# Impacts of the invasive fungus *Puccinia psidii* (myrtle rust) on three Australian Myrtaceae species of coastal swamp woodland

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Running headline: Individual and mixed-species level impacts of invasive forest fungi on native Australian flora

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## Declaration

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## Abstract

Exotic fungal pathogens can substantially affect individuals and populations of susceptible plant species, potentially resulting in changes in community structure and composition. Myrtle rust (*Puccinia psidii*) is a pathogenic fungus native to South America that affects species in the family Myrtaceae. The pathogen was introduced accidentally to Australia and first detected in NSW in April 2010. Ecological impacts have been poorly studied in the native range of myrtle rust and even less for Australian native communities. Two experiments were conducted to assess myrtle rust impacts in three co-occurring species of coastal swamp woodland that are known to be susceptible: *Melaleuca quinquenervia*, *Leptospermum laevigatum* and *Baeckea linifolia*. Plants of each species were grown individually (Expt 1) and in mixed species assemblages (Expt 2), with half inoculated with myrtle rust and the other half remaining as a controls. Infection level was assessed and its impact on seedling survival and growth was recorded. In both experiments *L. laevigatum* and *M. quinquenervia* seedlings were heavily infected and showed high degrees of susceptibility with negative effects on growth (height, biomass, number of leaves). In contrast no *B. linifolia* seedling presented visible symptoms of disease. *Melaleuca quinquenervia* seedlings had greater infection levels and suffered greater growth reductions than *L. laevigatum* in both experiments. Biomass allocation was largely unaffected, with the exception of increased stem mass fraction. As *M. quinquenervia* is dominant in coastal swamp communities and highly susceptible to myrtle rust, changes in community structure are likely. This study provides better understanding of the potential impacts of myrtle rust in this ecological community and has significant implications for the conservation and management of Australian Myrtaceae-dominated plant communities generally.

**Key words**: invasive pathogen, exotic fungus, forest fungi, Myrtaceae, Australian native communities.

## INTRODUCTION

Exotic fungal pathogens can have substantial negative ecological impacts including changes in vegetation community structure, composition and ecosystem processes, as well as threatening endangered species and communities worldwide (Ellison *et al*. 2005; Loo 2009; Roy *et al*. 2014). When the host trees of such pathogens are foundation species, significant landscape disruptions can be triggered, with severe consequences for herbivores, biotic interactions and ecosystem processes (Loo 2009). *Cryphonectria parasitica* (Chestnut blight) is an example of an exotic fungal pathogen that has had a dramatic impact on a tree species, killing nearly all mature chestnut trees in North America within 35 years of its introduction (Ellison *et al*. 2005; Brasier 2008; Loo 2009). Similarly *Cronartium ribicola* (the pine blister rust) has caused widespread mortality of pine species in North America which resulted in *Pinus albicaulis* being listed as an endangered species in Canada (Loo 2009; Roy *et al*. 2014). A devastating example of an exotic fungus in Australia is the soil-borne fungus *Phytophtora cinnamomi*, which particularly affects woody perennial plant species, causing substantial changes to the structure of vegetation communities such as the Jarrah forest (*Eucalyptus marginata*) of Western Australia (Ellison *et al*. 2005).

*Puccinia psidii* is a fungus in the order Uredinales native to Central and South America that was discovered in Brazil in 1884 by G. Winter. The pathogen was accidentally introduced to Australia, being first detected in the Central Coast region of New South Wales (NSW) in April 2010 (Carnegie *et al*. 2010a). At the beginning of the fungus incursion in Australia it was thought to be a pathogen different from guava rust, another rust fungus called *Uredo rangelii* (Carnegie & Cooper 2011). Given that the Australian fungus was determined from a *Myrtus communis* specimen, and in order to differentiate it from guava rust (Brazilian strain found in *Psidium guajava*), it became known as myrtle rust (Coutinho *et al*. 1998; Rayachhetry *et al*. 2001;, Carnegie *et al*. 2010a, Anderson 2012). This is the name which I will use to refer to the Australian pathogen in this study from now on.

Myrtle rust exclusively attacks species of the Myrtaceae family (Carnegie & Lidbetter 2012; Morin *et al.* 2014). It possess the capacity of shifting between hosts and attacking a wide range of species, greater than most of the known rusts (Grgurinovic *et al*. 2006; Carnegie *et al*. 2010a). The main target of myrtle rust infections are the young growing tissues of plants such as newly deployed leaves, floral buds, fruits and coppice, with stems and older foliage less frequently affected

(Coutinho *et al*. 1998; Glen *et al.* 2007). As the pathogen attacks apical buds, trees develop a shorter height and greater branching similar to a shrub physiognomy (Booth *et al*. 2000; Glen *et al*. 2007). Pustules can grow and coalesce causing the leaves and stems to become distorted (Carnegie *et al*. 2010a). Symptoms can range from no symptoms or purple flecks in resistant plants, through to yellow spore pustules on the leaves, stems and branches in susceptible species, to defoliation and even death in the most vulnerable species (Rayachhetry *et al*. 2001; Uchida *et al*. 2006).

*Myrtle rust impact globally*

Myrtle rust now occurs widely across the globe and is internationally recognised as an invasive fungus. It was found in Florida (USA) in 1977, only managing to disperse to Hawaii almost 30 years later (2005) (Marlatt & Kimbrough 1979; Burnett & Schubert 1985; Uchida *et al*. 2006). Once the rust succeeded in dispersing across the Pacific Ocean, it reached Japan in 2007 and China in 2009 (Kawanishi *et al*. 2009; Zhuang & Wei 2011).

Myrtle rust is considered one of the most serious threats to *Eucalyptus* forestry in Brazil and the worst globally (Coutinho *et al*. 1998, Alfenas *et al*. 2003; Moraes *et al*. 2014). The fungus obtained the common name of "eucalyptus rust" in Brazil when found infecting exotic *Eucalytpus* plantations (Coutinho *et al*. 1998; Booth *et al*. 2000; Alfenas *et al*. 2003; Carnegie 2012). The pathogen causes growth reduction of 25-35% in forestry eucalyptus trees (e.g. *E*. *grandis* in Brazil and *E*. *globulus* in Uruguay) (Takahashi 2002; Furtado *et al*. 2009). One of the most planted forestry species worldwide, *E*. *grandis*, is also one of the most vulnerable (Junghans *et al*. 2003), with all coppice regrowth highly susceptible to infection which can result in tree death (Furtado *et al*. 2009). Seedlings are highly susceptible, with the first outbreak of myrtle rust in Brazil recorded as killing 400,000 *E*. *grandis* seedlings in a nursery (Carnegie 2012). Nowadays, despite the widespread use in Brazil of myrtle rust resistant eucalyptus clones, the fungus still accounts for the death of 20% of the plantings (Quecine *et al*. 2014). A range of other commercial species are also vulnerable to myrtle rust. For example *Pimenta dioica* (allspice) distilleries in Jamaica were forced to close after myrtle rust infection in the 1930's (MacLachlan 1938; OCPPO 2007; Loope 2010). *Syzigium jambos* (rose apple) is native to south-east Asia but has been widely planted as an ornamental and fruit tree in Hawaii where whole stands have been defoliated and recruitment limited due to seedling infection and death (Uchida & Loope 2009, Silva *et al*. 2014). Even adult Rose apple trees up to 8-10 m die after suffering repeated infections (Loope 2010). Similarly, *Melaleuca quinquenervia* (Cav.) S. T. Blake is an Australian tree species that has been widely planted in Florida where it is now regarded as an environmental weed. Myrtle rust severely affects *M. quinquenervia* and has been considered as one of the useful components of this species' control in Florida (Rayachhetry *et al*. 2001).

Myrtle rust also affects commercial species even within their native distribution. *Psidium guajava* (guava) is native to Central and South America within the native distribution of myrtle rust, however it is highly susceptible to the pathogen, with flower and fruit production particularly affected, resulting in reduced yields of 80-100% (Junqueira *et al*. 2001; Ribeiro & Pommer 2002; Goes *et al*. 2004; Martins *et al*. 2014).

*Myrtle rust impact in Australia*

From where myrtle rust was first detected north of Sydney it spread quickly along the east coast of Australia, reaching Queensland (QLD) by the end of 2010 and Victoria one year later (Pegg *et al*. 2014a). The fungus has recently (February 2015) been detected in Tasmania (Biosecurity Tasmania 2015) and on Melville Island in Northern Territory (DPIF 2015). Bioclimatic mapping has been used to identify potential areas of risk of myrtle rust establishment in Australia (Booth *et al*. 2000; Booth & Jovanovic 2012; Elith *et al*. 2013). So far myrtle rust has established in climatically suitable areas on the Australian east coast, but has yet to reach climatically suitable areas in south-west Western Australia (Elith *et al*. 2013, Kriticos *et al*. 2013).

Taxonomic confusion began when Simpson *et al*. (2006) named the rust as *Uredo rangelii*, considering it a different species due to the presence of different urediniospores. Nevertheless, many authors disagreed with this criterion (Glen *et al*. 2007; Carnegie & Cooper 2011). Most researchers now agree that the rust fungus found on Myrtaceae in Australia should be called *Puccinia psidii sensu lato* and that the name *U. rangelii* should be removed from use (Carnegie *et al*. 2010a; Carnegie & Lidbetter 2012). Genetic studies suggested that the original strain present in Australia came from Hawaii, being different from those present in Florida (M.G. unpubl., cited in Morin *et al*. 2014). Currently it is thought that Australia possesses only one strain of myrtle rust, while in Florida three strains have been detected and there are at least five strains present in Brazil (Carnegie & Cooper 2011; Carnegie 2012).

The rust has a great dispersal capacity given that spores can travel by wind or attached to insects and animals, including humans (i.e. in shoes, clothes and cars) (Uchida *et al*. 2006). However, myrtle rust establishment is restricted by climatic conditions as the spores can only germinate in a limited range of temperatures, humidity and light: between 15-25º C, high humidity (leaf wetness) and 6-8 h darkness, sustained for 12-24 hours (Marlatt & Kimbrough 1979; Piza & Ribeiro 1988; Rayachhetry *et al*. 1997; Alfenas *et al*. 2003; Carnegie & Lidbetter 2012). A recent study has found lower optimum temperatures for the strain present in Australia, ranging from 12-20º C (Kriticos *et al*. 2013). In optimum conditions the fungus lifecycle is very fast, with experimentally infected leaves showing symptoms only 3-5 days after inoculation (Glen *et al*. 2007). Urediniospores can survive without a proper host up to 90 days depending on the conditions, losing viability afterwards (Glen *et al*. 2007; Lana *et al*. 2012).

Australian vegetation is likely to be highly vulnerable to myrtle rust infection. The native flora is dominated by trees and shrubs in the family Myrtaceae, such as the iconic eucalypts (including the genera *Eucalyptus, Corymbia* and *Angophora*), paper-bark and tea-trees (*Melaleuca* and *Leptospermum* spp.) and the bottlebrush (*Callistemon* spp.). Of the ~3000 Myrtaceae species recorded globally, more than half (1646 species in 70 genera) are native to Australia (Glen *et al*. 2007). There are also several endangered ecological communities including Castlereagh Swamp Woodland Community and Blue Mountains Swamps in the Sydney Basin Bioregion as well as Eucalypt forestry plantations across the continent that are likely to be affected by myrtle rust (NSW Scientific Committee 1999, 2000; Tommerup *et al*. 2003).

Very little is known about myrtle rust impacts on native communities in Australia (Carnegie & Cooper 2011; Carnegie & Lidbetter 2012). Several experimental as well as observational studies have been carried out to assess the vulnerability of Australian species to myrtle rust. Inoculation tests as well as field surveys have so far identified 231 species in 51 genera as susceptible to myrtle rust infection, 46 of which are rated as highly or extremely susceptible (Carnegie & Lidbetter 2012; Morin *et al*. 2012; Pegg *et al*. 2014b). Some of the most susceptible genera are *Agonis*, *Austromyrtus*, *Callistemon*, *Eucalyptus*, *Leptospermum*, *Melaleuca* and *Rhodamnia*. Forestry and horticultural species that have been identified as susceptible include *Agonis flexuosa* (willow myrtle), *Eucalyptus pilularis*, *E*. *cloeziana, E*. *grandis*, *E*. *agglomerata*, *Syzygium jambos* and *S*. *anisatum* (lemon myrtle) (Carnegie *et al*. 2010b; Carnegie & Lidbetter 2012). Most of the Myrtaceae species that coevolved with the fungus in its native range have evolved defences (Tommerup *et al*. 2003, OCPPO 2007). On the contrary, while many Australian Myrtaceae species are susceptible, some species possess both vulnerable and resistant individuals, as is the case of *M*. *quinquenervia* and several *Eucalyptus* species (Graça *et al*. 2011; Pegg *et al*. 2014a).

Field studies have found that some of the most susceptible species are the key rainforest species of the east coast *Rhodamnia rubescens* (brush turpentine) and *Rhodomyrtus psidioides* (native guava) and the wetland keystone species *M*. *quinquenervia* (broad leaved paperbark) (Carnegie & Lidbetter 2012, Giblin 2013). *R*. *rubescens* and *R*. *psidioides* are attacked from seedling stage to mature trees up to 5-12 m (Carnegie & Cooper 2011). Fruits suffer severe infection and whole stands are observed to be dying without new recruitment (A. Carnegie pers. comm.). Trees suffer around 75% defoliation, shoot death, repeated infections and dieback (Carnegie & Cooper 2011, Pegg *et al*. 2014a). *Melaleuca quinquenervia* has been found to suffer reduced growth and flower production, with a high proportion of dead seedlings and saplings (Carnegie 2012; Carnegie & Lidbetter 2012). Juvenile individuals are susceptible as well resulting in the development of a branched and stunted shrub physiognomy due to infection of apical meristems (Ruiz *et al*. 1989; Coutinho *et al*. 1998; Booth *et al*. 2000; Simeto *et al*. 2013, p. 45). The results of these studies of species' susceptibility has led to myrtle rust being recognized as one of the most serious threats to native and endemic flora in Australia, including endangered species. The 'Introduction and establishment of exotic rust fungi…" has been declared a Key Threatening Process under the *NSW Threatened Species Conservation Act* 1995.

*Coastal swamp woodlands*

Coastal swamp woodlands are sclerophyll communities dominated by a highly diverse combination of shrubs, trees, herbs, ferns and grasses, located from south-east QLD to Sydney (NSW) (Keith 2004). The swamp forests of the NSW north coast are considered endangered ecological communities, mainly as a consequence of land clearing for urban expansion (NSW Scientific Committee 2004). The special assets that distinguish these coastal floodplains include a canopy dominated by *E. robusta* (swamp mahogany), *M*. *quinquenervia* or *E*. *botryoides*, usually lacking the presence of other eucalypts, with occasional scattered trees or understorey plants, and a groundcover composed of large sedges and ferns. The more common species besides the ones already named are *Banksia oblongifolia*, *Callistemon salignus* (sweet willow bottlebrush), *C*. *linearis* (narrow-leaved bottlebrush), as well as *M*. *nodosa* and *Leptospermum juniperinum* (Keith 2004). Only a few relicts are conserved pristine and therefore free from plant biological invasions (NSW Scientific Committee 2004).

Coastal swamp woodlands are frequently burnt by wildfire and many of the constituent species are adapted to regenerate after fire, including the capacity to resprout or to establish from seedlings (Baird 1977; Trabaud 1987). The impact of myrtle rust in coastal swamp woodland may be exacerbated by fire as myrtle rust attacks young growing tissues, including regenerating seedlings and resprouting shoots (Grgurinovic *et al*. 2006; Glen *et al*. 2007). Myrtle rust could affect the recruitment of seedlings after fire, altering the outcome of competitive interactions between species. If the fungus attacks the dominant species in the landscape, non-dominant but more resistant species will eventually win the competition. Genetic variability can also be severely reduced given that the fungus attacks floral buds and fruits, reducing fecundity (Glen *et al*. 2007).

In this study I used glasshouse experiments to assess the impacts of myrtle rust on three common Myrtaceous species of the coastal swamp vegetation community of northern NSW: *M*. *quinquenervia*, *L. laevigatum* and *B. linifolia*. The objectives of the study were to 1) examine the susceptibility (infection level) and disease-severity (defoliation, dieback) manifestation of the three Myrtaceous species grown individually when inoculated with the rust fungus *P*. *psidii*, and 2) assess the impact of myrtle rust in mixed-species assemblages by assessing changes in abundance and dominance of species due to differential species' susceptibility to myrtle rust.

## METHODS

*Study species*

Three species were selected that are common in coastal swamp vegetation: *M. quinquenervia*, *L. laevigatum* and *B. linifolia*. This vegetation community type is described as Coastal Swamp Forest by Keith (2004) and occurs along the east coast of Australia. This vegetation community was chosen as a recent study at a Coastal Swamp Forest site located west of Lennox Head in Ballina Local Government Area, northern NSW suggested that myrtle rust may be having a significant effect on seedling recruitment after fire (G. Pegg, pers. comm.). The site is a coastal sclerophyll heathland on low nutrient soil dominated by *Banksia* spp., with wetland lower areas dominated by *M. quinquenervia* in association with the fern *Pellaea falcata* (Erskine *et al*., cited in Keith 2004). Different zones can be distinguished in relation to proximity to the swamp, with the zone in the area 11 – 15 m from the swamp edge dominated by the species *M. quinquenervia*, *L. laevigatum* and *B. linifolia* (G. Pegg pers. comm.).

*Melaleuca quinquenervia* is a keystone and dominant species in wetlands along the east Australian coast commonly known as 'melaleuca' or 'broad-leaved paperbark' (Carnegie 2012; Pegg *et al*. 2014a). Adult individuals can reach 12-20 m height (Myers 1983). Seedlings and saplings are highly susceptible to myrtle rust, with adult individuals being vulnerable but not severely affected (Rayachhetry *et al*.2001, Carnegie 2012). Defoliation and seedling dieback are observed symptoms in this species as well as reduced flower production and death (Pegg *et al*. 2014a). Variability exists in the susceptibility within the species with some individuals presenting resistance (Rayamajhi *et al*. 2010). The first infected individuals were recorded in Florida, USA, where this species has been introduced and become an environmental weed. Individuals are now recorded as severely affected by myrtle rust in Australian eastern native woodlands (Carnegie & Cooper 2011; Carnegie & Lidbetter 2012). *Leptospermum laevigatum* (Gaertn.) F. Muell is a small tree native to eastern Australia known as 'coastal tea tree' that can reach 8-12 m height (Burrell 1969). Due to its halophilic nature it is usually found on sandy soils. This species has been tested and found to be susceptible to myrtle rust, showing high variability within the species ranging from resistant individuals to very susceptible ones (Morin *et al*. 2012). Shoots can be infected as well as leaves and flowers, reducing fecundity (Pegg pers. comm.). *B*aeckea *linifolia* Rudge is a shrub native to NSW and Victoria (eastern Australia) usually known as heath myrtle or weeping Baeckea that can grow up to 2 m (Hose *et* *al*. 2014). No susceptibility tests have been performed on *B*. *linifolia*, although infected plants have been observed at the Lennox Head site (G. Pegg, pers. comm.). Interestingly this species has been reported as only becoming infected after other species at the site (i.e. *M*. *quinquenervia*) presented with severe impacts. The reason for this delay in infection time remains unknown.

## Pre-experiment trials

### *Seed germination*

A series of germination trials were performed to assess germination timing and percentage for each of the three study species *M*. *quinquenervia*, *B*. *linifolia* and *L*. *laevigatum*. This information was then used in order to grow uniformed plants of each species for the glasshouse experiments. *Melaleuca quinquenervia* and *L*. *laevigatum* seeds were obtained from the commercial seed supplier AustraHort Pty Ltd (QLD, Australia) and *B*. *linifolia* seeds from Royston Petrie Seeds Pty Ltd (NSW, Australia). For each species, 20 seeds were placed in each of five Petri dishes containing paper disk (filter paper), resulting in a total of 100 seeds. Seeds were set at ambient temperature and watered every two days with purified water (reverse osmosis), and germination (radicle emerged) was recorded. From these data the number of seeds and timing of seed germination required for the experiment were calculated for each species.

## Myrtle rust inoculation

Five different trials of inoculation of the experimental plants with *Puccinia psidii* spores were performed between the months of January and March 2015. The trials were performed in order to identify the best method to achieve the infection of the selected species prior to undertaking the experiments. Trials 1-4 were performed in the glasshouses at Macquarie University and trial 5 using both the Plant Breeding Facilities at The University of Sydney and the Macquarie University glasshouses.

Trial 1: Myrtle rust spores were obtained from the Queensland Department of Primary Industries (Dr Geoff Pegg). A reticulated microscope slide was used to calculate the spore concentration which was adjusted to 1 x $10^5$ spores $mL^{-1}$ (Pegg *et al*. 2014b).

For spore dilution a mix of purified water, mineral oil and a polysorbate surfactant was used (Sandhu & Park 2013). Plants were treated with the inoculum using a hand-held fine spray system (Pegg *et al*. 2014b). The temperature of the glasshouses were set for the optimum conditions for spore germination as described in the literature, being 18°C ± 1°C during night and 22°C ± 3°C during the day (MacLachlan 1938; Piza & Ribeiro 1988). Observations of the plants for the following two weeks determined that no plants had become infected.

Trial 2: For the second trial, the same protocol was used as for the first except that seedlings were covered with black plastic boxes to secure darkness for 24 h post-inoculation following the method of Carnegie *et al*. (2009). Observations of the plants for the following two weeks determined that no plants had become infected.

Trials 3 and 4: *Puccinia psidii* spores, diluted in light mineral oil at a concentration of 2 mg $mL^{-1}$, were obtained from The University of Sydney (Dr Karanjeet Sandhu). Seedlings were inoculated using a fine spraying system. Glasshouse temperatures were fixed at 20 ± 2° C with 80% relative humidity. The treated seedlings were kept in darkness for 24 h post-inoculation, using black plastic covering over the pots. A misting irrigation system in the glasshouses was used to maintain high humidity, although seedlings did not receive the mist spray directly. Only one seedling was observed to be infected over the following two weeks. This inoculation procedure was repeated but no further infection was obtained.

Trial 5: The same source of *Puccinia psidii* spores used in Trials 3 and 4 was used for the fifth trial. Plants were transported to the Plant Breeding Facilities at The University of Sydney where urediniospores were diluted in light mineral oil (Univar Solvent L naphtha 100, Univar Australia Pty Ltd) and seedlings inoculated using a fine spray system. The treated seedlings were kept in a humid chamber (95% RH) at 20°C in darkness for 24 hours before being transported back to the glasshouses at Macquarie University.

*Experimental design*

Both experiments used a fully factorial design with two factors: species (3 levels) and treatment (2 levels, control and infected) and for some variables a third factor (time, 7 levels) was included. I grew seedlings (10 replicates) of each species by treatment combination, with the treatment seedlings divided evenly between two glasshouses (two controls and two with infection treatment). Plants were inoculated with myrtle rust following the method devised in trial 5 above.

Experiment One: Species-level responses to myrtle rust

In order to test species' responses to myrtle rust, plants of each of the three species were grown individually in pots under equal glasshouse conditions, with half treated with *Puccinia psidii* inoculum and the other half left as controls. Plants were grown in pots 14 cm deep containing 1.8 L of soil (Sand – Soil Blend 80/20 purchased from Australian Native Landscapes Pty Ltd [NSW, Australia]). Seeds of each species were set to germinate on moist filter paper in Petri dishes, with maximum germination of all species timed to occur simultaneously based on the results from the germination trials. Three germinated seeds (radicle just emerged) of each species were planted approximately 3-5 mm deep in each pot, with all seeds planted on the same day. If two or more seedlings emerged, extra seedlings were removed to leave only one per pot after the first week. For each species, a total of 60 plants were grown with 15 pots randomly distributed in each of four glasshouses. After 6 weeks growth, seedlings in two of the glasshouses were treated with *Puccinia psidii* inoculum while plants in the other two glasshouses were left as controls. Several attempts at inoculation were conducted before successful infection was achieved (see above 'Myrtle rust inoculation'). Throughout the experiment a nutrient solution of 125 mg of fertiliser (Aquasol, Hortico Nurseries; 23N:3.95P:14K) dissolved in 125 mL of water was added weekly to each pot to promote plant growth and facilitate successful inoculation. Plants were watered using an automatic mist-spray irrigation system (four times daily for three minutes) before inoculation and all watering was conducted manually after inoculation. Plants were monitored every 2 weeks for 4 months for evidence of infection and details of growth and survival were recorded (see details below '*Rust infection and disease assessment*'). At the end of the experiment, all plants were harvested and washed free of soil then separated into leaf, stem and root components for biomass and allocation measurements (Poorter & Nagel 2000). The harvested biomass was oven-dried at 70° C for at least 48 hours before being weighed using a Sartorius analytical balance.

Shoot biomass was calculated as the sum of the biomass of leaves plus stems (aerial or above-ground biomass). Allocation measures were leaf mass fraction (LMF), stem mass fraction (SMF) and root mass fraction (RMF) calculated as the proportion of the total plant dry mass in leaves, stems and roots respectively (Pérez-Harguindeguy *et al*. 2013).

<u>Experiment Two: Mixed-species level responses to myrtle rust</u>

In order to test how competitive interactions among the seedlings of the three species may be mediated by infection with myrtle rust, plants were grown in mixed-species assemblages. Seeds were set to germinate on moist filter paper in Petri dishes, and planted at the stage of radicle emergence as described for Experiment One. Each assemblage consisted of five seedlings of each of the three study species located randomly in a rectangular arrangement of 3 x 5 rows, with plants spaced evenly 2 cm apart. Each mixed species assemblage was grown in a mesocosm measuring 26.5 cm length x 41.5 cm width x 64 cm depth containing 55 L of soil (Sand – Soil Blend 80/20; Australian Native Landscapes Pty Ltd [NSW, Australia]). A total of 20 mesocosms were created and were spread evenly among four glasshouses. After 6 weeks growth, mesocosms in two glasshouses were treated with *Puccinia psidii* inoculum, while the mesocosms in the remaining two glasshouses were treated as controls (see inoculation treatment details above). Watering and nutrient addition was conducted using the same protocol as for Experiment One. Plant infection, survival and growth were also recorded during the duration of the experiment (4 months) as for Experiment One. At the end of the experiment all surviving plants were harvested, separated into leaf, stem and root components and dried at 70° C for at least 48 hours before being weighed. As roots were unable to be separated between species these data were not included in the analyses. Relative abundance was calculated at the block-level (mesocosm) as the proportion of total shoot biomass represented by each of the species.

Both Experiments (One and Two) were conducted simultaneously in glasshouses at the Macquarie University Plant Growth Facility. The experiments were conducted between December 2014 and April 2015. Relative humidity (RH) ranged from 45% at the beginning to 80% RH in the last attempts. Temperature varied from 18°C night -

22°C day ± 3°C to a fixed 20°C ± 2°C at the end of the experiments. $CO_2$ concentrations were ambient (380-420ppm). No supplementary lighting was used.

**Rust infection and disease assessment**

Seedling growth and survival were monitored throughout both experiments. Seedling growth was measured as seedling height (soil surface to apical meristem), and the number of leaves and stems were counted. Defoliation was also assessed as number of fallen infected leaves. Seedlings were recorded as dead or alive. In order to assess the level of infection by myrtle rust, a combination of the systems of Sandhu & Park (2013) and Pegg *et al*. (2014a) was used. Infection level was assessed at the end of the experiments. The infection levels were based on: the presence of leaf spots characteristic of *P*. *psidii*, and presence of sori on leaves, shoots and stems. Plants were then classified into one of the following six levels of susceptibility:

0 *highly resistant* HR (no visible signs of infection)

1 *resistant* R (flecks or spots / necrosis but no sporulation)

2 *relatively tolerant* RT (small spots, sori on less than 10% of new leaves and shoots)

3 *moderately susceptible* MS (sori on 10-50% of young leaves and shoots)

4 *highly susceptible* HS (sori on 50-80% of young and older leaves, new stems and shoots. Stems and leaves show signs of blighting and distortion)

5 *extremely susceptible* ES (sori on more than 80% of the plant, including all new leaves, shoots and young stems. Foliage dieback and indication of shoot and stem dieback).

**Data Analysis**

I used Linear Mixed-Models (LMM) to test myrtle rust impacts on the different species through time (height and number of leaves). In the model, treatment, species and week were treated as fixed factors while repeated measures for each seedling, block and glasshouse were used as a random factor. For variables measured at the end of the experiments (i.e. total biomass, leaf mass fraction, stem mass fraction, root mass fraction and relative abundance), the factor “week” was not included. Block was not included as a factor for Experiment 1 as there were no significant differences between blocks. Variables were log-transformed [ln(x+1)] when necessary to fulfil the assumptions of normality and homoscedasticity. Model selection was performed comparing the complete LMM with one lacking the interaction or interaction (if not significant) plus factor of interest, performed with likelihood ratio tests (Chi-square tests). When lack of homoscedasticity was found, a model with different variances was used which tests fixed effects with F statistics. Multiple comparisons were performed when significant results were found using Tukey's post-hoc test of Honest Significant Differences (HSD). I also applied Chi square tests to assess for differences in myrtle rust infection and disease severity level (levels of infection and defoliation). Data analyses were performed using R 3.1.2 (R Core Team, 2014) and InfoStat statistical software (Di Rienzo *et al*. 2014). LMM models in R were fitted using the "lme4" package (Bates *et al*. 2011) and multiple comparisons were performed using the "multcomp" package ($\alpha$ = 0.05).

## RESULTS

<u>Myrtle rust inoculation</u>

The inoculation trials showed that only conditions of 95% RH at 20°C in darkness for 24 hours were successful in obtaining infection by myrtle rust. In both experiments *L. laevigatum* and *M. quinquenervia* developed disease symptoms of myrtle rust while no *B. linifolia* seedling presented visible symptoms of disease (dark spots on leaves or germinated yellow spores).

*Experiment One: Species-level responses to myrtle rust for individually-grown plants*

<u>Species' susceptibility</u>

There was variability between the two susceptible species (*L. laevigatum* and *M. quinquenervia*) in the level of myrtle rust infection. The proportional distributions of myrtle rust susceptibility levels differed between the species ($\chi^2$ (5, N = 45) = 17.36; p = 0.004), with *L. laevigatum* being more severely affected than *M. quinquenervia* (Fig. 1a). *Leptospermum laevigatum* presented a higher proportion of seedlings in the more severe susceptibility category (level 5, 28%) with only 10% healthy plants (category 0), compared to *M. quinquenervia* where no individuals registered a category of susceptibility level 5 (highly susceptible) and 44% of the individuals remained healthy (not showing symptoms of infection). Despite the difference in susceptibility to myrtle rust, defoliation was similar between the two species ($\chi^2$ (1, N = 44) = 1.64; p = 0.200).

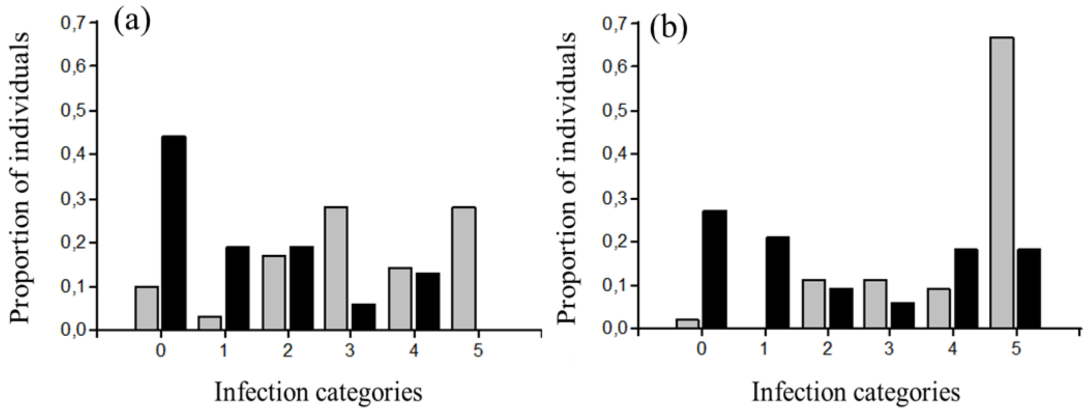


**Fig. 1.** Proportion of individuals in each of the **s**ix susceptibility levels for Experiments 1 (a) and 2 (b), where the susceptibility levels range from 0 (resistant) to 5 (highly susceptible). *L. laevigatum* (grey bars) and *M. quinquenervia* (black bars).

Effect on plant growth

The difference among the species for the effect of myrtle rust on seedling height through time was marginally non-significant (Interaction Treatment*Species*Week; $\chi^2$ (2) = 5.003; p = 0.0819; Fig. 2a). However, species that were affected by myrtle rust were shorter towards the end of the experiment compared to the control, with the difference in seedling height between control and treated seedlings increasing through time (Interaction Treatment * Week; $\chi^2$ (1) = 8.627; p = 0.003). Myrtle rust infection had a different effect for each species through time, with *L. laevigatum* having taller seedlings than *M. quinquenervia* in the first weeks of the experiment but becoming smaller than *M. quinquenervia* as time progressed (Interaction Species * Week; $\chi^2$ (1) = 30.123 ; p < 0.001). At the end of the experiment, average height of *L. laevigatum* in the myrtle rust treatment was 24% lower than in the control, while for the same period *M. quinquenervia* final height was reduced by 38% in the myrtle rust treatment. Interestingly, even though *B. linifolia* did not present symptoms of infection, final seedling height was reduced by 30% in the myrtle rust treatment in comparison with the control.

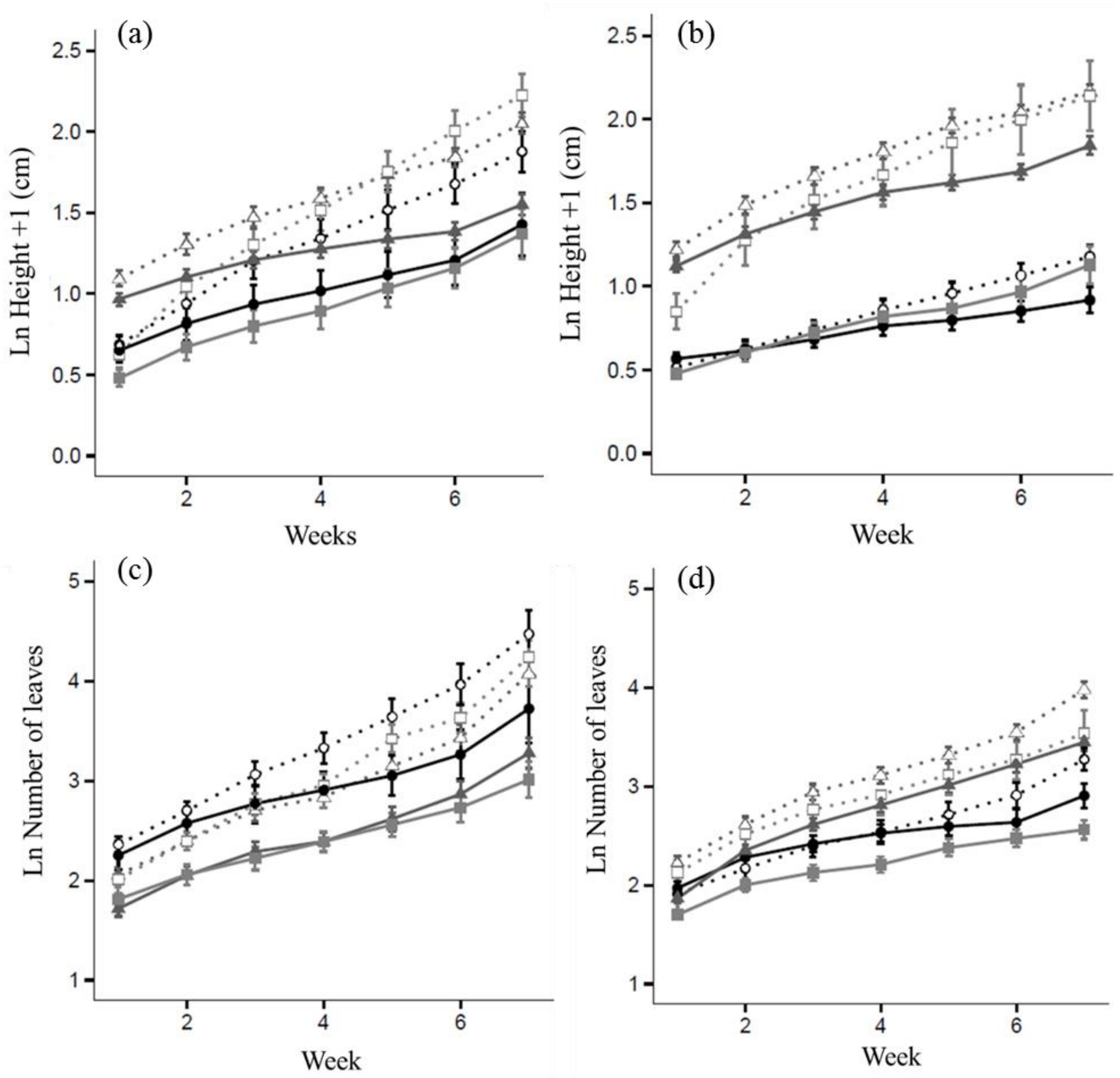


**Fig. 2.** Seedling height (log$_e$ (x+1), cm) and number of leaves (log$_e$ (x)) through time for *M. quinquenervia* (light grey, square), *L. laevigatum* (dark grey, triangle) and *B. linifolia* (black, circle) seedlings in control (dashed lines, open symbols) and myrtle rust treatment conditions (solid black lines and symbols ). Graphs (a) and (c) show results for seedlings grown individually (Experiment 1), graphs (b) and (d) show results for seedlings grown in mixed-species assemblages (Experiment 2). Vertical lines represent ± 1 SE.

Effect on leaf production

The result of the myrtle rust treatment on number of leaves was affected by the combination of the identities of the species and the time (Interaction

Treatment*Species*Week; $\chi^2$ (2) = 6.673; p = 0.036; Fig. 2c). *M. quinquenervia* and *L. laevigatum* had less leaves in the myrtle rust treatment compared with the control through time. However, *M. quinquenervia* showed a greater difference between control and myrtle rust treatment as time progressed, being the differences at the end of the experiment of 29% while 20% for *L. laevigatum*. Of the three species *B. linifolia* had the largest number of leaves both in the control and in the myrtle rust treatment compared to the other species, although leaf number was reduced (17% less) in the myrtle rust treatment. No differences between treatments were found for number of stems ($\chi^2$ (2) = 4.51; p = 0.105).

<u>Effect on biomass and biomass allocation</u>

There was a significant negative effect of myrtle rust treatment on seedling total biomass, root biomass and shoot biomass that was affected in all cases by the species identity (Interaction Treatment*Species; $F_{(2,114)}$ = 7.37, p = 0.001, Fig. 3a; $F_{(2,114)}$ = 5.593, p = 0.005, Fig. 3b and $F_{(2,114)}$ = 10.743; p = 0.0001, Fig. 3c, respectively). In all three measurements, *M*. *quinquenervia* and *L*. *laevigatum* seedling biomass was smaller in the myrtle rust treatment compared to control while no difference in total biomass was detected between control and rust fungus treatments for *B*. *linifolia* seedlings. For total biomass, *M*. *quinquenervia* had a reduction in total biomass of 75% while was 69% for *L*. *laevigatum*. Shoot (aboveground, leaves plus stems) biomass was also severely reduced in 79% for *M*. *quinquenervia* and 68% for *L*. *laevigatum* and similar values were assessed for root biomass (77% and 75% respectively).

Myrtle rust treatment significantly affected biomass allocation regarding SMF ($\chi^2$ (1) = 6.277; p = 0.0122, Fig. 3e), although this time with higher values for treatment. *Leptospermum laevigatum* SMF was 21% bigger for myrtle rust treatment in comparison with control, *M*. *quinquenervia* increased in 17% and *B*. *linifolia* in 12%. However, no effect of treatment was found for LMF or RMF ($\chi^2$ (1) = 0.017, p = 0.896 and $\chi^2$ (1) =0.478; p = 0.457, respectively).There were significant differences between species in LMF, SMF and RMF ($\chi^2$ (2) = 29.661, p < 0.0001, Fig. 3d; $\chi^2$ (2) = 51.652, p < 0.0001, Fig. 3e; and $\chi^2$ (2) = 33.992, p < 0.001, Fig. 3f, respectively).

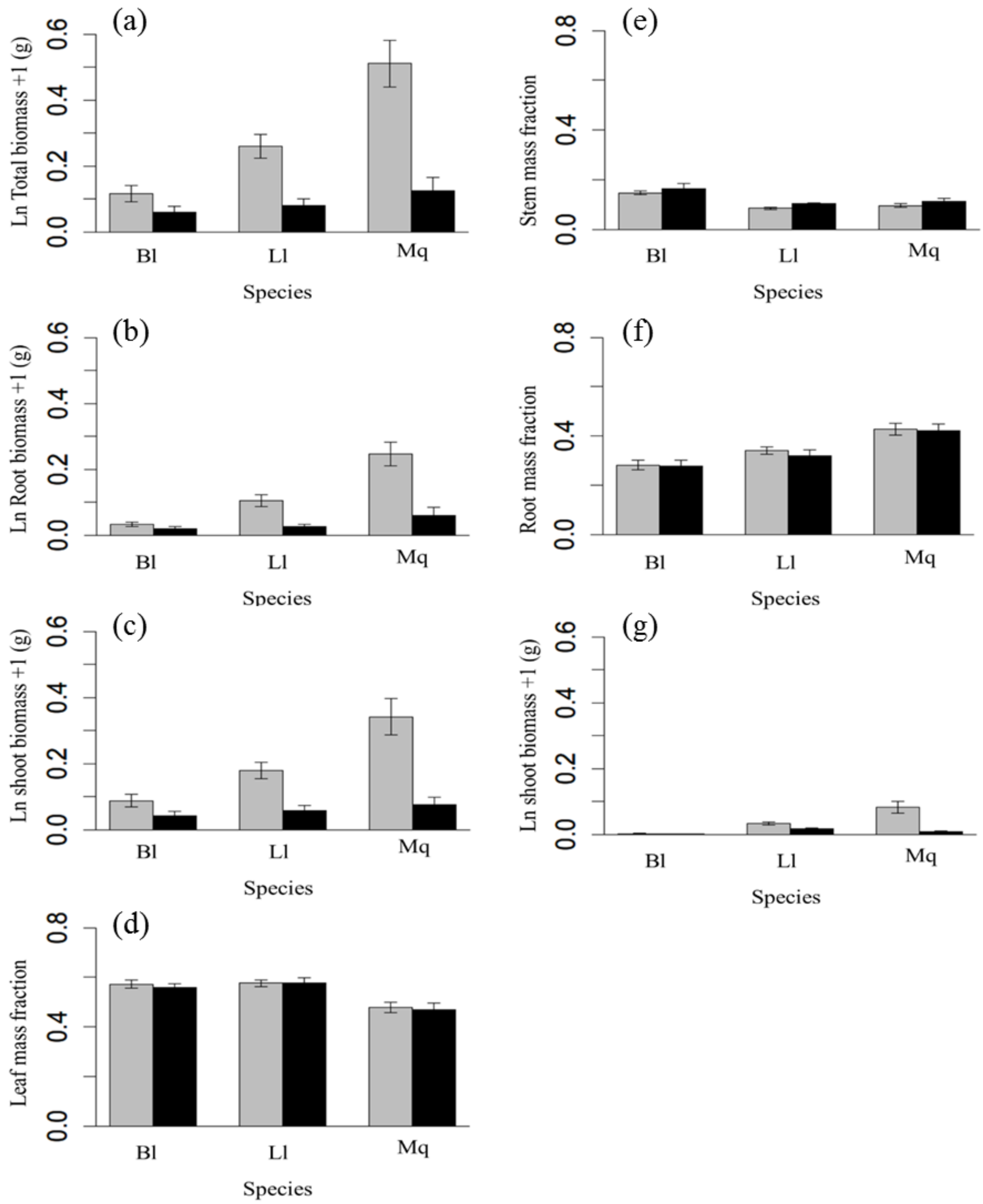


**Fig. 3.** Total biomass ($\log_e$ (x+1)(a), Root biomass ($\log_e$ (x+1)(b), Shoot biomass ($\log_e$ (x+1)(c, g), Leaf mass fraction (d), Stem mass fraction (e) and Root mass fraction ($\log_e$ (x+1)(f) of the three study species grown individually in pots (a, b, c, d, e, f) or grown in mixed-species assemblages (g). Species are denoted by codes Bl, (*B. linifolia*), Ll (*L. laevigatum*) and Mq (*M. quinquenervia*). Treatment conditions are indicated by the vertical bar colours: control conditions (grey bars) and myrtle rust treatment (black bars). Vertical lines represent ± 1 SE.

*Experiment Two: Species-level responses to myrtle rust for plants grown in mixed-assemblages*

Species' susceptibility

There were differences in susceptibility to myrtle rust among the three Myrtaceous species when grown in mixed-species assemblages that were consistent with the findings for the species-level responses found in Experiment 1. *Baeckea linifolia* did not show any visible evidence of myrtle rust infection. Both *L. laevigatum* and *M. quinquenervia* exhibited signs of infection in the myrtle rust treatment although there were significant differences in susceptibility between the two species ($\chi^2$ (5, N = 79) = 35.65; $p < 0.001$; Fig 1b). Almost all *L. laevigatum* seedlings were infected in the myrtle rust treatment, with two-thirds being heavily infected (category 5) whilst more than 25% of *M. quinquenervia* showed no signs of infection (category 0) and only 18% belonged to the highest category of infection. The level of defoliation was also significantly different between species ($\chi^2$ (1, N = 78) = 31.31; $p < 0.001$) with 72% of *L. laevigatum* seedlings having one or more fallen leaves due to infection, whilst 88% of *M. quinquenervia* individuals did not have any defoliation.

Effect on plant growth

Differences were found in the effect of the myrtle rust treatment on seedling height for the three species through time (Interaction Treatment*Species*Week; $\chi^2$ (2) = 11.351; $p = 0.003$; Fig. 2b). *Leptospermum laevigatum* had the tallest seedlings in the first weeks of experiment for both treatment and control compared with the other two species, although towards the end of the experiment its growth declined in the myrtle rust treatment, resulting in 15% shorter seedlings compared to the control. The impact of myrtle rust treatment on *M. quinquenervia* was substantial, with the difference in seedling height between control and myrtle rust treated seedlings increasing through time, resulting in a final reduction in seedling height of more than 45%. *Baeckea linifolia* was the smallest species of the mixed assemblage but also showed an increasing difference in seedling height between the control and treated seedlings having at the end of the experiment the seedlings under myrtle rust treatment 22% less height.

Effect on leaf production

Myrtle rust effect on number of leaves in mixed species assemblages was affected by the combination of the identities of the species and the week (Interaction Treatment*Species*Week; $\chi^2$ (2) = 6.552; p = 0.038; Fig. 2d). For all three species the number of leaves in the control treatment was greater than in the myrtle rust treatment. *Leptospermum laevigatum* and *M. quinquenervia* both showed increasing difference between control and myrtle rust treatments through time, although the effect was stronger for *M. quinquenervia*. *Baeckea linifolia* seedlings in the myrtle rust treatment had more leaves compared to the control in the first weeks of the experiment but the reverse was true towards the end of the experiment with a reduction of 11% in number of leaves in myrtle rust treatment compared to control. In contrast to the species-level experiment, in community assemblages *L. laevigatum* maintained a larger number of leaves than the other species during almost the entire experiment (except for the first week), increasing as time progressed, although being at the end of the study 13% smaller for myrtle rust treatment than for control. *Melaleuca quinquenervia* was the most affected species having a reduction of the number of leaves with myrtle rust infection of almost 30%. The number of stems was similar between control and myrtle rust treatments for the three species ($\chi^2$ (2) = 2.24; p = 0.135).

Effects on biomass

Root biomass was not obtained at the species-level as the roots of the three species were unable to be separated. Consequently only results for shoot (leaf and stem) biomass are reported. There was a significant interaction between species and treatment (Treatment*Species $F_{(2,170)}$ = 22.082; p < 0.0001; Fig. 3g) for shoot biomass with both *M. quinquenervia* and *L. laevigatum* showing strong negative impacts of myrtle rust treatment on biomass (85% and 54% reduction respectively) while *B. linifolia* seedlings were not significantly different between treatments.

Relative shoot biomass of the three species was then compared between treatments, where relative biomass was calculated at the block-level as the proportion of total shoot biomass represented by each species. Although there was a trend for increased relative abundance of *B. linifolia* and decreased relative abundance of *M. quinquenervia* under the myrtle rust treatment relative to the control, significant differences in relative abundance were found only between species ($\chi^2$ (2) = 68.798; p

< 0.001; Fig. 4) and not for the interaction Treatment*Species ($\chi^2$ (2) = 3.682; p = 0.158) or treatment ($\chi^2$ (1) = 0.0296; p = 0.863).

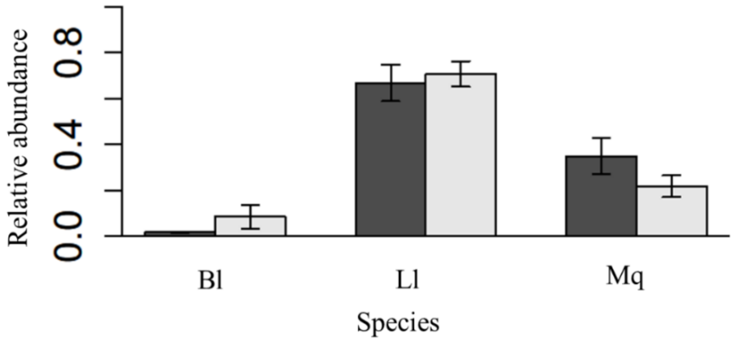


**Fig. 4.** Relative abundance of *B. linifolia* (Bl), *L. laevigatum* (Ll) and *M. quinquenervia* (Mq), grown in mixed-species assemblages under control conditions (dark grey bars) and under Myrtle rust treatment (light grey bars). Vertical lines represent ± 1 SE.

## DISCUSSION

Coastal swamp woodland species susceptibility to myrtle rust

Myrtle rust was found to have a significant impact on the three coastal swamp woodland species studied (*M*. *quinquenervia*, *L*. *laevigatum* and *B. linifolia*) although there was variation in susceptibility and growth outcomes among the species. *Melaleuca quinquenervia* and *L*. *laevigatum* seedlings were heavily infected and showed high degrees of susceptibility and negative impacts due to myrtle rust infection (with *L*. *laevigatum* being more susceptible than *M*. *quinquenervia*). In contrast, *B. linifolia* showed no visible symptoms of myrtle rust infection but showed some evidence of reduced growth. Different susceptibilities could be explained by possession of resistance genes (Rayamajhi *et al*. 2010; Pegg *et al*. 2014a). There are several potential explanations as to why *B*. *linifolia* showed no visible disease symptoms but had reduced growth in the myrtle rust treatments. One explanation is that the seedlings could be asymptomatic but infected, with the spores penetrating the plants' cuticle but not showing macroscopic symptoms (Hunt 1968). Alternatively, seedlings may be directing their resources towards defence against infection by myrtle rust at the expense of putting resources towards growth. Finally there may be a long time-lag between infection and visible symptoms of infection appearing. This last explanation is consistent with anecdotal reports of field studies where *B*. *linifolia* was slower than other species in showing visible symptoms of myrtle rust infection (G. Pegg, pers. comm., see below). Interestingly I found that *M*. *quinquenervia* susceptibility was increased for seedlings grown in mixed species assemblages, with lower susceptibility found in seedlings grown individually. For *L*. *laevigatum* there was some degree of resistance for plants grown individually, with 10% of seedlings showing no visible symptoms, but almost no resistance for seedlings grown in mixed species assemblages. There have been no previous studies published on susceptibility to myrtle rust for *B*. *linifolia*. Susceptibility tests have been performed mainly for forestry species, however susceptibility of non-commercial Myrtaceous species is poorly understood and few field studies have been conducted. The results of field surveys on two native Australian forest species *R. psidioides* and *R*. *rubescens* have shown severe damage by myrtle rust, leading to speculation that *R. psidioides* could be extinct in the wild in less than 5 years (A. Carnegie, pers. comm.). My results suggest that other native Myrtaceous species could be in the same critical situation.

Although *B*. *linifolia* did not showed visible symptoms or signs of infection in either of the two experiments, it is known to be susceptible to myrtle rust infection (G. Pegg, pers. comm.). In natural conditions at the Lennox Head site *B*. *linifolia* was observed to be infected after the other species already presented symptoms, showing an apparent time-lag in infection due to unknown causes. My results are consistent with this in that although there was no visible sign of infection, growth (seedling height and leaf number) of *B. linifolia* appeared to be reduced in the myrtle rust treatments. Future studies should investigate the mechanisms of this apparent time-lag in infection.

Similar patterns in species' susceptibility to *P*. *psidii* were found for seedlings grown individually and in mixed-species assemblages, however seedlings from mixed-species assemblages showed a higher proportion of individuals within the higher infection levels. It is plausible that micro-climatic conditions in the mixed species assemblages were more conducive to myrtle rust spore germination, with humidity likely to be higher than for seedlings grown in individual pots (Marlatt & Kimbrough 1979, Rayachhetry *et al*. 1997; Alfenas *et al*. 2003). In addition, potential re-infection from neighbours' spores could have contributed to the higher infection rates for the mixed species assemblages.

Defoliation levels were similar for *M*. *quinquenervia* and *L*. *laevigatum* when grown individually, however *L*. *laevigatum* suffered higher defoliation compared to *M*. *quinquenervia* when grown in mixed-species assemblages. The loss of leaf area for both species when infected with myrtle rust, due to either defoliation or sori and necrosis on leaves will result in a reduction of available photosynthetic area therefore reducing potential for growth (Furtado *et al*. 2009; Alves *et al*. 2011) and likely reducing plant fitness. Previous studies on P. *dioica*, *P*. *guajava* and *R*. *rubescens* have also showed that flower and fruit infection by myrtle rust reduces fecundity and can also reduce genetic variability (Burnett & Schubert 1985; Junqueira *et al*. 2001; Carnegie & Lidbetter 2012; Martins *et al*. 2014). To fully assess the impact of myrtle rust on the fitness of species selected for this study, future experiments would need to measure effect on reproduction.

<u>Effects on plant physiognomy</u>

Consistent with literature showing myrtle rust infection is associated with transformation of tree structure into shrub physiognomy with lower height and greater number of shoots (Coutinho *et al*. 1998; Booth *et al*. 2000; Simeto *et al*. 2013), both *M. quinquenervia* and *L. laevigatum* height was reduced in seedlings grown individually as well as in mixed-species assemblages. This result was dependent on time progression for both experiments with stronger differences between myrtle rust treatment and control as time progressed. *Melaleuca quinquenervia* showed the strongest reduction in seedling height when grown individually as well as in mixed-species assemblages, with height reduction most severe for the mixed-species assemblage. Surprisingly, *B. linifolia* height was also negatively affected (22-30%) by myrtle rust treatment, suggesting that this species is infected but there is a time-lag before visible symptoms are produced. In contrast to the other two species, *L. laevigatum* height was more affected in seedlings grown individually than in mixed-species assemblages (24% vs 15%). My results agree with what was found in literature concerning reduced growth for i.e. *E*. *globulus* in Uruguay with reductions of 20-35% (Takahashi 2002; Auer *et al*. 2010, Balmelli *et al*. 2013), similar to *B*. *linifolia* and *L*. *laevigatum* although smaller than the ones I found for *M*. *quinquenervia* (38-47%). There were no differences in the number of shoots between treatments for any of the three species. Most likely this variable is affected during later stages of plant growth and with repeated infection of plants.

The number of leaves was also affected by myrtle rust infection in both experiments (individual and mixed-species) but varied among species and increased through time. Again *M. quinquenervia* was the most affected species, suffering the biggest reduction in number of leaves with myrtle rust infection (30%) with similar values between individual and mixed-species growth conditions, while both *L. laevigatum* and *B. linifolia* were more affected when grown individually compared to in mixed-species assemblages (20% vs 13% and 17% vs. 11%, respectively).

<u>Effects on biomass and biomass allocation</u>

The pattern found for variation in myrtle rust impacts on species' morphological variables (height and leaf number) was consistent with the pattern found for biomass. *Melaleuca quinquenervia* was again the most affected species, suffering greater reductions than *L. laevigatum* in total biomass, shoot biomass and root biomass for seedlings grown individually while no impact of myrtle rust on biomass was found for *B. linifolia*. The pattern of biomass reduction for species grown in mixed-species assemblages was the same as for individually-grown seedlings although the differences between species in biomass reduction with myrtle rust infection were more pronounced i.e. *M. quinquenervia* reduction 85% compared to 54% for *L*. *laevigatum*. Biomass and morphological variables for *B*. *linifolia* were no consistent, showing reduced height and leaf number in myrtle rust treatment but not reduced biomass.

My results suggest that total biomass decline, at least for plants grown individually, is due to a combination of both above and below ground biomass, with total biomass being severely reduced in myrtle rust treatments. Part of the aerial biomass reduction could be explained by increased defoliation of infected plants, mainly for *L*. *laevigatum* which had the biggest proportion of individuals within infection category 5 where foliage dieback is expected (Pegg *et al*. 2014a). Previous studies on eucalyptus species have shown that repeated defoliation has a greater detrimental effect than an isolated defoliation event (see Balmelli *et al*. 2013).

My results also show that infection by myrtle rust results in reduced plant biomass but that biomass allocation is largely unaffected, with the exception of stem mass fraction. Commonly in commercial Myrtaceae plantations, infected plants recover from isolated infection episodes after the sori dry, however overall forest productivity is reduced due to reduced growth and biomass production (Silva *et al*. 2013).

<u>Mixed-species level impacts</u>

Although there was a trend in results for relative abundance from the mixed-species experiment, with an increase in *B. linifolia* and decrease in *M. quinquenervia*, the results were not significant. I could not find evidence of change in dominance of species at least at the seedling stage for this particular assemblage of species. At the Lennox Head site, adult *M*. *quinquenervia* individuals dominate presently, although there is almost no recruitment (G. Pegg, pers. comm.), probably because the species is highly susceptible to myrtle rust (Rayachhetry et al. 2001). If the trend in relative overall performance is realized in this coastal swamp forest vegetation type, for example as a consequence of repeated infections, *B*. *linifolia* could increase in dominance and *M*. *quinquenervia* decline, resulting in significant changes in the structure of the community.

## Implications for management

This study has shown that seedlings of three common Myrtaceous species of coastal swamp forest of eastern Australia (*M. quinquenervia*, *L. laevigatum* and *B. linifolia*) can be severely but differentially affected by infection with the myrtle rust pathogen. These results have significant implications for seedling recruitment after fire, which is a natural and frequent disturbance in Australian ecosystems. The impact of myrtle rust on plant response to fire is poorly understood. Observations at the Lennox Head site suggest that epicormic and regrowth shoots (new tissues highly susceptible to myrtle rust attack) of *M. quinquenervia* have been infected by myrtle rust (G. Pegg, pers. comm). Thus the combination of fire with conditions suitable for myrtle rust infection could have a dramatic effect on community dynamics in this vegetation type.

## CONCLUSION

This study has advanced our understanding of the impact of myrtle rust on three important species of coastal swamp woodland in eastern Australia. The impact of myrtle rust varied among seedlings of the three species, with *Melaleuca quinquenervia* most severely affected, followed by *Leptospermum laevigatum*, while *Baeckea linifolia* showed no visible signs of infection but showed slightly reduced growth. As *M. quinquenervia* is a dominant species in the community, myrtle rust may have significant ecological impacts, including changes in species composition and structure. Future studies should test the effect of myrtle rust infection on later stages of plant development of these key species, including flowers and fruits, as well as on seedling recruitment in natural communities in order to better understand the impacts of this important exotic pathogen. It is clear however that the invasion of this exotic pathogen has the potential to dramatically affect vegetation composition and structure, with flow-on effects to ecosystem function and processes, in Australia's Myrtaceous-dominated vegetation communities.

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