistance suggest that efforts to enhance this trait through breeding have reasonable prospects for success.

Eucalyptus species

Published as: Lee, D. J., Brawner, J. T., and Pegg, G. S. 2015. Screening *Eucalyptus cloeziana* and *E. argophloia* populations for resistance to *Austropuccinia psidii*. Plant Dis. 99:71-79.

Disease screening to determine the threat *Austropuccinia psidii* poses to plantation and native eucalypts in Australia was undertaken in half-sib families of two contrasting eucalypt species, *Eucalyptus cloeziana* and *E. argophloia*. Artificial inoculation with a single-lesion isolate of *A. psidii* was used to screen these species for resistance to the biotype of *A. psidii* established in Australia. The objective was to characterize resistance to *A. psidii* within these two distinct species: *E. argophloia,* a vulnerable species with a narrow distribution, and *E. cloeziana*, a species with a broad and extensive distribution in Queensland. Results for *E. cloeziana* indicate that inland provenances are more resistant to *A. psidii* infection than provenances from coastal regions. Heritability estimates for the two assessment systems used (resistance on a 1-to-5 ordinal scale verses resistance on a 0-to-1 binomial scale) were low to high (0.24 to 0.63) for *E. argophloia* and moderate to high (0.4 to 0.91) for *E. cloeziana*, indicating a significant level of additive genetic variance for rust resistance within the populations. This study demonstrates the potential to select resistant families within the tested populations and indicates that *A. psidii* could detrimentally affect these species in native forests, nurseries, and plantations.

Published as: Roux J, Germishuizen I, Nadal R, Lee DJ, Wingfield MJ, Pegg GS, 2015. Risk assessment for *Austropuccinia psidii* becoming established in South Africa. Plant Pathology 64, 1326-1335.

This study was conducted in Australia in collaboration with FABI in South Africa

The aim of this study was to consider the susceptibility of selected Eucalyptus genotypes, particularly those of interest to South African forestry, to infection by *A. psidii*. In addition, risk maps were compiled based on suitable climatic conditions and the occurrence of potential susceptible tree species. This made it possible to identify the season when *A. psidii* would be most likely to infect and to define the geographic areas where the rust disease would be most likely to establish in South Africa. As expected, variation in susceptibility was observed between eucalypt genotypes tested. Importantly, species commonly planted in South Africa show good potential for yielding disease-tolerant material for future planting. Myrtle rust is predicted to be more common in spring and summer. Coastal areas, as well as areas in South Africa with subtropical climates, are more conducive to outbreaks of the pathogen.

Published as: Butler, J. B. and Freeman, J. S. and Vaillancourt, R. E. and Potts, B. M. and Glen, M. and Lee, D. J. and Pegg, G. S. (2016) *Evidence for different QTL underlying the immune and hypersensitive responses of Eucalyptus globulus to the rust pathogen Austropuccinia psidii.* Tree Genetics & Genomes, 12 (3). ISSN 1614-2942

We studied the genetic basis of variation in rust resistance in *Eucalyptus globulus*, the main plantation eucalypt in Australia. Quantitative trait loci (QTL) analysis was undertaken using 218 genotypes of an outcross F2 mapping family, phenotyped by controlled inoculation of their open pollinated progeny with the strain of *A. psidii* found in Australia. QTL analyses were conducted using a binary classification of individuals with no symptoms (immune) versus those with disease symptoms, and in a separate analysis dividing plants with disease symptoms into those exhibiting the hypersensitive response versus those with more severe symptoms. Four QTL were identified, two influencing whether a plant exhibited symptoms (Ppr2 and Ppr3), and two influencing the presence or absence of a hypersensitive reaction (Ppr4 and Ppr5). These QTL mapped to four different linkage groups, none of which overlap with Ppr1, the major QTL previously identified for rust resistance in Eucalyptus grandis. Candidate genes within the QTL regions are presented and possible mechanisms discussed. Together with past findings, our results suggest that *A. psidii* resistance in eucalypts is quantitative in nature and influenced by the complex interaction of multiple loci of variable effect.

***Backhousia citriodora* – Lemon myrtle**

D. Lee, J. Doran, G. Pegg, D. Lea, P. Macdonell and F. Giblin. Myrtle Rust Screening in Lemon Myrtle Provenance Plantings. RIRDC Publication

The plant material used for this research was from a gene bank planting of *Backhousia citriodora* provenances, families and clones established near Beerburrum in south-eastern QLD in 1995-96 by CSIRO Forestry and Forest Products (now part of CSIRO NRCA) and Queensland Forestry Research Institute (now part of Qld DAF). Rooted cuttings from the range of clonal material set at the USC glasshouse were transferred to the Department of Agriculture and Fisheries glasshouse to undergo extensive testing for resistance to myrtle rust. The plants were inoculated with myrtle rust spores suspended in distilled water and 'Tween 20'. Immediately after inoculation, the clones were covered with a plastic sheet for 24 hours to maintain high humidity levels and leaf wetness in a controlled environment room set between 18 and 20^{o}C in the dark. After 24 hours plastic sheeting was removed and plants grown in a shade-house and hand watered as required. Disease symptom progression was monitored daily and assessed 12 to 14 days after inoculation using a five category disease rating score on new shoots and expanding leaves. In addition the percent leaf area with pustules (sori) was visually assessed to indicate the severity of the infection.

Austropuccinia psidii resistance screening in the glasshouse showed significant differences between provenances for the disease rating score, with all provenances tested being susceptible to the disease. The other method used to assess the severity of the disease was to assess the percentage of leaves affected by the disease. This method again indicated that there were significant differences between provenances: range from 15.9% for the Silver Valley clones to 35.3% for the Cathu clones. Disease incidence was also significantly different at the family level with a wide variation in disease incidence between families. The lowest incidence of leaf infection was recorded at 7.5% for family 1465 from the Woondum provenance, while the highest was 60% for family 1381 from Carlisle Island.

Susceptibility/resistance levels in populations of three broad leaved Melaleuca species from across their native Australian range

Three species of broad leaved *Melaleuca* were assessed for variability in susceptibility between different populations across their native range. Three month old seedlings were inoculated with *A. psidii* under controlled conditions and assessed 20 days post infection. Susceptibility was based on a 1 to 5 rating scale described in the methods and in Fig. 95-100.

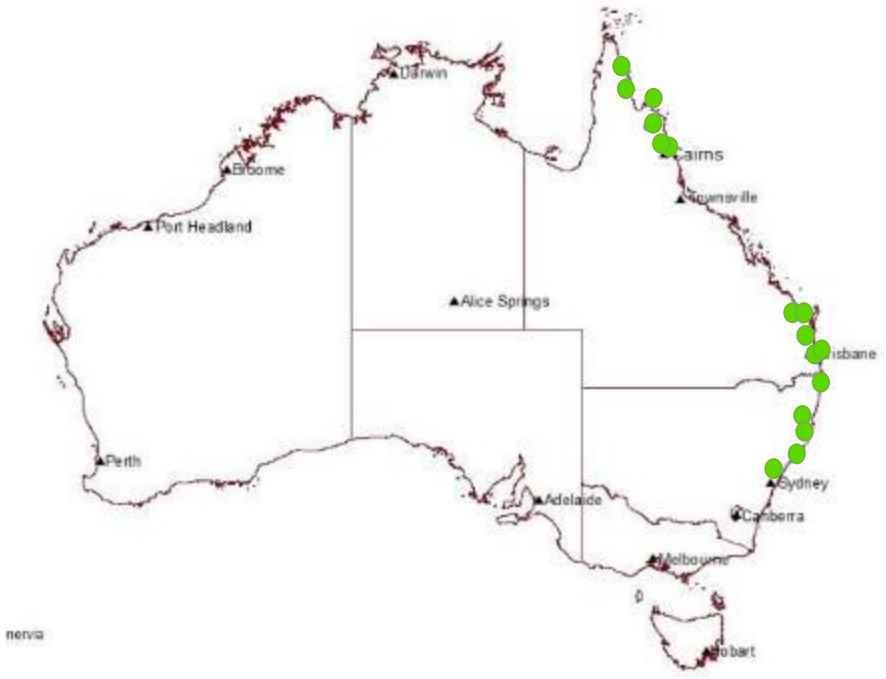


Figure 91 *Melaleuca quinquenervia* provenances tested for susceptibility to *Austropuccinia psidii*

Twelve provenances of *Melaleuca quinquenervia* from across the native range (Fig. 91) were assessed for susceptibility to *A. psidii*. Susceptibility was identified in all provenances tested. However, significant differences in levels of susceptibility (Table 17) were identified when comparing provenances. Seedlings from Rokeby National Park in Queensland were found to be most resistant. Queensland populations were in general found to have higher levels of resistant seedlings (Rating 1 & 2) in comparison to NSW provenances apart from Boggy Creek provenance. The Boggy Creek collection is the only collection taken from trees post myrtle rust detection in Australia and seed was collected from known resistant trees (Pegg *et al*. 2012; Fig. 92). All other provenances in NSW had less than 32% of seedlings showing resistance to *A. psidii*. The most susceptible provenance was Kuranda in far north Queensland with only 12.5% of seedling showing resistance to *A. psidii*.

Table 17 Susceptibility of *Melaleuca quinquenervia* provenances based on percentage of seedlings rated as resistant to *Austropuccinia psidii* and the average susceptibility rating based on a 1-5 rating scale as per Pegg *et al*. 2014.

***Melaleuca quinquenervia* provenance**	**Resistant seedlings (%) Ratings 1 & 2**	**Average myrtle rust susceptibility rating**
Rokeby NP, Qld	80	1.8 $^{\pm 0.176}$ a
Caloundra, Qld	77.5	1.825 $^{\pm 0.192}$ ab
Boggy Creek, NSW	67.5	2.452 $^{\pm 0.229}$ bc
Gympie, Qld	57.5	2.475 $^{\pm 0.256}$ c
Dohles Rocks, Qld	55.26	2.447 $^{\pm 0.252}$ c
Teddington, Qld	53.85	2.59 $^{\pm 0.289}$ c
Bribie Island, Qld	52.78	2.694 $^{\pm 0.258}$ c
Tozers Gap, Qld	52.5	2.4 $^{\pm 0.208}$ bc
Moreton Island, Qld	38.46	2.949 $^{\pm 0.229}$ cd
Mt Molloy, Qld	37.14	2.8 $^{\pm 0.2}$ c
Long Jetty, NSW	31.58	3.395 $^{\pm 0.222}$ de
Tuggerah Lake, NSW	30.77	3.59 $^{\pm 0.237}$ e
Julatten, Qld	29.73	2.838 $^{\pm 0.162}$ cd
Worrel Ck, NSW	29.73	3.432 $^{\pm 0.231}$ de
Hawks Nest, NSW	29.41	3.559 $^{\pm 0.25}$ de
Port Macquarie, NSW	20.51	3.667 $^{\pm 0.218}$ e
Kuranda, Qld	12.5	3.475 $^{\pm 0.148}$ de

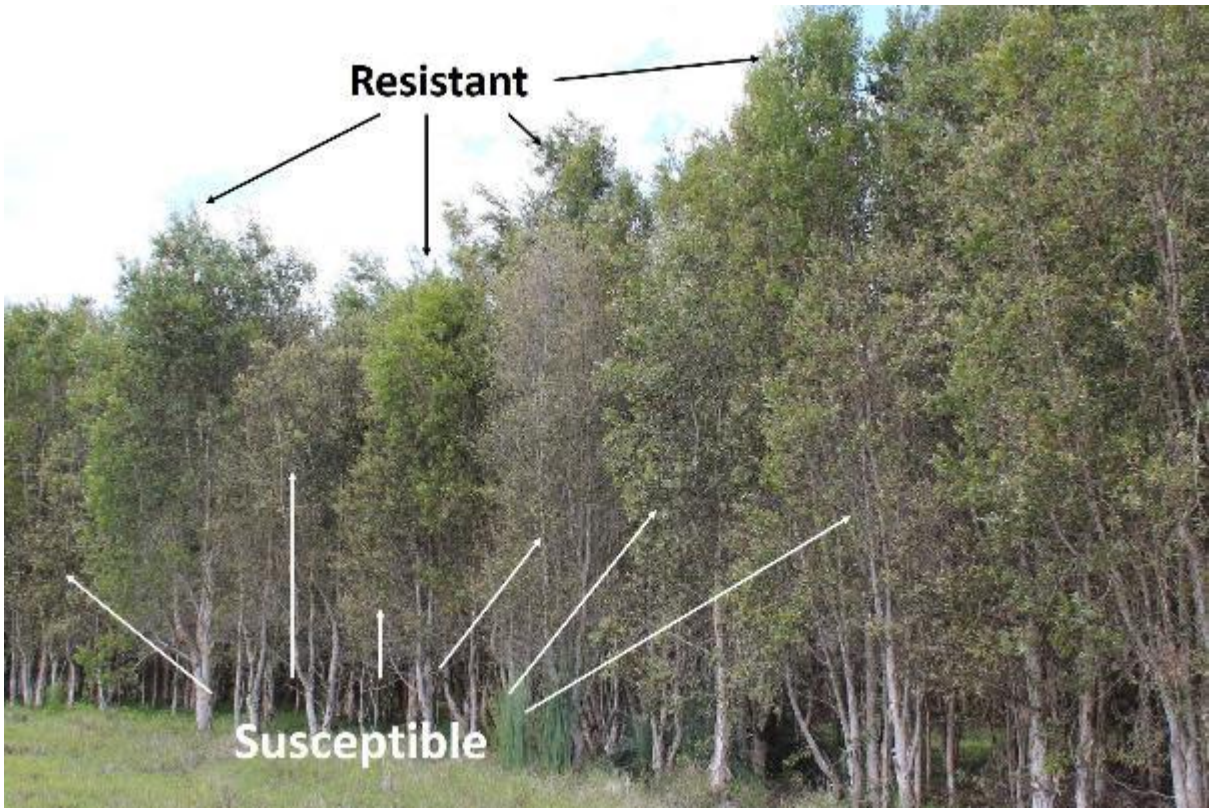


Figure 92- *Austropuccinia psidii* resistant and susceptible *Melaleuca quinquenervia* at Boggy Creek trial site in northern New South Wales.

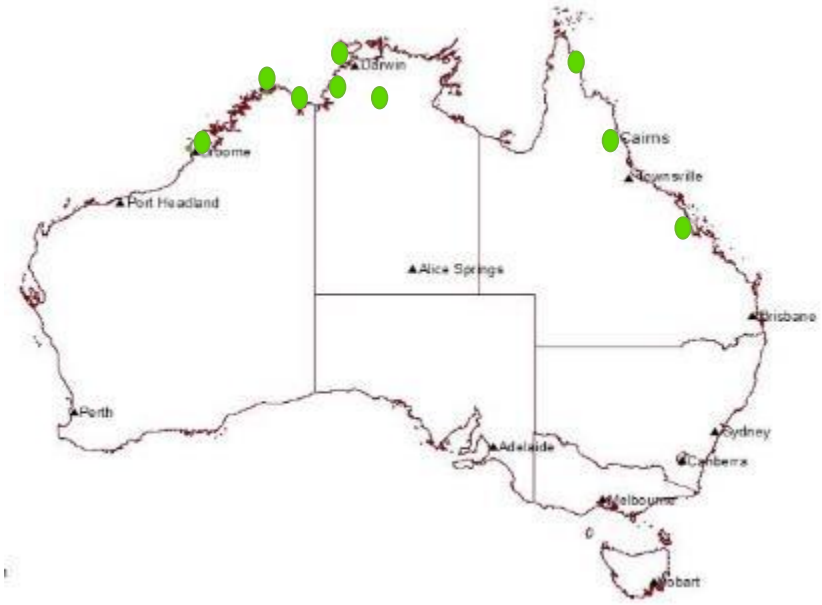


Figure 93 *Melaleuca leucadendra* provenances tested for susceptibility to *Austropuccinia psidii*

Provenance of *M. leucadendra* from Queensland, Northern Territory and Western Australia were assessed for susceptibility to *A. psidii* (Fig. 93). Susceptible seedlings were identified in all provenances with no resistant seedlings found from Nimalaica Calypan provenance in Western Australia. Mareeba and Cambridge Gulf provenances had the highest percentage of resistant seedlings and lowest average susceptibility ratings (Table 18). Other provenances tested all had less than 20% of seedlings showing resistance and average susceptibility levels greater than 3.8 (Table 18).

Table 18 Susceptibility of *Melaleuca leucadendra* provenances based on percentage of seedlings rated as resistant to *Austropuccinia psidii* and the average susceptibility rating based on a 1-5 rating scale as per Pegg *et al*. 2014.

***Melaleuca leucadendra* provenance**	**Resistant seedling (%) Rating 1 & 2**	**Average myrtle rust susceptibility rating**
Mareeba, Qld	76.92	2.051 $^{\pm 0.244}$ a
Cambridge Gulf, WA	65	2.125 $^{\pm 0.169}$ a
Iron Range, Qld	17.5	4 $^{\pm 0.179}$ b
St Lawrence, Qld	17.5	3.925 $^{\pm 0.18}$ b
King River, NT	15.79	3.816 $^{\pm 0.176}$ b
Wangi, Litchfield NP, NT	15	3.9 $^{\pm 0.159}$ b
Buffalo Ck, NT	12.5	3.8 $^{\pm 0.18}$ b
Kalumburu Mission, WA	6	4.256 $^{\pm 0.102}$ b
Nimalaica Claypan, WA	0	4.2 $^{\pm 0.096}$ b

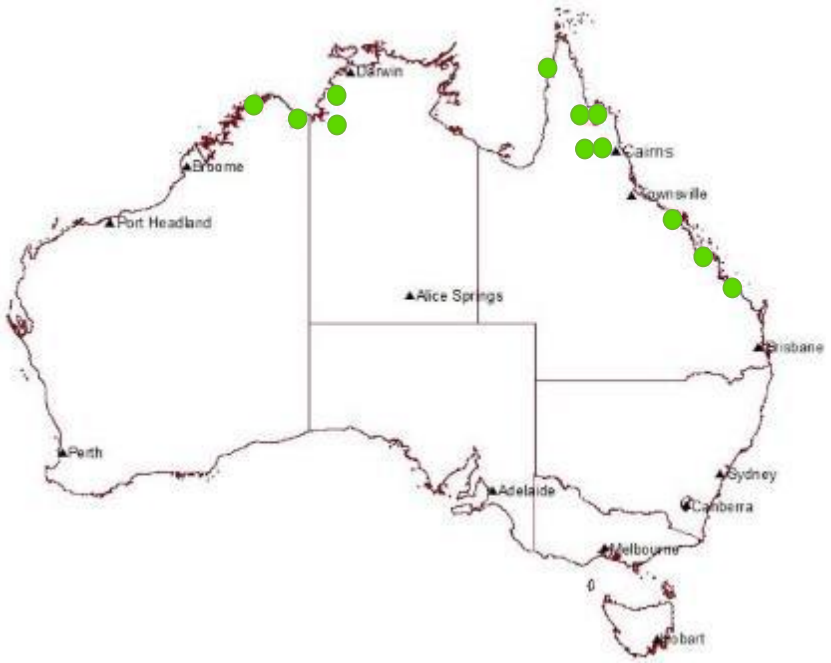


Figure 94 *Melaleuca viridiflora* provenances tested for susceptibility to *Austropuccinia psidii*

Twelve provenances of *M. viridiflora* from Queensland, Western Australia and Northern Territory were assessed for susceptibility to *A. psidii* (Fig. 94). No provenance was identified as being completely resistant to *A. psidii*. Rockhampton provenance had the highest percentage of resistant seedlings (72.97) and Weipa the lowest with only 8.3% (Table 19). All other provenances had less than 50% of seedlings rate as resistant to *A. psidii*.

Table 19 Susceptibility of *Melaleuca viridiflora* provenances based on percentage of seedlings rated as resistant to *Austropuccinia psidii* and the average susceptibility rating based on a 1-5 rating scale as per Pegg *et al*. 2014.

***Melaleuca viridiflora* provenance**	**Resistant seedling (%) Rating 1 & 2**	**Average myrtle rust susceptibility rating**
Rockhampton, Qld	72.97	2.216 ±0.206 a
Round Hill Head, Qld	45.71	2.914 ±0.254 b
Chillagoe, Qld	45	2.775 ±0.244 ab
Wangi, Litchfield NP, NT	43.59	2.949 ±0.249 b
Prosperpine, Qld	32.5	3.275 ±0.256 bc
North Kennedy River, Qld	27.5	3.275 ±0.232 bc
Lakeland, Qld	25.64	3.59 ±0.223 c
Theda Station Kalumbura, WA	25.64	3.205 ±0.181 bc
Laura, Qld	24.32	3.459 ±0.228 bc
Ningbing Range Rd, WA	19.44	3.639 ±0.236 cd
East Baines River, NT	13.16	3.605 ±0.194 cd
Weipa, Qld	8.3	4.167 ±0.197 d

Figure 95 Rating 1 – Resistant rating with no evidence of *Austropuccinia psidii* sori (pustules) or necrotic lesions; clear or chlorotic flecks can often be seen on new growth flush and

Figure 96 Rating 2 – Resistant rating with evidence of necrotic lesions (hypersensitive reaction) with no evidence of *Austropuccinia psidii* sori (pustules)

Figure 97 Rating 3 – Susceptible - Small lesions (pustules) with or two uredinia present; lesion size <0.8mm in diameter

Figure 98 Rating 4 – Susceptible – Medium sized lesions (pustules) with multiple uredinia; lesions 0.8-1.6mm diameter

Figure 99 Rating 5 – Susceptible – large lesions (pustules) > 1.6mm diameter with 20 or more uredinia on leaves, petioles, shoots and/or juvenile stems

Figure 100 Rating 5 – Susceptible – large lesions (pustules) > 1.6mm diameter with 20 or more uredinia on leaves, petioles, shoots and/or juvenile stems

6. Discussion

Geographical spread of Austropuccinia psidii

Since first being detected in Australia in April 2010, *A. psidii* has continued to spread and is now well established in native ecosystems along the east coast of New South Wales and Queensland. Disease reports extend west to the Great Dividing Range, including towns like Toowoomba, but only a few detections, apart from nursery stock, have been made further west including Warwick in south east Queensland and Chillagoe in far north Queensland. While the disease is frequently detected in parks and gardens in Victoria, there have been no reports of impact in native ecosystems (Pers. Comm. David Smith). Similarly, in Tasmania, detections have been restricted to residential gardens and nurseries (dpipwe.tas.gov.au). A detection on Melville Island in May 2015 represents the most western distribution of the disease, with further detections on the mainland in Darwin in the Northern Territory occurring soon after (environment.gov.au/nt.gov.au). At the time of writing this report, there have been no detections of *A. psidii* in Western or South Australia.

Globally *A. psidii* has also spread, with the most recent detections in Sumatra on *Melaleuca leucadendra* and *Rhodomyrtus tomentosa* (McTaggart et al. 2015). While the disease distribution in Sumatra and other surrounding Islands in Indonesia is unknown, it would appear likely that it is becoming widespread with further reports of the disease on *Rhodomyrtus tomentosa* across its native range in high elevation regions (Pers. Comm. Mathew Purcell CSIRO). Additional detections have also been made in Singapore, also on *R. tomentosa* (Pers. Comm. Mathew Purcell CSIRO; McTaggart Unpublished). While it is known that the isolate detected in Sumatra is identical to the one detected in Australia (McTaggart et al. 2015), the sequencing of the isolate from Singapore had not been completed at the time of completion of this report. It would appear likely that *A. psidii* is widespread in parts of Asia. *Austropuccinia psidii* has also continued to spread in South Africa and the particular strain that is present there differs from what is in Australia, and is indeed considered unique with no similar biotypes or strains previously reported (Roux et al. 2016). The additional threat that this strain, and indeed others identified in South America, pose to Myrtaceae in Australia is unknown. Da Silva et al. (2013) found variable levels virulence when testing the susceptibility of *Metrosideros polymorpha* to five different strains from Brazil.

Disease impact

Within Queensland and New South Wales the effects of *A. psidii* can be observed in a range of different ecosystems from the NSW/Victoria border to Cape York Peninsula including temperate, subtropical and tropical regions (both wet and dry tropics). Impact on different species has been recorded in coastal heath, littoral, subtropical and tropical rainforest, wet and dry sclerophyll and sand island ecosystems. The host range of *A. psidii* in Australia continues to grow with 347 species now reported as susceptible from 57 genera (Giblin & Carnegie 2014). It is likely that this number will increase over time as the pathogen continues to spread into new environments. Impact on plant species ranges from minor leaf spotting, varying levels of defoliation and dieback and death of trees/shrubs in all life stages including seedlings, saplings and mature trees. Infection of coppice regrowth, either as a stress response to repeated rust infection or a mode of regeneration following disturbance, has been recorded for a range of species. Flower and fruit infection has been identified on 32 hosts resulting in reduced fecundity or in some cases, total loss of fecundity with seedling regeneration for species like *Rhodamnia rubescens* not observed since study of the impact of *A. psidii* commenced. Regeneration of *Rhodomyrtus psidioides* has been observed in the form of root suckers but, in sites assessed to date, all of these have shown impact from *A. psidii* infection. Indirect effects on flowering have also been recorded with a link to dieback levels, as a result of repeat infection, and reduced flowering rates. The effects of the disease on pollinator behavior due to changes in host density or species fragmentation and flowering rates is unknown.

Assessment methodologies - glasshouse

One of the main outputs for this project was focused on developing assessment methods for controlled (glasshouse) disease screening and for determining susceptibility levels and impact on species and plant communities under field conditions. Glasshouse screening methodologies were first developed for *A. psidii* to select resistance within key eucalypt species (Junghans et al. 2003) and has been used successfully as a standard process in on-going eucalypt breeding programs. The methodology used for this project has been adopted from this process. From an industry perspective, glasshouse screening is an effective and rapid process allowing for the testing of large numbers of individuals under controlled conditions. It can be cost effectively applied for identifying resistant species and varieties as well as comparing provenances, families and individuals within a species. The method can be applied to seedlings, cuttings and larger plants as long as they are actively growing and producing susceptible flush and they are not nutrient limited. Pruning of more mature plants and inoculating the new growing tips as they emerge is preferable and been found successful in our studies (results not reported). Like all glasshouse studies, the method removes external factors that may impact on disease development which can result in “escapes” and incorrect identification of resistance.

There are two distinct resistant reactions which are consistent across different species and genera (*Eucalyptus*, *Corymbia and Melaleuca*), one with clear flecking and absence of any necrotic or hypersensitive reaction and the other with hypersensitive reaction but an absence of any *A. psidii* sori (pustules). The severity of necrotic lesions can vary. However, it is uncertain if there is a genetic relationship to this or if leaf age/stage of development at the time of infection is influencing symptom expression. Studies by Butler *et al*. (2016) identified four QTL, two influencing whether a plant exhibited symptoms (Ppr2 and Ppr3), and two influencing the presence or absence of a hypersensitive reaction (Ppr4 and Ppr5). Pustule size can also be influenced by leaf age (K. Ireland Unpublished) as can the type of spore. The development of telia and production of teliospores has been found on a range of species (*Eucalyptus globulus*, *Rhodamnia rubescens*, *Melaleuca quinquenervia*) but has generally been restricted to foliage that is in a more advanced stage of development at the time of inoculation (Pegg unpublished). While teliospores, and associated basidiospores, have been identified from a range of host species (Pegg et al. 2014) their role in sexual recombination within populations is unknown. There is still some uncertainty regarding the life cycle of *A. psidii*.

Most of the disease screening work that has been done as part of this project has been aimed at examining large populations for on-going breeding programs in forestry (Pegg et al. 2014; Lee et al. 2014) or research into resistance mechanisms (Bala et al. 2013, Butler et al. 2016). The level of detail used when conducting glasshouse assessments is somewhat dependent on the required outcomes. From a nursery industry perspective simply distinguishing resistance from susceptibility of a species or cultivar is quite likely sufficient. Obviously selecting resistance would be of great commercial benefit, not only as a result of improved sales, but through reduced reliance on fungicides both from the producer and end-user perspective. Selecting individuals that produce no symptoms are also more likely to be aesthetically appealing. From an environmental regeneration perspective where large numbers of seedlings are often required, the removal of highly susceptible individuals and then using both resistant and tolerant planting material may be more appropriate. However, all this is dependent on their being an affordable screening process available or, in the longer term, a well-established tree breeding program for key species selecting resistant parent material. Certainly in this study we have identified the potential to do this for species of the broad-leaved *Melaleuca* across the range of provenances. One additional factor to carefully consider is that rust selections should not be done in isolation from other pests and disease issues known to affect the selected plant species. In studies on spotted gum, it was identified (Pegg *et al*. 2014; Pegg & Lee Unpublished) that individuals found to be resistant to *A. psidii* were in many cases susceptible to the endemic foliage pathogen *Quambalaria pitereka* and vice versa. While the infection process of *Q. pitereka* differs (Pegg *et al*. 2009), the conditions for infection are very similar to what is required for *A. psidii* and therefore there is considerable overlap in distribution of the two pathogens.

There is no doubt that using glasshouse screening is effective for examining species resistance/susceptibility but care must be taken in using this to predict disease impact in either commercial plantation systems or native ecosystems. For example *Melaleuca alternifolia*, the main species used for the production of tea tree oil, is highly susceptible when tested under glasshouse conditions (Morin *et al*. 2012, Giblin & Pegg unpublished). However, while the disease can be detected under field conditions, the impact is generally low and to date no widespread control measures have been required (Pers. Comm. P. Entwistle). Likewise the disease has not been reported from native stands of *M. alternifolia*. Similarly screening of eucalypts has identified susceptibility within three species of spotted gum (Pegg *et al*. 2014) and a range of commercially and environmentally significant *Eucalyptus* species (Lee *et al*. 2014; Roux *et al*. 2014), but few detections have been made in plantation (Carnegie 2014) or native stands. There is a need to better understand the rating system and how it relates to species reactions under field conditions and indeed should be coupled with field survey work to better understand impact.

Assessment methodologies - field

Methods for assessing disease susceptibility and to capture impact on species and plant communities over time have been developed as part of this and the previous PBCRC project. The method developed and reported by Pegg *et al*. 2014 for assessing levels of susceptibility under field conditions has primarily been used in Queensland. As reported in this study, the method of using disease levels on new flush ranging from relatively tolerant (RT) to extremely susceptible (ES) is not only a simple method to apply but, as our studies have shown, is effective in predicting impact over time, particularly those rated a highly (HS) or ES. This scale of assessment should be adopted as part of the procedure when reporting new hosts of *A. psidii* or reports on host in regions where the disease has only recently spread. This method doesn't necessarily require a detailed knowledge of the pathogen and symptoms of impact on different hosts and could be very easily utilised more widely by people working in areas of vegetation management. Its adoption by a wider community will improve our ability to not only identifying new species and their relative susceptibility but help gain vital information on potential variability in susceptibility within host species and options for selecting resistance both from a commercial and environmental perspective. From an industry perspective it can also be used to select individuals from which cuttings or seed can be propagated. Similar rating scales have also been developed in Hawaii to monitor disease levels on *Metrosideros* and *Syzygium jambos* (Uchida et al. 2008). However, there is no indication as to who is using these methods developed or how effective they have been.

Methods to assess impact of *A. psidii* on species and plant communities have transformed over time as we have gained a better understanding of the host-pathogen interactions and individual host species characteristics. Unfortunately, for many of the species we have studied, there is very little published information, apart from taxonomic descriptions, on growth habits, importance in the ecosystem and even less on other pests and pathogens that might affect these species. Our work has highlighted the difficulties associated with field assessments for *A. psidii*. When assessing *R. rubescens* across 43 sites, a strong correlation was identified between crown transparency and incidence of disease on old leaves and also the disease rating score, but there was no correlation with incidence of disease on the new leaves. Crown transparency is an effective measure of the loss of foliage due to repeated infection by *A. psidii*. The incidence of *A. psidii* on old leaves can be used to identify previous infection events that have not as yet resulted in leaf loss and can also help identify differences in reactions to infection by different host species. The disease rating score measures the impact of recent infection events on new growth flush, but is imperfect if the conditions have not been conducive to disease development or symptoms are yet to present.

When assessing incidence of *A. psidii* on new leaves, there are compounding factors that affect whether the score obtained accurately provides an indication of disease impact or not. For example, if a new leaf flush coincides with an infection event several weeks prior to assessment then the true impact of *A. psidii* is obtained (i.e. a high incidence score). However, if a new flush event does not coincide with an infection event, then this could give a misleading score (i.e. a low or zero incidence score). Furthermore, if there is no flush event at the time of assessment, even if there were conditions conducive for an infection event, no incidence score is able to be assessed. Another more recent observation is the fact that repeated infection is resulting in a change in flush morphology, which is also influencing disease development. In many cases flush size is smaller and leaves appear thicker and, while still susceptible to infection, severity of symptoms can differ from what was first observed when *A. psidii* was first detected.

As we have gained a better understanding of how some species react to repeated infection we have identified that crown transparency, when assessing from directly under the tree, can provide an underestimate of the true impact score. In the case of a species like *Rhodamnia rubescens*, defoliation and dieback is initiated in the lower canopy, often with a "healthy" flush of growth occurring in top 25% of branches; thus assessing trees from directly underneath underestimates crown transparency compared to assessing from the side. This impact can be captured by including a canopy health score where the percentage of dead branches, branches with evidence of dieback and healthy branches are assessed. In other tree species dieback is limited to the outer growing tips with little immediate evidence on canopy density apparent. We recommend a combination of crown transparency as well as assessing tree canopy health (branch death and branch dieback) and disease ratings on new flush when present be used to assess impact of *A. psidii* in native environments. Moreover, we recommend frequent assessments of sites to gain a better understanding of disease impact on different species over time.

In studies where we have established short and long term monitoring plots, we have been able to modify assessment methods to suit the questions we have posed. In the case of *Melaleuca quinquenervia*, one of our primary questions has been to determine the impact of repeated infection on new growth flush, on epicormic and coppice establishment, growth of seedlings and coppice shoots and flowering as well as interactions between *A. psidii* and native insect pests. With an increase in frequency of assessments we have applied methodologies commonly used in plant pathology including disease incidence and severity to monitor disease progressions and assess impact. We have developed a disease severity score based on an understanding of the different symptoms occurring on *M. quinquenervia* but have found that these can easily be transferred to a range of species. However, we have also identified some anomalies where the rating system would need to be varied. For example on both *Leptospermum liversidgei* and *Baeckea frutescens*, infection was restricted primarily to juvenile stems with little or no evidence of symptom developments occurring on foliage making the use of the before mentioned disease severity rating difficult. Using levels of branch dieback and death do however, seem to be applicable across all areas.

Impact of Austropuccinia psidii *on species of Myrtaceae*
Impact of Austropuccinia psidii *on common species,* Rhodamnia rubescens *and* Rhodomyrtus psidioides*, across their native range*

The disease exclusion trial at Olney SF unequivocally showed that repeated, severe infection by *A. psidii* resulting in a reduction in foliage production, severely affects crown health, and can lead to tree death. It also revealed that myrtle rust is capable of killing mature trees in a native forest ecosystem in fewer than four years. This provided strong supporting evidence for our conclusions that the severe crown loss, dieback and tree mortality we observed in *R. rubescens* and *R. psidioides* across their native range was a result of repeated infection by *A. psidii.* There is no other plausible causal agent. This is supported by previous studies (Pegg et al. 2014; www.brushturkey.com.au).

Austropuccinia psidii has caused significant damage in commercial plantations and orchards in South and Central America (to both exotic and endemic species), to invasive weed species in Florida and Hawai'i, and to endangered endemic species in Hawai'i, and is now causing significant damage to endemic Myrtaceae in natural ecosystems in Australia. Severe infection and crown loss, dieback and tree mortality were observed in our indicator species—*R. rubescens* and *R. psidioides*—across their entire native range. *R. psidioides* has been particularly affected, with deaths of over half the trees in many stands, including mature trees up to 12 m tall, within two-to-three years of *A. psidii* establishing. This species is now undergoing a process of rapid decline across its range as a result of *A. psidii* invasion: of the 297 trees across 18 stands that we assessed, over half of them were dead, with all but three sites having exceptional levels of tree mortality. This level of decline has continued with follow-up surveys in 2016 at selected sites identifying even greater levels of tree mortality. We know from observations of botanists and seed collectors that these stands were healthy prior to *A. psidii* establishing (e.g., www.brushturkey.com.au). Thus, based on our data, *R. psidioides* has undergone a population decline of greater than 50% in less than five years. Similar impact has been observed in Hawai'i to endangered *Eugenia koolauensis* and mature trees of the exotic *S. jambos* (Uchida and Loope 2009; Loope 2010), but not previously to an abundant endemic species. The damage to *R. rubescens* is just as extensive but less severe, with 11.5% of trees assessed as dead in our study, and tree mortality observed in fewer than half the stands. After our initial assessments it appeared that *R. rubescens* could cope better with the disease because it managed to produce some flush even after substantial defoliation. Still, based on our surveys, *R. rubescens* numbers have declined by 11% in less than five years. This common species is also undergoing significant decline across its range and our most recent (2016) assessment of selected sites suggests that this decline rate may be accelerating.

Our quantitative findings on both species are supported by field botanists who have conducted extensive surveys of these species during routine botanical surveys and seed collecting over many years: "...all sites of *R. rubescens* visited since 2010 are in serious decline...with no flowering or seed observed" (Doug Beckers, Senior Botanist, National Parks & Wildlife Service, pers. comm., May 2014); "*R. rubescens* and *R. psidioides* are seriously threatened, with significant decline in all stands visited...the worst area in the Bellinger Valley [NSW] where hundreds of plants have died..." (Richard Johnstone, Seed Bank Officer/Botanist, The Australian Botanic Garden Mount Annan, pers. comm., July 2014); "Neither *R. rubescens* or *R. psidioides* have flowered since 2010, with at least half of *R. rubescens* dead and all known *R. psidioides* dead at monitoring sites" (Deb Holloman, Bush Regeneration Coordinator, National Parks & Wildlife Service, May 2015). Monitoring of *R. rubescens* and *R. psidioides* stands in northern NSW—which prior to 2011 appeared to be vigorous and in robust health—revealed devastating effects, with 75% tree mortality in some areas (Smith, M., National Parks & Wildlife Service, 2014, unpublished). The impact of *A. psidii* on these hitherto widespread species, neither of which is legislatively 'listed' under state and federal legislation, is likely to be sufficient to justify a change in their status to 'threatened'. Other highly susceptible species currently listed as 'threatened', such as *R. angustifolia* (Pegg et al. 2014), are likely to be elevated to higher extinction-risk categories following similar field investigations.

The assessments of *R. rubescens* in Olney SF allowed us to gain information not only on the effects of the disease on *R. rubescens* but on the progression of disease and rate of decline in the plant population. This revealed how quickly the crown declines (within 6 months) due to repeated infection of immature leave and subsequent defoliation, but also fluctuations in incidence and severity of disease over time. The study using the image processing software QUANT (Vale et al. 2003) provided more rigorous data on the effects of *A. psidii* on *R. rubescens* at Olney SF. For the immature leaf class, we saw a significant difference in both disease severity and leaf area between treatments, indicating a causal relationship between disease and reduced leaf area. We had hypothesized that reduced leaf size would be associated with increased crown transparency on trees: fewer leaves resulting in less photosynthesis leading to a gradual decline in carbohydrates for leaf production. However, we did not see a significant correlation between these traits. This may be an artifact of the trial design, as there were only three assessments (August, November, December 2011) of crown transparency prior to sampling leaves (February 2012). The data did, however, show that untreated trees had more disease, smaller leaves and higher crown transparency compared to treated trees. We surmise that the gradual decline in foliage retention on diseased trees resulted in a reduction in the surface area of new leaves produced, leading to decreased photosynthesis capability. Over time this likely resulted in the depletion of stored carbohydrates, affecting further leaf development and foliage replacement. Like *P. dioica* in Jamaica (MacLachlan 1938) and *S. jambos* in Hawai'i (Uchida and Loope 2009), *R. rubescens* and *R. psidioides* are severely defoliated by *A. psidii,* resulting in the production of highly susceptible new growth, which in-turn becomes severely infected and defoliated. Repeated defoliation leads to reduced foliage re-growth, affects reproduction, and ultimately causes tree morality, likely due to carbohydrate depletion (McPherson and Williams 1998).

The overall impact of an invasive species can be measured by the total area occupied (range), the abundance across that range, and the damage on individual plants (Parker et al. 1999). Our work showed that *A. psidii* has expanded across the entire natural range of our two study species, was found on every plant surveyed (669 *R. rubescens* and 297 *R. psidioides*), and the damage to individual plants was generally high to extreme. Thus, based on the metrics proposed by Parker *et al*. (1999), the impact of *A. psidii* on these two endemic species in natural ecosystems in Australia is severe. Our study, however, only investigated damage to individuals within populations, and as such more research is required to gain an understanding of the effects on plant communities and ecosystem processes (Parker *et al*. 1999). The short-term ecosystem-level impacts of *A. psidii* are likely to include a reduction in photosynthesis and productivity, stimulation of decomposition and changes in microclimate and light condition in the forest due to crown loss and mortality of highly susceptible species (Lovett *et al.* 2006). Longer term effects are likely to be related to a change in species composition, due to local extirpation of highly susceptible species, and subsequent changes of forest structure, productivity, and nutrient cycling. Already we are observing changes in plant community structure, with native grasses and exotic weeds (e.g. *Lantana camara*) colonizing gaps provided by mortality of *R. psidioides* stands (authors, pers. obs.). The rapid decline in both species has been further demonstrated when selected sites were revisited two years after the initial assessment. In all cases there was a dramatic increase in number of dead trees for both species. Seedling germination at the sites assessed has been absent. However, in some *Rhodomyrtus psidioides* sites, root sucker regeneration has been observed. Unfortunately the majority of these are already showing severe levels of decline as a result of *A. psidii* infection. This further highlights the need to quickly implement a conservation program for these rapidly declining species with opportunities to capture vital germplasm diminishing.

Impact on other species

Detailed studies on the impact of *A. psidii* needs to expand with our studies only representing a small proportion of those likely to be significantly affected by *A. psidii*. A further 49 species have been ranked as either HS or ES. While much of the data on the impact of *A. psidii* on these hosts has been collected from ex-situ plantings (Botanic Gardens), it should perhaps be seen as an important guide to prioritizing species for not only further study but for development of conservation strategies. To some degree this is already happening with studies of *Chamelaucium uncinatum* (Geraldton Wax) to examine for resistance within the natural population and study the potential impact of *A. psidii* if it did arrive in Western Australia (Tobias *et al*. 2015). Unfortunately for this species no resistance was identified. Other studies are also now beginning to focus on prioritising species based on host susceptibility, identified as part of this project, and environmental factors within their native range that are suitable for high disease incidence and severity levels. The current conservation status and potential to conserve are also being considered.

Following some initial surveys conducted as part of this project, Sunshine Coast Regional Council staff are conducting more extensive assessments and establishing long term monitoring programs for the Threatened *Lenwebbia* sp. Blackall Range. Populations of *Lenwebbia* sp. Blackall Range are restricted to south-east Queensland and were already threatened by urban development. Significant *A. psidii* impact has been recorded on all life stages including decline and death of mature trees, saplings and seedlings and infection of flowers and fruit. This rapid decline in tree health should warrant increasing the status of this species to Critically Endangered. Another species with limited distribution is *Rhodamnia maideniana*. Just prior to the detection of *A. psidii* in Australia *Rhodamnia maideniana* was deemed no longer Threatened (Pers. Comm. G. Guymer). Unfortunately this species is highly susceptible with severe impact on all life stages including flowers and fruit. While assessments have not been completed across the entire range, albeit restricted, indications are that this species is likely to become critically endangered in the very near future.

Impact on fecundity

Austropuccinia psidii infection has been identified on flowering and fruiting structures of 32 species. While the direct effects of rust are easier to report, estimating the indirect effects repeated infection have on flower production, seed/fruit development and seed viability is more complex, particularly given the absence of detailed data on most species in relation to what is deemed normal. The small study we did investigating the impacts of *A. psidii* on fruit development of *R. rubescens* at Tucki Tucki NR revealed some interesting results. There was no significant difference between mean numbers of fruits on treated versus untreated branches after the treatment began, however, there was an observable difference at the final assessment (34.7 versus 17.6, respectively); a higher proportion of fruits fell from untreated branches (71%) compared to treated branches (47%). The time-series plots reveal a sharp increase in infection on untreated fruit at the second assessment (82.5%), but a decline in percentage infected fruit at the final assessment (62.4%). This indicates that more diseased than un-diseased fruit had been shed resulting in a greater proportion of un-diseased fruit being retained. Fruit on untreated branches began to fall immediately and at a steady rate, while there was a delay in fruit drop on treated branches of two weeks. Fruit of *R. rubescens* are likely to fall once they have matured, and so the decline in number of fruit on treated branches is expected. We surmise that the delay in fruit fall of treated branches in the early period of the study indicates a fruit maturation period; fruit are maturing on the tree before they are later naturally shed. The lack of such a delay in the untreated trees indicates that a proportion of fruit did not go through this maturation period before being shed, indicating that disease was the cause of premature fruit drop. Seed collected from under treated trees was larger/heavier than fruit collected from under untreated trees, indicating an impact of disease. Other studies have indicated that disease affects fruit maturation and seed viability (Assefa et al. 2014). Although not conclusive, this study indicates an impact of *A. psidii* on the regeneration capacity of *R. rubescens.* The lack of fruit on this species observed by us and others since 2010 supports this.

When assessing *Melaleuca nodosa* regeneration following the wildfire event in the coastal heath, those trees showing high levels of dieback produced lower numbers of seed capsules. Given that we didn't identify any infection on flower or fruiting structures would suggest that this was more related to an indirect effect. Similarly, lower flowering rates were observed on *Baeckea frutescens* and *Leptospermum liversidgei* showing stem dieback in comparison to those with no symptoms of rust infection. However, this was not quantified. Pegg et al. (2012) previously reported the effects of *A. psidii* infection on juvenile stems and shoots of *Melaleuca quinquenervia* with flowering restricted to trees showing resistance or infection on foliage only. Further research is required on a range of species examining direct and indirect effects of repeated *A. psidii* infection on species fecundity. Trials using fungicide to eliminate or limit *A. psidii* infection would be required to effectively study these impacts. The effects of reduced plant density or fragmentation of populations and flowering rates on pollinators, both mammals and invertebrates, and any long term implications on genetic diversity is unknown.

Both native stingless bees (*Tetragonula* sp.) and European honey bees (*Apis melifera*) have been observed actively foraging *A. psidii* urediniospores, particularly on *Syzygium jambos*. While not quantified, bees were seen visiting rust covered leaves in the absence of any apparent flower buds. This has been reported previously in Jamaica where bees were seen foraging spores on *Pimenta dioica* (allspice) trees. Examination of bee loads found they consisted purely of *A. psidii* urediniospores (Chapman 1963). When inspecting a nearby hive orange deposits within the comb were found to contain *A. psidii* spores. The effects on hive health for both *Apis melifera* and *Tetragonula* species here in Australia is unknown.

Impact of Austropuccinia psidii on plant communities and species composition – recovery of coastal heath following wildfire

Our research is the first to examine and report on the impact of *A. psidii* in different plant communities. The first study examined impact on regeneration following wildfire in coastal heath environments in which a range of Myrtaceae are considered common. While fire is considered as an important selection agent in the development of Australia's native flora (Gill 1975), the development of new coppice and young seedlings en-masse are ideal conditions for the development and rapid spread of *A. psidii*. *Austropuccinia psidii* infection was found on all species of regenerating Myrtaceae in the coastal heath environment following wildfire. *Melaleuca nodosa* and *Melaleuca quinquenervia* were both significantly impacted with repeated *A. psidii* infection causing severe dieback, and in some cases, death of coppice with infection starting soon after regeneration was first detected. However, for *M. quinquenervia* we identified that around 30% of trees were resistant to *A. psidii*. This was not the case for *M. nodosa* where all trees assessed showed some level of infection and dieback but some individuals appeared more tolerant to disease producing flowers and seedpods. The viability of the seed was not determined.

Melaleuca nodosa regeneration following wildfire was significantly affected by *A. psidii* with repeat infection on susceptible new growth flushes resulting in dieback and reduced fecundity. *Melaleuca nodosa* occurs on the coast and tablelands of Queensland and New South Wales often forming dense thickets in heathland environments (Brophy *et al*. 2013). *Austropuccinia psidii* infection of this species has previously been reported, but not quantified, in both Queensland (Pegg *et al*. 2014) and northern New South Wales by the authors with infection on established and mature trees in coastal heath environments as well as areas further inland (e.g. south of Casino, northern NSW). The impact of *A. psidii* on this species across its natural range of distribution needs further investigation.

This study does provide some evidence of indirect impact of *A. psidii* on tree fecundity for *M. nodosa* with trees showing high levels of dieback producing a lower number of flowers/seed pods or no flowers/seed pods. However, without control treatments this impact is difficult to quantify. Over time as decline rates continue, an additional impact that may occur could be related to a reduction in abundance of this species in an ecosystems considered species rich. The decline in number of a species like *M. nodosa*, despite there being some individuals with higher tolerance to *A. psidii*, could result in a further reduction of pollination rates. *Melaleuca* species are pollinated by a wide suite of generalist insect vectors, including native and honey bees, beetles and flies (Beardsell et al. 1993). As a result of this it is believed that low densities of *Melaleuca* spp. in species rich or diverse sites can result in limited reproductions through pollinators losing pollen as they forage between other plant species. At the Lennox Head site it was evident, based on frequency of coppice regeneration, that *M. nodosa* was previously present in relatively concentrated patches but with the repeated infection by *A. psidii* causing dieback the species inability to compete with other non-Myrtaceae (e.g. *Banksia* spp.) may result in further decline of the species with additional interference to pollination processes further exacerbating the problem.

Further decline of this species may be evidenced by the fact that during our study period no seedling germination of *M. nodosa* was observed either within the established plots or in the areas surveyed. While reported previously as regenerating via coppice or epicormics following fire, *M. nodosa* primarily reproduce from seed. In a study conducted by Hewitt *et al*. (2014) they identified that recruitment of *M. nodosa* was continuous with seedlings found throughout the three-year study. Their study was conducted from 2009 to 2012 but there is no mention of myrtle rust found on *M. nodosa* within their study plots. In our case it is uncertain if seed viability was affected as a result of the intensity of the fire or as a result of previous rust infection events pre-fire.

Impact of *A. psidii* was also evident on species of *Leptospermum* and *Baeckea frutescens* but there appeared to be relatively high levels of resistance within the populations as well. *Leptospermum liversidgei* was the most susceptible, with stem and shoot dieback recorded and evidence of this dieback resulting in reduced fecundity levels. This species, while a common coastal shrub in eastern parts of Australia, has become a serious weed in South Australia and Western Australia (Kloot 1985; Lam & Etten 2002). *Leptospermum trinervium*, while not within the study plots, was identified as a species on which *A. psidii* could have significant impacts, particularly with regards to regeneration following disturbance. *Austropuccinia psidii* infection on new flush growth and juvenile stems initially resulted in defoliation followed by shoot and branch dieback and complete death of all coppice shoots on some trees. Of the trees assessed all showed some level of decline as a result of repeated infection. Further studies on this widespread species are required.

To draw conclusion on the influence of *A. psidii* on species composition within this environment we would have ideally established fungicide control plots. This would have allowed us to compare rates of establishment and recovery over time as well as examine the effects *A. psidii* may be having species composition and ability of Myrtaceae to compete with non-Myrtaceae or resistant species. Gaining an understanding of species composition pre-fire as well as comparing impacts of rust under different fire regimes (e.g. wildfire v prescribed burn) would have added to the value of our studies and perhaps provided insight into appropriate fire management strategies that might limit *A. psidii* impact. In an attempt to overcome this additional plots have been established in sites where prescribed burns were planned. However, despite considerable time and effort in establishing these plots (2014/15) these areas to-date remain unburnt.

Varying levels of *A. psidii* impact were observed on *Melaleuca quinquenervia* coppice regeneration with about 70% of trees assessed within our transect studies showing some degree of susceptibility. This is a similar level of susceptibility identified in previous studies (Pegg et al. 2012). The effect on seedling survival was also demonstrated with *A. psidii* infection identified on *M. quinquenervia* seedlings soon after emergence. At the conclusion of the study, the more resistant *Lophostemon suaveolens* was the dominant species despite the fact, based on presence of adult trees, that it was not as common as *M. quinquenervia* or even *Leptospermum polygalifolium* or *L. whitei* pre-fire. While not all seedlings of *M. quinquenervia* were killed, the effects of *A. psidii* infection on those susceptible were obvious, with repeated loss of apical dominance resulting in "shrub-like" growth characteristics. However, it must be stated that without data on recruitment in the absence of *A. psidii* in this ecosystem we cannot make definitive statements on the impact.

The impact of *A. psidii* on coppice regeneration of *M. quinquenervia* following wildfire in the swamp ecosystem varied from minor leaf spots to repeated death of coppice shoots leading to eventual death of the entire tree. Coppice regeneration was also affected by mirid bugs attacking the new shoots, initially in combination with *A. psidii* but also during periods when the rust fungus was absent. Disease and mirid bug impact levels at the site declined over time as tree growth rates appeared to slow after approximately 12 months. It is uncertain if this is a site characteristic or due to the impact of rust and insect damage affecting host vitality. It is also not clear if the presence of the mirid bugs had an additive effect to decline from rust or if the presence of one affected the ability of the other to infect/attack the host tissue. It is also unclear if the presence of *A. psidii* on susceptible hosts resulted in an increase in attack levels from mirid bugs on rust resistant trees. In studies conducted in Florida (Rayamajhi *et al*. 2005), where *M. quinquenervia* is a weed, *A. psidii* and psyllids showed a better ability to co-attack the same leaf tissues in comparison to the *A. psidii* and the weevil *Oxyops vitosa*. To better understand this the interactions between other native insects that attack *M. quinquenervia*, particularly when regenerating, we conducted a further study where we could examine the effects in more detail.

Impact of Austropuccinia psidii *on Melaleuca quinquenervia coppice regeneration and interaction with native insect pests*
In the trial established to examine the effects of *A. psidii* on coppice regeneration of *M. quinquenervia* and the interaction with insects, the combined effect of insect herbivory and rust infection resulted in higher levels of stump mortality and coppice damage and reduced tree growth rates. These results are similar to the findings from Rayamajhi *et al*. (2010) in Florida although our types and number of insect species differed. Insect damage consisted of a combination of weevil damage on stems, chewing from Chrysomelids and locusts, tip sucking bugs and damage to new growth flush from mirid bugs. Insect activity was restricted to warmer months of the year and strongly correlated with an increase in both maximum and minimum temperature. On the other hand the occurrence of *A. psidii* was independent of any climatic factors and is perhaps more likely to be closely linked with the availability of susceptible growth flush on host species. While other studies have found links to duration of leaf wetness (Zauza et al. 2014) this was not the case in our study. Perhaps climatic conditions in northern New South Wales are generally more conducive to year-round infection by *A. psidii*.

Despite monthly application of fungicide and insecticide, total elimination of *A. psidii* and insects did not occur. This is despite Carnegie *et al*. (2015) demonstrating this frequency of fungicide application was sufficient to prevent *A. psidii* symptom development on *Rhodamnia rubescens*. This may be due to the fact that *M. quinquenervia* is a faster growing species and the site was in an open forest and "monoculture" of evenly developing *M. quinquenervia* with no other tree species competing or shading the trees. Control of both insect pests and *A. psidii* appears to have improved in the latter half of the experiment and maybe due to a slow-down in recorded growth rates. However, fortnightly application of treatments would be recommended for future studies.

The effects of repeated infection on *M. quinquenervia* trees was just beginning to become more apparent in the last couple of assessments. Leaf area measurements were lower (but not significantly so) in insecticide treated plots in comparison to fungicide only treated plots. Similar to findings from Carnegie *et al*. (2015), one of the impacts of repeated infection on host species is a decline in leaf size and leaf area. Indeed, on many of the susceptible trees, foliage loss was significant and stem dieback was beginning to become more apparent. Significant insect damage on fungicide treated trees, in the absence of rust, has led to a dense growth habit with higher average leaf area and was the only treatment to increase in growth rate over the last 6 months of the trial. *Austropuccinia psidii* incidence and severity levels increased over this period on the other treatments.

Previous studies in Australia have identified a reduction in reproductive structures with increasing severity of *A. psidii*, particularly when shoot infection or death occurs (Pegg *et al*. 2012). Similar findings have been made in relation to *Oxyops vitiosa*, a weevil used for biological control of *M. quinquenervia* in Florida. Pratt *et al*. (2005) found that with *O. vitiosa*, which exclusively feeds on seasonal flushes of developing foliage at branch apices, undamaged trees were 36 times more likely to produce flowers and seed pods than damaged trees. They concluded that *M. quinquenervia* compensates for damage by producing new stems and foliage but this results in a substantial reduction in reproduction. At the time of this report flowering had not occurred on any of the treatments but there is evidence of frequent shoot dieback on untreated control trees and insecticide-only treated trees. There is no evidence of this occurring in fungicide or fungicide + insecticide treated trees. It is hoped that we can continue this experiment until flowering data is captured.

While the impact of *A. psidii* on *M. quinquenervia* is apparent, there is also evidence of resistance within the field populations studied. At sites studied to date it would appear that there is around 30% of trees that are either resistant or tolerant to *A. psidii*. However, to determine if this is representative of the species across its native range we conducted glasshouse screenings of seedlings from provenances in Queensland and New South Wales. All seed, apart from a single collection from our trial site (Boggy Creek), was collected prior to the detection of *A. psidii* in Australia. Levels of resistance varied between provenances with some showing high levels of resistant seedlings within the populations. Interestingly all populations from New South Wales had 30% or fewer seedlings resistant to *A. psidii*, similar to what our field observations have been. However, seed taken from known resistant parents (identified & collected by G. Pegg) showed a much higher percentage of resistance (67.5%) suggesting potential for a more resistant future generation. However, it is unclear what effects this change may have on genetic diversity within populations or what the reduced flowering rates may have on pollination frequency.

Given that *Melaleuca quinquenervia* ecosystems in Australia are already considered under threat from urban expansion and agricultural activities, the additional impact that *A. psidii* may have in relation to not only tree survival but flower production should not be underplayed. *Melaleuca quinquenervia* are key to maintaining and improving water quality (McJannet 2008), as well as being important to a range of wildlife. The species is unique in that the trees can be temporarily inundated with water for up to three to six months of the year but can also tolerate fire (Laroche 1999). They provide valuable nesting or roosting sites for a number of bird and bat species (Grover & Slater 1994) and are a very important food source for migratory birds. The species is a significant Autumn/Winter flowering plant which provides shelter and breeding sites for water-birds, amphibians and insects, and nectar for species such as the gliders and scaly-breasted lorikeet (*Trichoglossus chlorolepidotus*). The grey-headed flying fox (*Pteropus poliocephalus*) and little red flying-fox (*P. scapulatus*) also consume the flowers. *Melaleuca* trees can flower all year round, providing an almost constant source of nectar and pollen, which is particularly important in winter for insects, birds and bats. While impact of *A. psidii* and associated decline of *M. quinquenervia* stands has been demonstrated as part of this study, there is a clear potential to implement either a program to select resistant individuals for current regeneration programs or establishment of seed orchards to enable long term provision of *A. psidii* resistance and breeding programs to help regain lost genetic diversity.

In addition to examining resistance patterns in *M. quinquenervia* we assessed provenances from across the native range of two other broad-leaved *Melaleuca*; *M. leucadendra* and *M. viridiflora*. Similar to *M. quinquenervia*, variability in levels of resistance were identified. This is the first reported study of these species and has identified that *A. psidii* has the potential to significantly impact on these two species, particularly in areas of the Northern Territory and Western Australia where low levels of resistant seedlings were recorded. While we have clearly demonstrated the susceptibility of these species under controlled conditions, the impact of *A. psidii* in their natural environments is unknown. Both species are ecologically significant and play an important role in fragile ecosystems such as the Kakadu National Park. An understanding of *A. psidii* impacts under environmental conditions such as those experienced in the tropical regions of Australia is crucial. Further studies on larger populations of these *Melaleuca* species are needed to not only help predict impact but to prevent impact though use in revegetation programs.

Impact of Austropuccinia psidii *in wet sclerophyll environments with rainforest understorey*

Our studies are the first to identify the significant impact of *A. psidii* on plant communities in a wet sclerophyll ecosystems where the majority of rainforest understory species are Myrtaceae. In Queensland, wet sclerophyll forests are mostly found in the south-east but also occur as narrow ecotones bordering the western edge of rainforests in the wet tropics (Peeters & Butler 2014). These ecosystems are unique to Australia (Ashton 1981) and the understory may be comprised of rainforest plants or be grassy with sparse shrub layer or a combination of both. Species composition within the site may vary depending on climate, topography, soil type and previous land management practices. In the absence of fire or other disturbances, many of the wet sclerophyll sites will transition to rainforest with a dense understory reducing light levels preventing further recruitment of eucalypt species (Ashton & Atwill 1994). In the absence of *A. psidii* it would appear that this process was well underway at our study site.

While the overstorey Myrtaceae, *Eucalyptus grandis* and *Lophostemon confertus*, showed no evidence of *A. psidii* impact, all mid- and understorey Myrtaceae were impacted upon. Only *Acmena smithii* showed variability in levels of susceptibility to *A. psidii* and impact and it is now the dominant regenerating species. *Archirhodomyrtus beckleri*, *Decaspermum humile*, *Gossia hillii* and *Rhodamnia maideniana* were the most common mid- and understorey species but all were significantly impacted upon with dieback recorded on all trees. It is likely that in the very near future these species will become extinct from this location with no evidence of resistance. This is a rapid change in the plant community structure, given that the disease was only detected in the region five years prior to our assessment (Pegg et al. 2014; Carnegie et al. 2015). Unfortunately what we have found at this site appears also to be representative of sites in the immediate area that have similar species compositions and are at similar stages of establishment. However, further surveys are required to determine if this type of impact is restricted to the Tallebudgera Valley or extends into other areas in Queensland and northern New South Wales, including the World Heritage Listed Gondwana Rainforest. It is also unknown if the same level of impact can be identified in more established rainforest ecosystems in the region.

Time will tell if the "disturbance" caused by *A. psidii* is enough to prevent the site transitioning to a rainforest ecosystem. Indications are that *Acmena smithii* will become a dominant species but there may also be other non-Myrtaceae that will inhabit the site. However, it will still result in a dramatic change in species composition and may well reduce species diversity within the site. Studies in other ecosystems have shown that a reduction in biodiversity increases ecosystem vulnerability to invasive plant species and also enhances the spread of plant fungal diseases and alters the richness and structure of insect communities (Johannes *et al.* 1999). Conversely, a loss in the highly susceptible species may also see a reduction in disease pressure through lower *A. psidii* inoculum levels resulting in reduced disease incidence and severity levels.

Given that species like *Archirhodomyrtus beckleri*, *Gossia hillii* and *Decaspermum humile* and considered widespread, it is possible that the impact we have seen at the site in Tallebudgera is an indicator of the potential long term impact *A. psidii* may have on these species. However, the influence of different climatic conditions may prevent the level of damage seen at this site from occurring. The impact on *Syzygium corynanthum*, also considered common, is significant and has not been reported previously. Likewise species considered Threatened, such as *Syzygium hodgkinsoniae*, seem likely to be pushed closer to extinction with impact occurring both on regenerating saplings and, albeit slower, mature trees. *Rhodamnia maideniana* has a restricted range and exists within regions that seem ideal for *A. psidii* and should be considered for immediate conservation action. Significant impact by *A. psidii* on this species has now been identified (Pegg unpublished) in a range of different sites in south-east Queensland.

7. Conclusion

A more in depth understanding of the long term effects of repeated *A. psidii* infection on host species and plant communities in ecosystems outside of where we have focused our studies (subtropical regions) is required. The effects of tropical and temperate conditions in Australia on disease incidence and severity are to date unknown. Additionally, data on the long term impact on species considered MS or RT is still required. Evidence of dieback in the ex-situ plantings have given some indication that decline will not be as rapid in these species but the effects on plant health and ability to reproduce and even compete in different plant communities may be compromised. It must also be remembered that these ex-situ plantings are often only made up of a handful of individuals and may not be representative of the broader populations. Studying impact in different ecosystems in which some of these species exist will help identify species at greatest risk and prioritise conservation efforts.

In just the short time that *A. psidii* has been established in Australian natural ecosystems, we have observed significant damage and tree mortality. There are few exotic diseases in Australia that threaten a wide range of Australian flora. The most significant of these is *Phytophthora cinnamomi,* which is associated with mortality of a wide range of overstorey and understorey species in multiple families including Myrtaceae, Proteaceae, Epacridaceae and Papilionaceae (Wills 1992; Weste 1994). *Phytophthora cinnamomi* is associated with significant ecological impact in plant communities in south-eastern and south-western Australia, with declines in species richness, plant abundance and percentage cover (Wills 1992; Weste 1994). Fauna dependent on these plant communities are also affected. While *A. psidii*-associated mortality of dominant overstorey trees has not yet been recorded (although effects on vegetative and seedling recruitment of these remain unknown), over time we are likely to see significant alterations to understorey plant communities due to *A. psidii*.

There are numerous examples of invasive forest pathogens causing landscape-level ecological impacts (Ellison et al. 2005; Loo 2009), including chestnut blight (*Cryphonectria parasitica*) in North America (Anagnostakis 1987), Dutch elm disease (*Ophiostoma ulmi* and *O. nova-ulmi*) in Europe and North America (Gibbs 1978) and phytophthora dieback (*Phytophthora cinnamomi*) in Australia (Wills 1992; Weste 1994). The greatest impacts occur when invasive pathogens cause mortality of foundation species (Ellison *et al*. 2005; Loo 2009). Death of foundation species are also often very dramatic, garnering government and public attention, e.g. phytophthora dieback, Dutch elm disease and chestnut blight. Although receiving government and public attention prior to reaching Australia (e.g., O'Neill 2000; Grgurinovic *et al.* 2006), and during the emergency response following detection (e.g. Carnegie & Cooper 2011; Makinson 2012), interest in *A. psidii* in Australia has waned, partly because the "mycological firestorm" that "environmentalists predicted" does not appear to have eventuated (according to McRae 2013); there has been no large scale tree mortality and minimal affects to industries so far. Our studies, while currently limited, have shown that *A. psidii* is severely affecting key species in natural ecosystems, and likely to be significantly affecting a wider range of species. Local extirpation of highly susceptible species is likely, potentially leading to species extinction. The dramatic decline of multiple species in the wet sclerophyll sites in Queensland is the first report of impact at a plant community level. The extent to which this level of damage extends is unknown. However, our studies have demonstrated, at a species and plant community level, the potential for *A. psidii* to negatively affect Australia's biodiversity.

Our selected species have proven useful in illustrating the potentially severe impact of *A. psidii* on other highly or extremely susceptible species in an ecologically critical family (Myrtaceae) that constitutes about 10% of the Australian flora by species—about half of which occur in climatic zones identified as conducive to *A. psidii* naturalisation (Kriticos et al. 2013). Information on susceptibility and impact under field conditions has only been collated for a small percentage (approx. 50%) of host species but this has been primarily through examination of hosts in ex-situ plantings. Even fewer species have been studied in detail across their native range. Pegg et al. (2014) considered 48 species in Queensland alone to be highly or extremely susceptible to *A. psidii*. We recommend a greater range of species with a broader variation in susceptibility be monitored, including both currently 'listed' threatened species and 'non-listed' species. Understanding the variability in species susceptibility is critical in order to optimize scarce resources for potential species recovery plans. Such monitoring will also assist in detecting changes in disease severity due to local and regional variation in climate and potentially herald the incursion of new strains of *A. psidii* (e.g. Loope 2010). The introduction of new strains of *A. psidii* into Jamaica (MacLachlan 1938) and Florida (Rayachetry *et al*. 1997) resulted in devastating epidemics not previously seen in those counties. Furthermore, it is imperative that monitoring of plant communities and ecosystems are initiated to fully understand the long term impact of this devastating invasive pathogen.

The effects of *A. psidii* on some species of Myrtaceae will be less obvious in the short term with impacts likely not recognisable for many years, especially in situations where flower and fruit infection occurs in the absence of significant tree dieback. We have observed this phenomenon on species like *Austromyrtus dulcis* and *Rhodamnia sessiliflora* where disease on foliage presents as minor but when flowers and fruit are produced they become infected and senesce prematurely. Additionally, where there is a decline in a species from rust related dieback (e.g. *Melaleuca nodosa*) resulting in fragmentation, impacts to population survival may result from reduced pollination rates. This may be more the case for species relying on generalist pollinators (e.g. *Melaleuca* spp.). Without human intervention regaining lost genetic diversity within some species populations may not be possible.

An additional outcome of this study should be to highlight the threats alien invasive pests and pathogens pose to Australian native plant species and plant communities. Managing these threats once they become established is challenging. There is a need to better understand these exotic pests and pathogens to improve processes that can be implemented to help prevent future incursions.

As a result of our studies, the following outcomes have been achieved:

Outcomes and impact of research - *Plant industries*

1. Host list and susceptibility rating system identifying:
    - Resistant/tolerant species for future commercial development
    - Highly/extremely susceptible species from an environmental perspective

2. Full host list on-line and susceptibility ratings published

- Giblin FR & Carnegie AJ (2014) *Austropuccinia psidii* (myrtle rust) - Australian host list. http://www.anpc.asn.au/myrtle-rust
- Pegg GS, Giblin FR, McTaggart AR, Guymer GP, Taylor H, Ireland KB, Shivas RG, Perry S (2014) *Austropuccinia psidii* in Queensland, Australia: disease symptoms, distribution and impact. Plant Pathology 63, 1005–102

3. Identification of resistance for implementation into current and future breeding programs - Eucalypt species
   - Pegg, G. S., Brawner, J. T., and Lee, D. J. 2014. Screening *Corymbia* populations for resistance to *Austropuccinia psidii* Plant Pathology 63: 425-436.
   - Lee, David, Brawner, Jeremy, and Pegg, Geoff 2014. Screening *Eucalyptus cloeziana* and *E. argophloia* populations for resistance to *Austropuccinia psidii*. Plant Disease 99, 71-79.
   - Butler J. B., Freeman J. S., Vaillancourt R. E., Potts B. M., Glen M., Lee D. J., Pegg G. S. 2016. Evidence for different QTL underlying the immune and hypersensitive responses of *Eucalyptus globulus* to the rust pathogen *Austropuccinia psidii* Tree Genetics & Genomes 12(3).

4. Assessment of clonal collection of lemon myrtle germplasm – collaborative project with A/Prof. David Lee, University of the Sunshine Coast (RIRDC project)
   - 392 clones screened
   - D. Lee, J. Doran, G. Pegg, D. Lea, P. Macdonell and F. Giblin 2015. Myrtle Rust Screening in Lemon Myrtle Provenance Plantings. RIRDC Publication

5. Proposed industry publications
   - Publication in Horticulture journal and industry magazine articles - April 2017

Outcomes and impact of research - *Environment*

1. Myrtaceae species susceptibility recorded – 180 species assessed
   - Highly and extremely susceptible species identified
     - Pegg GS, Giblin FR, McTaggart AR, Guymer GP, Taylor H, Ireland KB, Shivas RG, Perry S. (2014). *Austropuccinia psidii* in Queensland, Australia: disease symptoms, distribution and impact. Plant Pathology 63, 1005–102
2. Impact assessments conducted on selected species and plant communities
   - Impact across selected host species natural distribution
     - Angus J. Carnegie, Amrit Kathuria, Geoff S. Pegg, Peter Entwistle, Matthew Nagel, Fiona Giblin, 2015. Environmental impact of the invasive rust *Austropuccinia psidii* on Australian native Myrtaceae. Biological Invasions DOI 10.1007/s10530-015-0996-y

   - Impact on regeneration for multiple Myrtaceae – coastal heath and *Melaleuca quinquenervia*
   - Impact on plant communities – wet sclerophyll environments in south east Queensland
   - Proposed publications - 2017
     - Impact of *Austropuccinia psidii* on regeneration of *Melaleuca quinquenervia* and interaction with native insect pests
     - Impact of *Austropuccinia psidii* on Myrtaceous rich plant communities in wet sclerophyll environments in south-east Queensland
     - Impact of *Austropuccinia psidii* on regeneration of Myrtaceae in coastal heath following wildfires

Impacts from myrtle rust research:

- Myrtle rust declared as a Threatening Process in NSW and application submitted for national listing
- Data used for species conservation listing and risk modelling
- *Rhodomyrtus psidioides* and *Rhodamnia rubescens* submitted for listing as Critically Endangered
- *Myrtle rust identified as a priority for NESP projects*

Data used for Briefing the federal Department of Environment Division Heads and Head of Australian Environment Agencies (recommendation that the issue of myrtle rust be taken to a future COAG meeting).

Collaborations developed as part of this project

1. **Forest & Agriculture Biotechnology Institute (FABI), University of Pretoria, South Africa**

Research aims:

Identifying risk of myrtle rust to production forestry in South Africa

Understanding defence responses to myrtle rust

2. **University of Tasmania**

Research aims:

Genetics of *Eucalyptus/Corymbia* disease susceptibility: The relationship between susceptibility to native pathogens and the introduced myrtle rust pathogen *A. psidii*

QTL study - evidence that the symptomless and hypersensitive responses to *A. psidii* infection are under independent genetic control

Identifying susceptibility/resistance of key eucalypt species used in revegetation programs in Tasmania – *Eucalyptus pauciflora*, *E. ovata*

3. **University of the Sunshine Coast**

Potential impact of myrtle rust of eucalypt species of commercial significance to Queensland forest industry

Identification of resistance patterns to *A. psidii* in spotted gum species and interaction with *Quambalaria pitereka*, a significant native fungal pathogen

Identification of myrtle rust resistance patterns in *E. cloeziana* and *E. argophloia* (Endangered)

Examination of myrtle rust resistance across provenances of *Backhousia citriodora* (Lemon myrtle) for commercial production

4. **Griffith University**

PhD student (Tamara Taylor) supervision – commenced 2014 – APA/CSIRO funding

Research:

The impact of *Austropuccinia psidii* (myrtle rust) disease on fleshy-fruited Myrtaceae in Queensland, Australia

5. **University of Queensland**

PhD student (Emily Lancaster) supervision – commenced 2015 – CRCPB funding

Research:

Epidemiology, impact and management of myrtle rust in lemon myrtle plantations

6. Macquarie University

PhD student (Laura Fernandez) supervision – commenced 2015 – CRCPB funding

Research:

Ecological impacts of invasive fungus in Australian native plant communities

8.0 Recommendations:

As a result of our studies we recommend the following:

Plant industries

- Improve awareness within the nursery industry with regards to the threat that myrtle rust poses with an aim to reduce the number of susceptible host species being utilized
    - Promote opportunities to select for myrtle rust resistance in popular nursery and garden species
- Promote development/use of more resistant species/varieties in production nurseries
- Promote opportunities to introduce rust resistance screening programs for species used in revegetation programs removing susceptible individuals (e.g. *Melaleuca quinquenervia*)
- Ensure that industries reliant on Myrtaceae (oil, food and fibre) have well established tree breeding programs to reduce current impact of *A. psidii* and also reduce risk from other exotic pests

Environment

- Adoption of rating systems developed in this project as standard when reporting new host species to help identify at risk species
- Adoption of rating systems to assess impact on species and plant communities allowing for uniform data collection
- Prioritise species for conservation efforts based on:
    - Distribution in relation to areas climatically favourable to *Austropuccinia psidii*
    - Current *Austropuccinia psidii* susceptibility rating and impact status
    - Current conservation status and potential to conserve a species based on ease or ability to propagate or store seed
- Determine species for conservation status reclassification based on the impact data provided from this project. This may include capturing additional data from across the natural distribution of selected species before compiling applications. Species that should be included based on our studies are:
    - *Archirhodomyrtus beckleri*
    - *Decaspermum humile*
    - *Gossia hillii*
    - *Gossia inophloia*
    - *Gossia myrsinocarpa*
    - *Eugenia reinwardtiana*
    - *Lenwebbia sp.* Blackall Range
    - *Melaleuca nodosa*

- *Syzygium hodgkinsoniae*
- *Rhodamnia maideniana*

- Establish long term monitoring plots and reporting programs to enable impact of *Austropuccinia psidii* on species and plant communities over time to be determined
    - Transition the assessment and reporting of these plots to environmental agencies

- Identify opportunities to develop tree breeding programs for selected species (e.g. *Melaleuca* spp.) to promote genetic diversity where resistance to *A. psidii* is limited and impacts are likely to cause species fragmentation

Policy and research development

- Establishment of an ongoing myrtle rust working group to help prioritise areas of research and focus conservation strategies

- Promote the findings from this research to State, Territory and Federal Environment Departments. This may be in the form of briefings or series of "Roadshows" throughout Australia provided to State and local government groups, mining companies, revegetation groups etc. demonstrating the impact of myrtle rust and potential management options

- Engage mining companies as an opportunity to help support species conservation and utilization of myrtle rust resistant species/individuals in revegetation programs

- An increase in awareness and emphasis on environmental biosecurity through publication of our research outcomes in popular and scientific articles

- Examine the threat that other *Austropuccinia psidii* strains/biotypes pose to industries reliant on Myrtaceae and the environment

    - Develop international collaborations and using outcomes from this project examine differences in host range/virulence of different strains

- Using lessons learnt from dealing with myrtle rust there is a need to promote the importance of environmental biosecurity with prevention of incursion and improved post-border surveillance a priority area
    - Develop an environmental biosecurity strategy

Identify exotic threats and determine possible pathways of entry into Australia

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9. Published papers

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Conference abstracts

Louise Shuey, Geoff Pegg, Sanushka Naidoo 2015. *Eucalyptus grandis* defence responses against the myrtle rust pathogen, *Austropuccinia psidii* – insights from RNA-SEQ transcriptome profiling. APPS conference September 2015

Louise Shuey, Geoff Pegg, Sanushka Naidoo 2015. *Eucalyptus grandis* defence responses against the myrtle rust pathogen, *Austropuccinia psidii* – insights from transcriptome profiling. IUFRO Tree Biotech

Pegg GS, Carnegie AJ, Giblin FR, Perry S, 2015. Myrtle Rust, impacts on Myrtaceous diversity in Australia. Australasia Plant Pathology Conference, Fremantle, Western Australia September 2015.

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