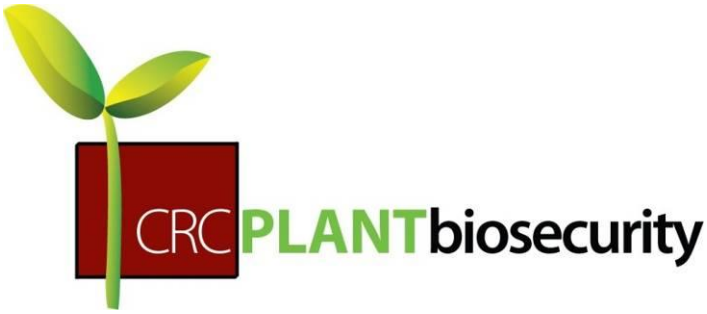

Cooperative Research Centre
for National Plant Biosecurity

Final Report

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Understanding myrtle rust epidemiology and host specificity to determine disease impact in Australia

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1. Executive Summary

Myrtle rust (eucalyptus/guava rust) caused by the fungus *Puccinia psidii* affects plants in the Myrtaceae family, which includes many Australian natives such as eucalypts, paperbark, bottlebrush, tea tree and lilly pilly. The disease is native to South America and was first described in 1884 in Brazil affecting guava, and more recently affecting eucalypt plantations (hence the common names guava and eucalyptus rust). *P. psidii* was identified as a 'high to extreme risk' biosecurity threat to Australia prior to its introduction here, due to the potential impacts on plant industries that rely on myrtaceous plants, and the significance of Myrtaceae in the Australian environment. A strain of *Puccinia psidii* (referred to as myrtle rust) was first detected on the central coast of New South Wales (NSW) in April 2010 and then quickly spread to Queensland (in December 2010) and Victoria (in December 2011), affecting more than 200 host species.

To determine the impacts of myrtle rust in Australia, it is necessary to have a thorough understanding of how the disease will manifest under our environmental conditions, and with the vast number of native myrtaceous hosts available; in plant production, environmental, amenity planting and backyard situations in Australia. The aims of this project have been to provide preliminary data on the host range and impact of myrtle rust on native Myrtaceae in Australia; identify factors influencing spread and severity of *P. psidii* infection; and establish a baseline for pathogen population studies of the Australian strain of *P. psidii*.

The full impact of myrtle rust on individual species of Myrtaceae, plant populations and ecosystems is not yet understood, especially given that the disease has only been present in Australia for two years. However, data collected in this study indicates that the disease has already impacted on growth, flowering, fruiting and seed viability for a number of myrtaceous species. Thirty one species are currently listed as having high or very high susceptibility levels to myrtle rust. In some cases (e.g. *Rhodamnia* spp.) there has been no evidence of resistance within plant populations, while a range of susceptibility levels is apparent within others (e.g. *Melaleuca quinquenervia*).

To date, most epidemiological data available for *P. psidii* comes from studies of the disease affecting eucalypt plantations in Brazil and very little published information is available on *P. psidii* affecting the native environment. The information generated in this project is the first in Australia to investigate factors influencing *P. psidii* disease spread and severity. This data suggests that the disease will be active most of the year round under subtropical conditions but with reduced levels of disease over the drier winter months. However, climate data in relation to disease spread and severity has only been collected for less than one year, and further data would need to be collected over a longer period of time to use this information to develop predictive models or for disease forecasting systems. In addition, now that myrtle rust has extended its geographic range into temperate and tropical regions, these epidemiological studies need to be expanded.

A collection of isolates of *P. psidii* has been compiled from a range of different host species and locations in Australia. These isolates will be included in future population studies and provide important baseline data for detecting change in pathogen populations over time or a new disease incursion in Australia. Host range testing of these isolates will guide future host resistance screening programs. In addition, resistance screening protocols have been optimised on populations of key eucalypt species, provenances and families used in production forestry in Queensland. Continuation of these studies, in addition to

supplementary host genetics research, will be a key to identifying species and ecosystems at greatest risk of significant impact from myrtle rust.

It is not yet known or understood how the disease as a 'new encounter' interaction will play out in Australia and the subsequent impacts on our native Myrtaceae. However, it is generally well understood that this disease will have wide ranging impacts for a large number of different stakeholders across industry, government and community.

We are already observing the short-term impacts of myrtle rust, such as the effects on plant production and trade; the cost and efficacy of chemical control measures; the loss of street and amenity trees; the cost of tree removal and replacement of susceptible species.

Some of the anticipated longer-term impacts include loss of plant species, loss of biodiversity, reduced flowering and seed production, and the subsequent impact on plant regeneration and ecosystem damage resulting in the loss of ecosystem functions such as climate regulation and carbon sequestration.

The baseline data that we have collected in this project is critical to minimise both the short and long term impacts of this disease incursion in Australia.

In addition, the data and information gathered in this project and the subsequent research outcomes, will contribute to our scientific understanding of *P. psidii* internationally.

2. Aims and objectives

Myrtle rust poses a serious threat to Australian plant industries dependent on myrtaceous hosts and the Australian environment. There is likely to also be a significant social impact with many public amenity areas and backyard gardens being affected, combined with the community concern for damage to iconic species in the Australian environment.

To properly assess the economic, environmental and social impacts of myrtle rust in Australia, it is necessary to have a good understanding of the disease epidemiology and host specificity. While there are many other aspects of myrtle rust that will also require research and development; in this project disease epidemiology and host specificity were priority areas for research, to help understand how this disease is going to behave under Australian environmental conditions and enable accurate impact assessments.

Developing our knowledge and understanding in these research areas will also help provide informed options for disease management in Australia. The most appropriate management strategies (i.e. deployment of resistance, chemical control) for particular industries will depend on answers to questions focussing on the behaviour of the rust pathogen in Australia; its life cycle, epidemiology and host-pathogen interactions.

The aims of this project were:

1. Impact of *Puccinia psidii* on native Myrtaceae and changes in key ecosystems

To determine the impact of *P. psidii* on individual species of Myrtaceae and the subsequent changes in ecosystems, by assessing the impact of myrtle rust on growth and survival of a range of species within Myrtaceae; regeneration; flower and fruit production; development and survival; and seed viability of key species of Myrtaceae.

2. Pathogen variability and host specificity

To determine differences in pathogenicity or host specificity of *P. psidii* collected from a range of host species (to examine changes in host specificity of isolates); locations (as the disease spreads) and times (to examine for changes in the pathogen).

3. Disease epidemiology - factors influencing disease development

To determine factors influencing spread and severity of *Puccinia psidii* in different environments of Australia.

3. Key findings

1. Impact of *Puccinia psidii* on native Myrtaceae and changes in key ecosystems

1.1 Host range and susceptibility to myrtle rust

The current (May 2012) host range exceeds 200 species nationally from more than 35 genera. Susceptibility ratings, developed for assessing species in Queensland (Table 1), identified 31 species rated as being highly or very highly susceptible. Included in these are species of commercial (e.g. *Backhousia citriodora*, *B. anisata*, *Chamelaucium* spp.) and environmental significance (e.g. *Melaleuca* spp.) and species commonly used in urban and peri urban areas. At least two species (*Rhodamnia angustifolia*, *Gossia gonoclada*) are listed as rare and endangered and both are highly or extremely susceptible to myrtle rust. *Rhodamnia maideniana*, common in the Springbrook National Park area, was recently removed from the threatened list but is likely to be reinstated given its susceptibility to myrtle rust and the lack of evidence of any existing resistance within the surveyed populations.

Symptoms of myrtle rust range from restricted leaf spots on less susceptible species to severe shoot and stem blight (Fig 1) impacting on growth. Repeated infection has been observed to result in stem dieback, stem cankers and distorted 'shrub-like' growth (Fig 1). *Rhodamnia rubescens*, a common species ranging from Batemans Bay area in New South Wales to the Fraser Coast in Queensland, is extremely susceptible to myrtle rust with infection causing shoot and stem dieback as well as flower death and premature senescence of fruit. Seventeen other species have been identified as having infection present on flowering and fruiting structures (Table 1; Fig 2) including species listed as moderate through to those of high to very high susceptibility. It is likely this list will expand over time as the geographic range of myrtle rust in Australia increases and more surveys are targeted specifically for fruiting and flowering periods.

Table 1. Host range and susceptibility of Myrtaceae to myrtle rust in Queensland as of May 2012. Low susceptibility = restricted leaf spot or spots only; Moderate susceptibility = blight symptoms on new shoots and expanding foliage; High susceptibility = blight symptoms on new shoots and expanding foliage and juvenile stems; Very high susceptibility = death of new shoots and severe blighting on all foliage types, shoot and stem dieback. Susceptibility ratings are based on observations to date and may change with time.

Disease rating	Host name	Host name
Low	*Acmena hemilampra*	*Metrosideros thomasii*
	Acmena ingens	*Myciaria cauliflora*
	Acmena smithii	*Myrtus communis*
	Asteromyrtus brassii	*Pilidiostigma glabrum*
	Austromyrtus tenuifolia	*Rhodamnia acuminata*
	Backhousia angustifolia	*Ristania waterhousei*
	Backhousia bancroftii	*Syzygium argyropedicum*
	Backhousia sp. 'Prince Regent'	*Syzygium armstrongii*
	Backhousia sciadophora	*Syzygium australe*
	Choricarpia subargentea	*Syzygium boongee*
	Corymbia henryi	*Syzygium canicortex*
	Corymbia torelliana	*Syzygium coryanthum*
	Corymbia citriodora subsp. variegata	*Syzygium dansiei*
		Syzygium forte ssp. *forte*
	Gossia bidwillii	*Syzygium forte* ssp. *potamophilium*
	Gossia floribunda	*Syzygium luehmanii*
	Gossia myrsinocarpa	*Syzygium moorei*
	Gossia punctata	*Syzygium nervosum*
	Lenwebbia sp. Blackall Range	*Syzygium paniculatum*
	Leptospermum luehmannii	*Syzygium rubrimolle*
	Leptospermum petersonii	*Syzygium tierneyana*
	Leptospermum semibaccatum	*Syzygium wilsonii*
	Lindsayomyrtus racemoides	*Syzygium wilsonii x S. luehmannii*
	Lophostemon sauveolans	*Tristaniopsis laurina*
	Melaleuca formosa	*Uromyrtus tenella*
	Melaleuca linarifolia	*Waterhousea floribunda*
	Melaleuca nesophila	*Waterhousea hedraiophylla*
	Melaleuca pachyphylla	*Waterhousea mulgraveana*
	Metrosideros collina	*Xanthostemon chrysanthus*
	Metrosideros kermadecensis	
Moderate	*Acmenosperma claviflorum*	*Rhodamnia australis*
	Backhousia myrtifolia	*Rhodamnia glabrescens*
	Backhousia oligantha	*Rhodamnia pauciovulata*
	Eucalyptus carnea	*Rhodomyrtus canescens**
	Eucalyptus cloeziana	*Rhodomyrtus pervagata**
	Eucalyptus curtisii	*Rhodomyrtus sericea*
	Eucalyptus grandis	*Rhodomyrtus tomentosa*
	Eucalyptus planchoniana	*Rhodomyrtus trineura* ssp. *capensis*
	Eucalyptus tindaliae	*Sphaerantia discolor*
	Eugenia zeyheri	*Syzygium angophoroides*
	Gossia fragrantissima	*Syzygium corynanthum*
	Gossia macilwraithensis	*Syzygium cumini*
	Gossia punctata	*Syzygium eucalyptoides subsp. eucalyptoides*
	Leptospermum liversidgei	
	Melaleuca fluviatilis	*Syzygium luehmannii x S. wilsonii*
	Melaleuca saligna	*Syzygium xerampelinum*
	Melaleuca viminalis	*Sphaerantra discolor*
	Mitrantia bilocularis	*Tristania neriifolia*
	Rhodamnia argentea	*Waterhousea unipunctata*

	*Rhodamnia arenaria**	*Xanthostemon youngii*
High	*Austromyrtus dulcis**	*Melaleuca quinquenervia**
	Backhousia citriodora	*Melaleuca leucadendra**
	Backhousia/Anetholea anisata	*Melaleuca nodosa*
	Callistemon viminalis	*Melaleuca polandii*
	*Chamelaucium ciliate**	*Melaleuca viridiflora*
	*Chamelaucium uncinatum**	*Rhodamnia costata*
	Choricarpia leptopetala	*Rhodamnia dumicola*
	Decaspermum humile	*Rhodamnia sessiliflora**
	Gossia acmenoides	*Rhodamnia spongiosa**
	Gossia hillii	*Rhodomyrtus tomentose**
	Gossia gonoclada	*Rhodomyrtus trineura subsp. capensis*
	Lenwebbia lasioclada	*Syzygium oleosum*
	*Lenwebbia prominens**	*Tristania neriifolia*
	Melaleuca fluviatilis	*Xanthostemon oppositifolius*
Very High	*Agonis flexuosa*	*Rhodamnia rubescens**
	Backhousia oligantha	*Rhodamnia maideniana**
	*Eugenia reinwardtiana**	*Rhodomyrtus psidioides**
	Gossia inophloia	*Syzygium jambos**
	*Rhodamnia angustifolia**	

Teliospores identified
*Disease recorded on flower buds, flowers or fruit of hosts

Figure 1. Repeated infection of new shoots and juvenile stems (a. *Melaleuca quinquenervia* & b. *Xanthostemon oppositifolius*) by myrtle rust results in stem dieback and shrub-like growth (c. *M. quinquenervia* & d. *Decaspermum humile*).

Figure 2. Myrtle rust infection on fruit and flowers of *Rhodamnia sessiliflora* (a & b), *Eugenia reinwardtiana* (c) and *Chamelaucium uncinatum* (d).

Figure 3. Progress of symptoms on *Rhodamnia angustifolia* over a three month period when (a) infection levels were low and restricted to less than 5% of new shoots and leaves; (b) 100% of new shoots and new leaves; (c) death and premature senescence of all new leaves and shoots with branch dieback occurring.

1.2 Monitoring the impact of myrtle rust in native forests

Monitoring plots were established in native forest on the Central Coast of NSW to quantify the impact of myrtle rust on foliage production, flower and fruit production and survival of brush turpentine (*Rhodamnia rubescens*) and to gather preliminary data on epidemiology of myrtle rust in Australia. The site (Olney State Forest) has a recorded history of severe myrtle rust, including when the rust was first detected in the area (October/November 2010).

1.2.1 Methods

Plots were established in a native stand of *R. rubescens* (plants of varying ages and from 0.5 m to 4.0 m in height) in August 2011. Seedlings were also planted (August 2011) at the site to obtain data on the impact of rust on seedlings/young plants. In the native stand, twenty plants were selected along a line-transect and randomly assigned as treated (sprayed monthly with the fungicide triadimenol [50 ml/100 L]) or untreated (not sprayed); 10 plants each. Four seedlings were planted along the same transect in each of five plots and one seedling was treated monthly with fungicide.

Three branches per plant in the native stand were randomly (height and compass bearing) selected and tagged 30 cm from the growing tip. Each branch was assessed monthly for (1) total number of leaves [initially divided into old and new leaves]; (2) incidence [number affected] and severity [% leaf area affected] of myrtle rust; and (3) number of new shoots and incidence of myrtle rust on new shoots. Each plant was assessed for crown transparency, generally every second month, using standard methods (USDA-FS, Figure 3). For the planted seedlings, each plant was assessed monthly for (1) total number of leaves; (2) incidence and severity of myrtle rust; and (3) number of new shoots and incidence of myrtle rust on new shoots. For both plants in the native stand and for planted seedlings, the proportion of leaves each month that were newly emerged/developed was also recorded. Height of each plant and seedling, and branch length on tagged plants, was measured annually. Development/production of flowers (generally August-October) and fruit (generally October-December) (Floyd 1989), and incidence of rust on each, will be collected for plants in the native stand during 2012 (no flower production was observed in the area in 2011).

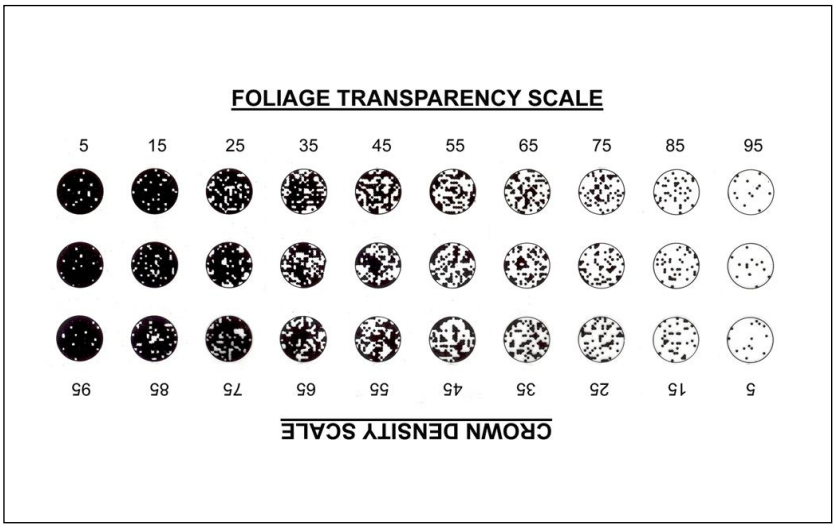

Figure 4. Foliage Transparency Scale (USDA Forest Service, http://www.srs.fs.usda.gov/pubs/27730).

A data logger collected temperature, RH and dew point and leaf-wetness logger was installed.

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 myrtle rust. Six leaves of each leaf-phase from each tree in the native stand were sampled in February 2012. Three branches per tree were randomly selected (but not the tagged branch) and 2 'old' leaves were removed and placed in paper envelopes (i.e. 2 leaves x 3 branches = 6 leaves). This was repeated for 'intermediate' and current/new foliage (Fig 8). Leaves were scanned and then the program QUANT (Vale et al. 2003) was used to quantify the size (area) of leaves and the percentage of rust from fungicide-sprayed and unsprayed plants.

1.2.2 Results to date

Initial data analysis (August 2011 to March 2012 assessments) reveal significant differences ($P<0.05$) in leaf production (mean number of leaves), incidence and severity of myrtle rust on leaves and crown transparency between sprayed and unsprayed trees (Fig 4). There were significant differences in myrtle rust damage and foliage production between sprayed and unsprayed trees (Fig 5 & 6). For the sprayed trees there was virtually no new rust recorded after ~2 months of fungicide application. Fresh rust was recorded on unsprayed trees in each month from ~September. Leaf production (new leaves and new shoots) was recorded on both treatments generally each month since ~September. Results indicate monthly applications of fungicide controlling the rust, with treated plants responding by producing significantly more foliage than unsprayed plants (Fig 5 & 6).

Data analysis for the quantification of leaf size and rust severity on leaving using QUANT is ongoing. Preliminary analysis indicates significant differences in damage from myrtle rust between sprayed and unsprayed trees (Fig 8) as well as a reduction in size of newly produced leaves on unsprayed (diseased) plants.

This trial is planned to continue until at least the end of 2012 to assess the impact of rust on flower and fruit production. Spatial analysis of the 20 plants will be conducted and it is planned that a greater sample of *R. rubescens* will be assessed (e.g. several 0.1 ha plots) to obtain a better understanding of the spatial distribution of the impact of rust in this stand.

Incidence values were first changed to the percentage incidence by dividing the leaves with infestation by the total number of leaves. The averages were then taken at the branch level to get the tree level incidence percentage.

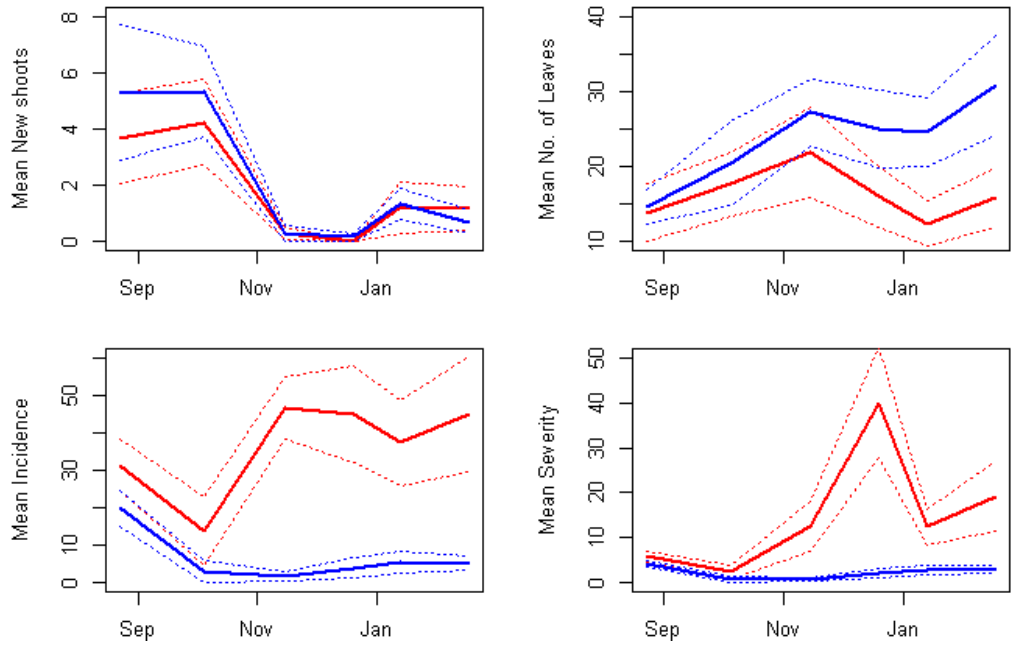

Figure 5. Plot of the mean values (solid lines) for mean number of new shoots, mean number of leaves, mean incidence and mean severity against the measurement time. Red is for the control trees and Blue for the treated trees. The dotted lines are the 95% confidence interval around the mean values (Carnegie unpublished).

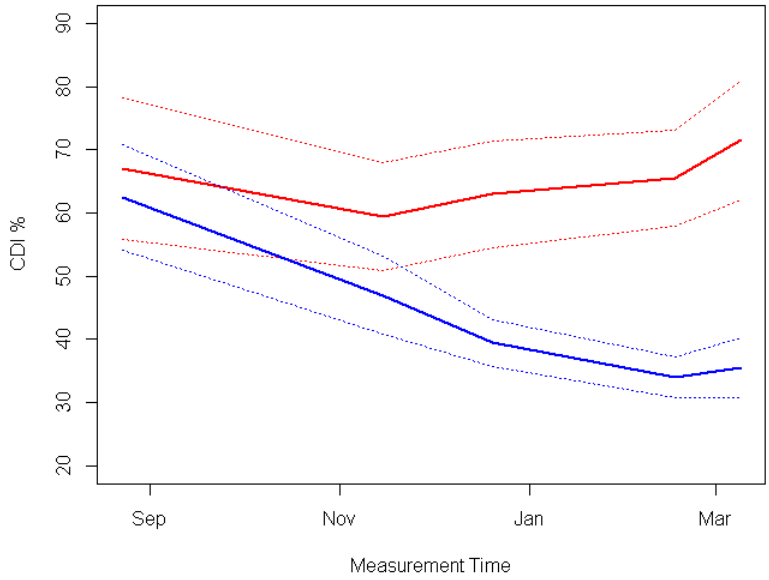

Figure 6. Plot of the mean CDI% against the measurement time. Red is for the control trees and Blue for the treated trees. The dotted lines are the 95% confidence interval around the mean values (Carnegie unpublished).

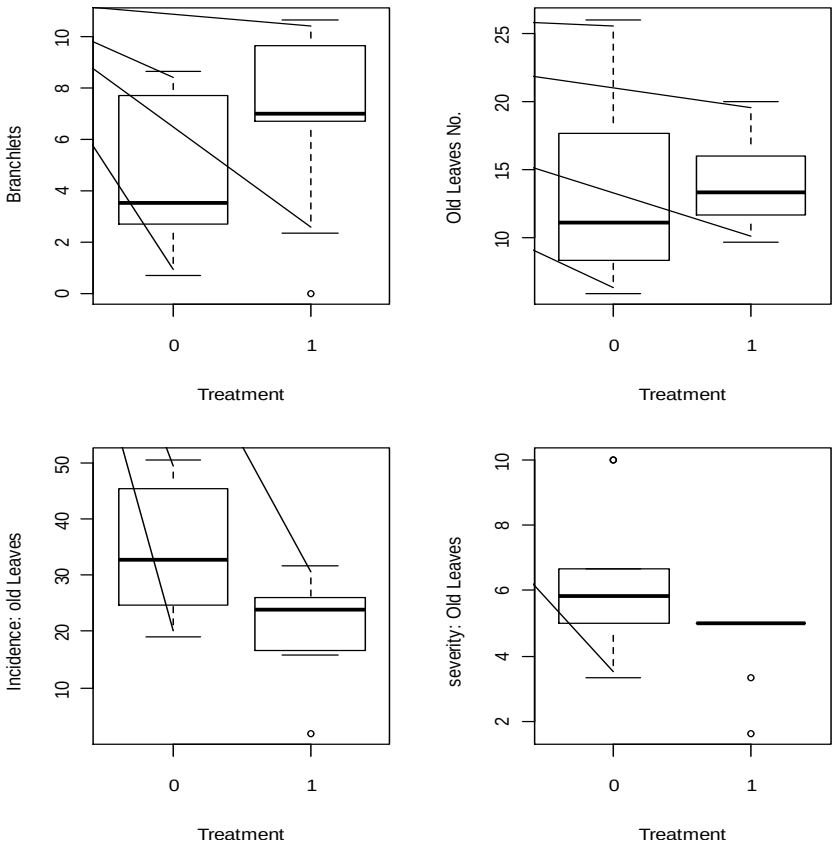

Figure 7. Boxplot of number of branchlets, number of old leaves, old leaves incidence and severity on old leaves at the start of the experiment. The bottom and top edges of the boxes indicate the sample 25th and 75th percentiles. The solid line is for the median value, the whiskers are for the range excluding the extreme values which are represented by the small circles (Carnegie unpublished).

Wilcoxon rank-sum test also known as the Mann-Whitney U test, is a non parametric test that uses ranks of the sample data from two independent populations to test if the median values of the two populations are equal. As the data for all the variables considered for the baseline comparisons is non-normal this is a more appropriate test to use than the t-test. The results of the test are presented in Table 2 along with the mean and standard error values for the baseline variables.

Table 2. Summary statistics for the base line variables. Values in the brackets with mean values are the standard errors (SE). The WRS test presents the results of Wilcoxon rank-sum test (Carnegie unpublished).

	Mean		
Variable	**Control**	**Treated**	**WRS test**
No. Of Branchlets	4.5 (0.91)	6.67 (1.06)	-1.29(P=0.20)
No. Of old leaves	13.2 (2.05)	13.8 (1.00)	-0.76 (P= 0.45)
Incidence%: Old leaves	34.07(3.68)	20.79 (2.61)	2.46(P = 0.01)
Severity: Old leaves	6.33 (0.69)	4.5 (0.36)	2.14(P=0.03)

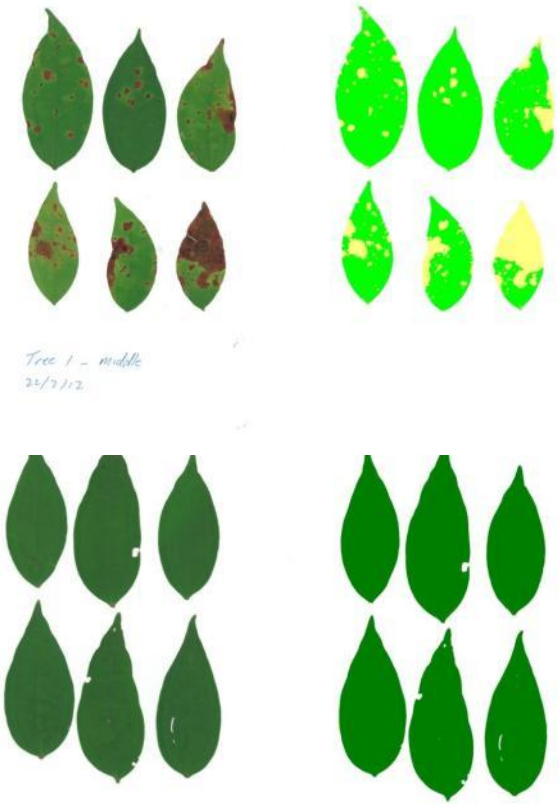

Figure 8. Difference in necrosis caused by myrtle rust on unsprayed (above) and fungicide-sprayed (below) leaves (Carnegie unpublished).

1.3 Monitoring the impact of myrtle rust on regeneration – *Melaleuca quinquenervia*

A monitoring site was established within a naturally regenerating stand of *M. quinquenervia* at Woodburn in northern New South Wales to determine the impact of myrtle rust on tree growth and survival and to examine the impact of infection on flower development and seed viability.

1.3.1 Methods

A plot of 100 trees was selected at random in August 2011 and is being assessed for incidence and severity of myrtle rust infection on a monthly basis. The impact on tree growth is being assessed through measurement of trees at the time of commencement six and 12 months after assessments were initiated and is to be analysed in relation to disease data. The presence of flowers and the number of flowers per tree is also being assessed.

Disease levels were recorded as:

1. Incidence at the stand level (percentage of trees infected)

2. Incidence at the plant level (incidence of disease on new foliage of individual trees)

3. Severity at the plant level (disease severity based on a 0-6 rating scale)

- 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
- 6 = All new shoots and stems dead; shrub like growth appearance with total loss of apical dominance

1.3.2 Results to date

At the time of the first assessment in August 2011, 80% of trees were infected with this level increasing over the next two months peaking at 96% in October 2011. A decline in

rust levels occurred following flood periods in January 2012. In February 2012, new growth flush was absent during the period due to attack by mirid bugs causing death and premature senescence of new flush and young leaves. In March 2012, 42% of trees had some evidence of myrtle rust infection, increasing in April and May to 79 and 90% respectively.

Average disease incidence and severity levels on trees followed a similar fluctuating pattern. In August 2011, 45% of the trees infected had a disease incidence level of 50% or greater (Fig 9 & 10) increasing to 83% in October 2011.

Measurements taken six months after the site was first assessed indicated significant differences in tree growth. Growth on trees with average disease severity levels greater than 3 was significantly reduced in comparison to trees with no infection or those with only minor foliage infection. Detailed analysis of growth data will be completed after 12 months assessment.

Flower production was first detected in April 2012 and was identified on 10% of trees assessed with only 7% having more than two flowers. Of the 10% of trees producing flowers, 2% had infection detected on the flower buds or flowers (Fig 11). Seed will be collected from these trees to determine viability levels and to investigate resistance levels within progeny. Correlations are yet to be completed for this data but flowering was not observed on any trees exhibiting evidence of stem infection or averaging a severity score of 3 or greater.

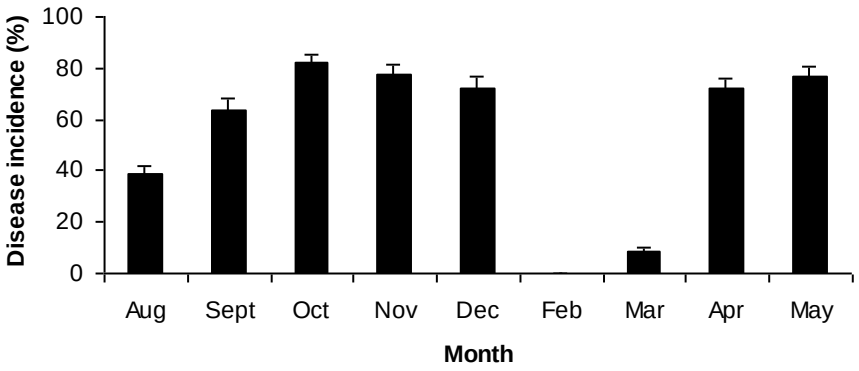

Figure 9. Average disease incidence levels on new shoots and leaves of *M. quinquenervia* seedlings at Woodburn in northern NSW (Pegg et al. unpublished).

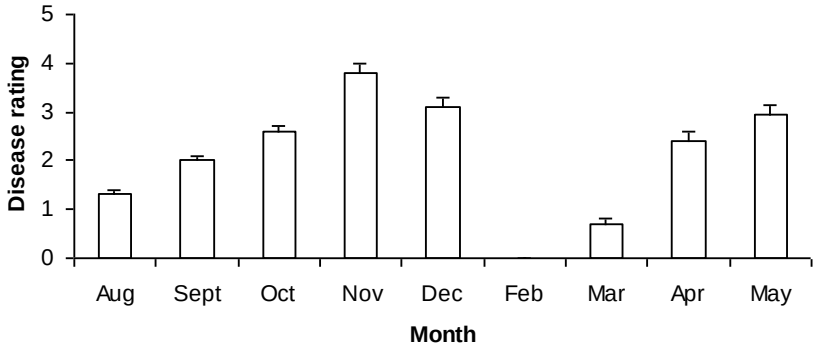

Figure 10. Average disease severity rating levels (0-6) on new shoots and leaves of *M. quinquenervia* seedlings at Woodburn in northern NSW (Pegg et al. unpublished).

Figure 11. Myrtle rust infection (arrow) on *Melaleuca quinquenervia* preventing full flower development (Photo Pegg).

1.4 Impact of myrtle rust on flower and fruit production and survival

To determine the impact of myrtle rust, *Rhodamnia sessiliflora* and *R. angustifolia*, both rated as highly or extremely susceptible, were assessed for the rate and level of infection on flower & fruit production and survival.

1.4.1 Methods

Six branches with flower buds were labelled from each species and assessed on a daily basis for the number of flowers and fruit present and those showing evidence of myrtle rust infection. The rate of fruit infection was assessed on a *R. sessiliflora* only as no fruit has been observed on *R. angustifolia* since infection was first detected. Insufficient numbers of trees are available to investigate differences between treated and untreated trees.

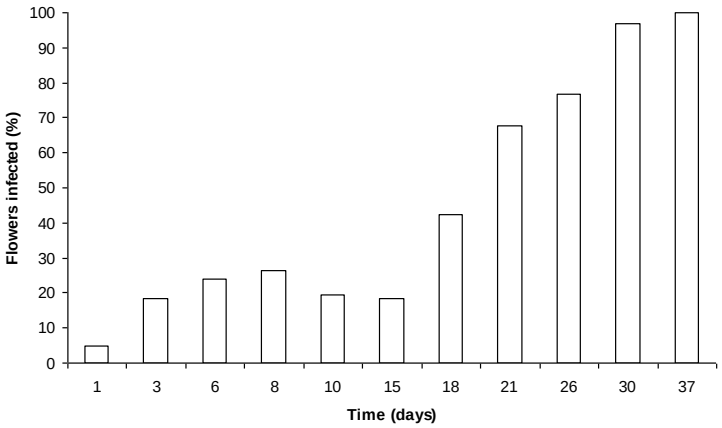

Figure 12. Percentage of *Rhodamnia sessiliflora* flowers infected by myrtle rust over days since first assessment (Pegg et al. unpublished).

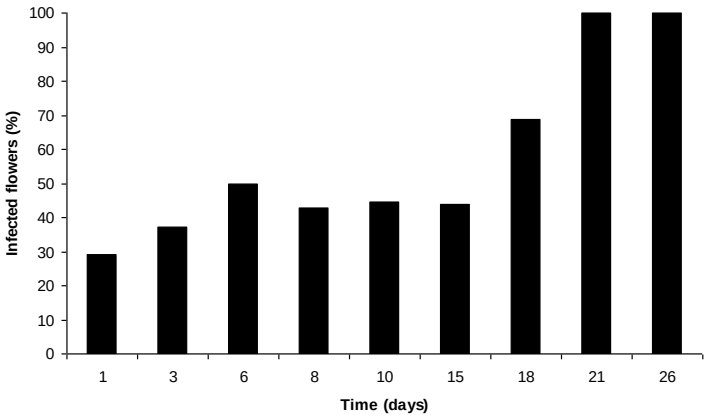

Figure 13. Percentage of *Rhodamnia angustifolia* flowers infected by myrtle rust over days since first assessment (Pegg et al. unpublished).

1.4.4 Results to date

Myrtle rust infection occurred on both species at the bud stage of flower development with 100% of flowers infected within 37 days on *R. sessiliflora* (Fig 12) and 21 days on *R. angustifolia* (Fig 13). No fruit development occurred from any infected flowers with premature senescence occurring for all flowers.

Myrtle rust infection occurred on green fruit of *R. sessiliflora* with 100% of fruit infected 24 days after assessment commenced (Fig 14, 15, 16). All fruit prematurely senesced before reaching maturity.

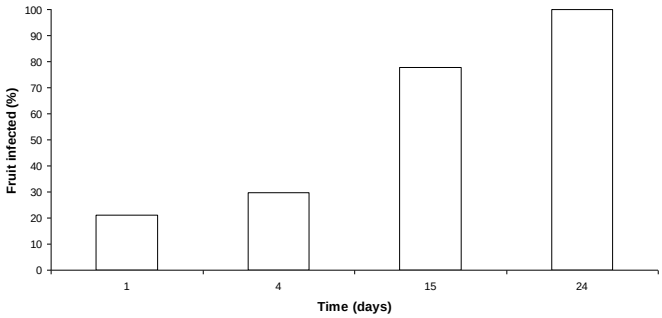

Figure 14. Percentage of *Rhodamnia sessiliflora* fruit infected by myrtle rust over days since first assessment (Pegg et al. unpublished).

Figure 15. Photographic sequence of myrtle rust infection on *Rhodamnia rubescens* fruit (Photo Pegg).

Figure 16. Myrtle rust infection on immature (green) and mature (black) fruit of *Rhodamnia sessiliflora*. Fruit of this species are a known food source for birds such as the Cassowary (Photo Pegg).

2. Pathogen variability and host specificity

2.1 Isolate collection

Isolates of *P. psidii* have been collected from a range of hosts and locations from New South Wales and Queensland. For each isolate (except the first four, Table 3), three "field" pustules have been stored immediately for future molecular studies. If enough spore was available, spores from each of these isolates were transferred onto *Rhodamnia rubescens* or *Syzygium jambos* seedlings in order to increase the isolate for storage at -80^{o}C (see inoculation methodology below). The majority of these increased isolates are of a bulk collection of urediniospores, although six single pustule isolates have been increased during the life of the project, four of which are in current cultivation. For each isolate which has been successful in cultivation a minimum of three collections of increased urediniospores has been made and stored at -80^{o}C (see collection and storage methodology below). Where spores were still available following the bulk inoculation for increase, a collection of field urediniospores was made for storage at -80 ^{o}C. Following initial and regular 14-21 day re-inoculations (as per inoculation procedures below), isolates were kept at a distance from one another in Controlled Environment Rooms (with no air flow, 18/26 ^{o}C night/day cycle) for the duration of their cultivation to maintain the integrity of the separate isolates and prevent cross-contamination.

In summary:

- 76 isolates of *Puccinia psidii* are in storage
  - 71 with field pustules stored
  - 16 with field collections of urediniospores stored
  - 30 with cultivated (increased) urediniospores stored
- Six single pustule isolates are in storage, four of which are in current cultivation
- Seven isolates are currently in cultivation (four of these are the single pustule isolates)
- The 76 isolates encompass:
  - 31 locations
  - Four local government areas in NSW
  - Nine local government areas in QLD
  - 40 hosts from 17 genera

Table 3. *Puccinia psidii* isolate collection from a range of locations and host in Queensland and New South Wales (Ireland et al. unpublished).

Isolate	Host	State	Local Government Area	Bulk isolate increased	Single pustule isolates	Field pustules stored	Field urediniospores stored	Primary transfer for increase successful	Urediniospores stored at -80
11-001	*Melaleuca quinquenervia*	QLD	Gold Coast				✓		
11-002	*Rhodamnia*	QLD	Gold Coast				✓		
11-003	*Syzygium jambos*	QLD	Gold Coast				✓		
11-004	*Syzygium jambos*	QLD	Gold Coast				✓		
11-005	*Rhodamnia rubescens*	QLD	Brisbane		**1**	✓	✓		
11-006	*Lenwebbia prominens*	QLD	Brisbane			✓	✓		
11-007	*Backhousia sciadophora*	QLD	Brisbane	✓	**2**	✓		✓	✓
11-008	*Syzygium coryanthanum*	QLD	Brisbane	✓	**2**	✓	✓	✓	✓
11-009	*Rhodamnia arenaria*	QLD	Brisbane			✓	✓		
11-010	*Melaleuca quinquenervia*	QLD	Sunshine Coast			✓			
11-011	*Lophostemon suaveolens*	QLD	Sunshine Coast			✓			
11-012	*Melaleuca quinquenervia*	QLD	Brisbane			✓	✓		
11-013	*Eugenia reinwardtiana*	QLD	Brisbane			✓	✓		
11-014	*Eugenia reinwardtiana*	QLD	Bundaberg			✓	✓		
11-015	*Rhodamnia rubescens*	NSW	Hunter	✓		✓		✓	✓
11-016	*Backhousia spp.*	NSW	Hunter			✓		✓	
11-017	*Syncarpia glomulifera*	NSW	Hunter		**1**	✓		✓	
11-018	*Rhodamnia rubescens*	NSW	Hunter	✓		✓		✓	✓

11-019	*Rhodamnia rubescens*	NSW	Hunter	✓	**2**	✓		✓	✓
11-020	*Eucalyptus aglomerata*	NSW	Hunter	✓		✓		✓	✓
11-021	*Eucalyptus aglomerata*	NSW	Hunter			✓			
11-022	*Eucalyptus aglomerata*	NSW	Hunter	✓		✓		✓	✓
11-023	*Eucalyptus curtisii*	QLD	Gold Coast			✓			
11-024	*Eucalyptus grandis*	QLD	Gold Coast			✓			
11-025	*Rhodamnia rubescens*	QLD	Redlands			✓			
11-026	*Chamelaucium uncinatum*	NSW	Clarence			✓		✓	
11-027	*Rhodamnia argentea*	NSW	Clarence			✓			
11-028	*Melaleuca quinquenervia*	NSW	Bellingen			✓			
11-029	*Rhodamnia rubescens*	QLD	Redlands	✓		✓		✓	✓
12-001	*Rhodamnia arenaria*	QLD	Brisbane	✓		✓	✓	✓	✓
12-002	*Rhodomyrtus sericea*	QLD	Brisbane	✓		✓		✓	✓
12-003	*Rhodomyrtus pervagata*	QLD	Brisbane			✓	✓		
12-004	*Rhodomyrtus tormentosa*	QLD	Brisbane			✓	✓		
12-005	*Melaleuca quinquenervia*	QLD	Brisbane			✓			
12-006	*Syzygium australe*	QLD	Brisbane	✓		✓		✓	✓
12-007	*Eucalyptus planchoniana*	QLD	Brisbane	✓		✓		✓	✓
12-008	*Syzygium francissii*	QLD	Brisbane	✓		✓			✓
12-009	*Syzygium jambos*	QLD	Brisbane	✓		✓		✓	✓
12-010	*Syzygium oleosum*	QLD	Brisbane	✓		✓		✓	✓
12-011	*Lenwebbia prominens*	QLD	Brisbane			✓			
12-012	*Corymbia henryii*	QLD	Brisbane	✓		✓		✓	✓
12-013	*Rhodamnia rubescens*	QLD	Brisbane	✓		✓	✓	✓	✓

12-014	*Eugenia reinwardtiana*	QLD	Rockhampton			✓			
12-015	*Eucalyptus tindaliae*	QLD	Brisbane			✓			
12-016	*Eucalyptus carnea*	QLD	Brisbane	✓		✓		✓	✓
12-017	*Rhodamnia dumicola*	QLD	Brisbane	✓		✓		✓	✓
12-018	*Eucalyptus planchoniana*	QLD	Brisbane			✓			
12-019	*Melaleuca leucadendra*	QLD	Brisbane	✓		✓		✓	✓
12-020	*Austromyrtus dulcis*	QLD	Brisbane			✓			
12-021	*Leptospermum liversedgii*	QLD	Brisbane			✓			
12-022	*Eugenia reinwardtiana*	QLD	Rockhampton			✓			
12-023	*Melaleuca leucadendra*	QLD	Cairns			✓			
12-024	*Syzygium jambos*	QLD	Brisbane	✓	**1**	✓		✓	✓
12-025	*Homoranthus virgatus*	NSW	Ballina	✓		✓		✓	✓
12-026	*Decaspermum humile*	QLD	Gold Coast	✓		✓		✓	✓
12-027	*Melaleuca alternifolia*	NSW	Bellingen	✓		✓		✓	✓
12-028	*Melaleuca quinquenervia*	NSW	Bellingen	✓	**1**	✓		✓	✓
12-029	*Eugenia reinwardtiana*	QLD	Gladstone			✓			
12-030	*Backhousia citriodora*	QLD	Gold Coast			✓			
12-031	*Syzygium jambos*	QLD	Gold Coast			✓			
12-032	*Gossia fragrantissimo*	QLD	Gold Coast			✓			
12-033	*Lenwebbia prominens*	QLD	Brisbane			✓			
12-034	*Syzygium australe*	QLD	Brisbane			✓			
12-035	*Rhodamnia sessiliflora*	QLD	Brisbane			✓			
12-036	*Eugenia reinwardtiana*	QLD	Brisbane	✓		✓		✓	✓

12-037	*Eucalyptus curtisii*	QLD	Gold Coast	✓		✓		✓	✓
12-038	*Melaleuca nodosa*	QLD	Gold Coast	✓		✓		✓	✓
12-039	*Syzygium ingens*	QLD	Gold Coast			✓			
12-040	*Eucalyptus grandis*	QLD	Gold Coast	✓	**1**	✓		✓	✓
12-041	*Gossia gonoclada*	QLD	Brisbane			✓			
12-042	*Honey lemon myrtle?*	QLD	Toowoomba	✓		✓		✓	✓
12-043	*Melaleuca quinquenervia*	QLD	Toowoomba			✓	✓	?	
12-044	*Syzygium jambos*	QLD	Redlands			✓			
12-045	*Rhodamnia rubescens*	QLD	Redlands			✓			
12-046	*Melaleuca quinquenervia*	QLD	Redlands			✓			
12-046	*Melaleuca quinquenervia*	QLD	Redlands			✓			
12-047	*Eugenia reinwardtiana*	QLD	Mackay	✓		✓	✓	✓	

2.2 Disease screening methodology and testing

Studies have been conducted to test inoculation, incubation and assessment methodologies for adoption in host screening and host/pathogen studies.

1.2.2 Collection & storage of spores

Spore collection from field and glasshouse infected plant material is done using a vacuum pump system and collected into glass pipettes before being dried in a desiccator for a minimum of 48 hours. Spores are then transferred into Nunc tubes and stored at -80^{o}C.

There is a need to investigate the viability of spores stored for various durations and to test the efficacy of drying methods and their influence on spore viability.

1.2.3 Inoculation & incubation

Spore suspensions are made up in sterile distilled water (SDW) to which Tween or a mineral oil is added. Spore counts are conducted prior to inoculation using a haemocytometer.

To test spore viability and germination levels, a sample of the spore suspension is placed onto water agar blocks on a glass slide, maintained at 100% relative humidity (RH) and placed in the controlled environment room (CER) along with inoculated plants.

The spore suspension is applied to seedlings using a fine mist spray (2.9 kPa pressure) generated by a compressor driven spray gun (Iwata Studio series 1/6 hp; Gravity spray gun RG3, Portland, USA), to the upper and lower leaf surfaces of the seedlings to a stage

where complete coverage of the leaf was achieved but avoiding runoff so as to reduce loss of inoculum. In order to rapidly increase and maintain high relative humidity, plants were then placed in plastic bags or under plastic sheeting to which hot water was added at the base to ensure rapid increase in RH. Trays of plants were then placed into a CER set at 18-20^{o}C for 24 hours under dark conditions. Plants were then removed from the CER and placed in shade house under natural light conditions.

1.2.4 Disease assessment

Symptom development varies between and within host species and it is important to monitor disease levels to identify the optimum time to assess susceptibility levels. Using DAFF Queensland (Forestry Science) germplasm for three key eucalypt species, Spotted gum including *C. variegata*, *C. citriodora* and *C. henryi*, *Eucalyptus argophloia* (Chinchilla white gum) and *E. cloeziana* (Gympie messmate), studies were conducted looking at resistance patterns at species (Fig 17), provenance and family level as well as the applicability of susceptibility rating methods developed in Brazil (Fig 18). These species were used as the collections within DAFF Queensland represent a well structured population including provenances from the entire native range of each species. For many other Myrtaceae affected by myrtle rust seed collections are often restricted and in many cases lacking. Those species used in nursery propagation are often collected from random sources with little or no details or collection information. This makes studying resistance patterns and any heritability levels within progeny difficult.

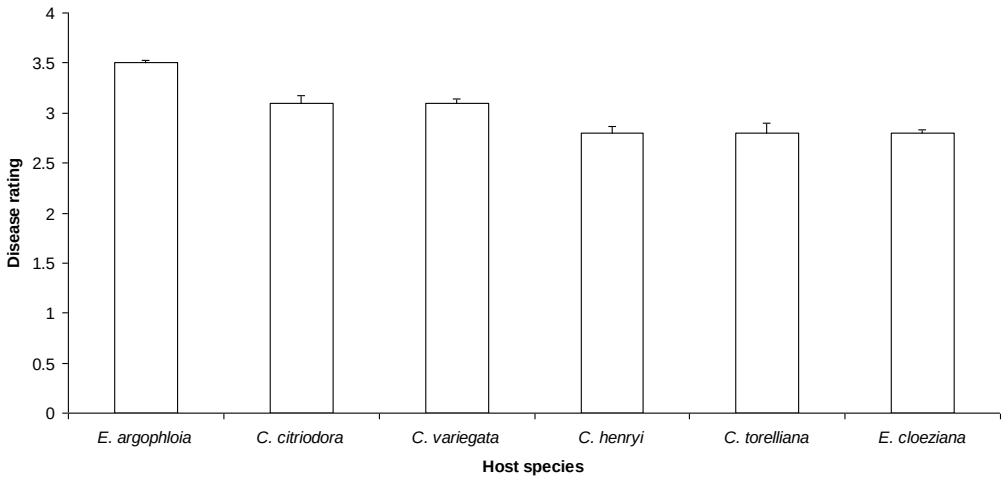

Figure 17. Average disease susceptibility rating for Queensland eucalypts (*Eucalyptus* and *Corymbia* spp.) (Pegg et al. unpublished).

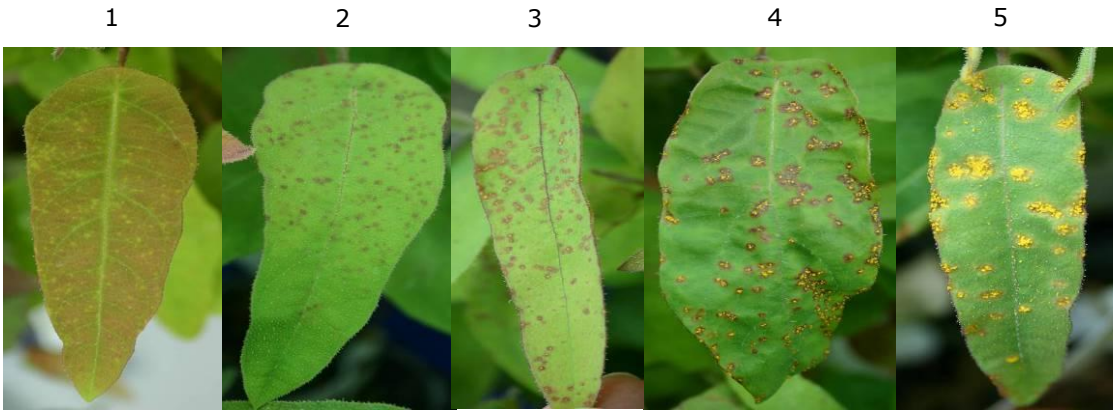

Figure 18. Spotted gum rating based on the Brazilian susceptibility rating system.
1 = 'immune' – no evidence of disease or yellow flecking apparent
2 = Hypersensitive reaction (HR) – no evidence of pustules present
3 = Lesion size <0.8mm diameter, 1-2 pustules on some lesions
4 = lesion size 0.8-1.6mm diameter, multiple pustules per lesion
5 = lesion size <1.6mm diameter, large number pustules per lesion; lesions with pustules can be present on shoots and stems

It is likely, when studying detailed genetics of resistance that further categories could be including distinguishing variations within the above categories. Some seedlings lack any evidence of infection with no flecking apparent. Similarly some seedlings can have a high number of HR lesions per leaf while others only have one or two HR spots per leaf and yet they are rated at the same level.

2.3 Differential host set

To identify variability in current fungal populations and to test for future changes or new introductions it is essential that a range of individuals, both resistant and susceptible, from a range of hosts are identified for establishment and use in differential host set. Ideally this host set would be tested regularly to identify any changes in the pathogen population for quick incorporation into any screening program, similar to what is used for cereal rust breeding programs (McIntosh *et al.* 1995). Current studies, both field and glasshouse, have identified individuals within species showing ranges of susceptibility. This includes *M. quinquenervia, Eucalyptus* and *Corymbia* species. Ideally cloning susceptible and resistant individuals from each species would be used in establishing a differential set. However, spotted gum species and *E. cloeziana* are difficult to clone.

Future work needs to focus on developing a differential set from a wide range of Myrtaceae and include these as part of an international approach to indentifying variation within the current *P. psidii* population and any future changes in the pathogen.

3. Disease epidemiology - factors influencing disease development

To determine factors influencing the rate and severity of myrtle rust on susceptible hosts, studies investigating the impact of rainfall, temperature, relative humidity and dew point have been conducted. *Rhodamnia sessiliflora* has been used as the host in this study.

3.1 Infection rate

3.1.1 Methods

Within the first five days of each month all new shoots (made up of a pair of leaves) were labelled and sequentially numbered. Labelled shoots were then examined daily for the presence of pustules typical of *P. psidii* infection. The date of detection and the number of pustules per shoot were recorded. The number of pustules on each infected leaf was also recorded over time until no further increase could be detected.

Weather data is collected using data loggers, capturing temperature, relative humidity (RH) and dew point on an hourly basis.

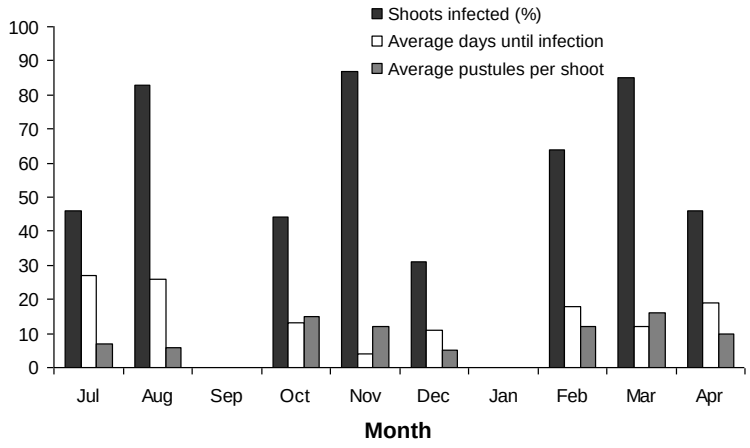

Figure 19. Rate and level of *Puccinia psidii* infection on *Rhodamnia sessiliflora* shoots from July 2011 to April 2012. (Pegg et al. unpublished)

3.1.2 Results to date

The number of shoots infected has fluctuated monthly showing a harmonic-like pattern with disease levels building to a peak before declining (Fig 19). This pattern was repeated three times over the period of assessment. September is the only month when infection of new shoots did not occur despite there being a comparatively large number produced within the first five days. In January no shoots were produced with heavy flower set the likely cause.

The factors influencing these peaks and troughs is not yet clear and requires continued studies extended over a further 12 month period and using additional host species. When comparing climate data, the only significant difference between months is in September when night time RH levels were comparatively lower influencing dew point which is likely to impact directly on spore germination levels and host infection. Leaf wetness loggers have now been placed and will provide more accurate data recording duration of leaf wetness in relation to disease development.

The length of time for infection to occur and the number of pustules per shoot is closely linked but correlation data needs to be examined to confirm this. Leaf age is an important factor in disease development with susceptibility declining as leaves age. The influence of the number of shoots produced at a time and the level of inoculum present at each month

needs further investigation. Spore trapping data will help quantify spore levels in relation to rate and severity of infection. Additionally an understanding of factors triggering spore production will assist in developing a disease forecasting system.

Preliminary spore trapping studies (Fig 20) indicated that higher levels of spore were produced and released from pustules early in the morning through until midday and during periods when dew point was more likely to have been reached. However, this work needs to be repeated over an extended period capturing data daily in relation to different climatic conditions.

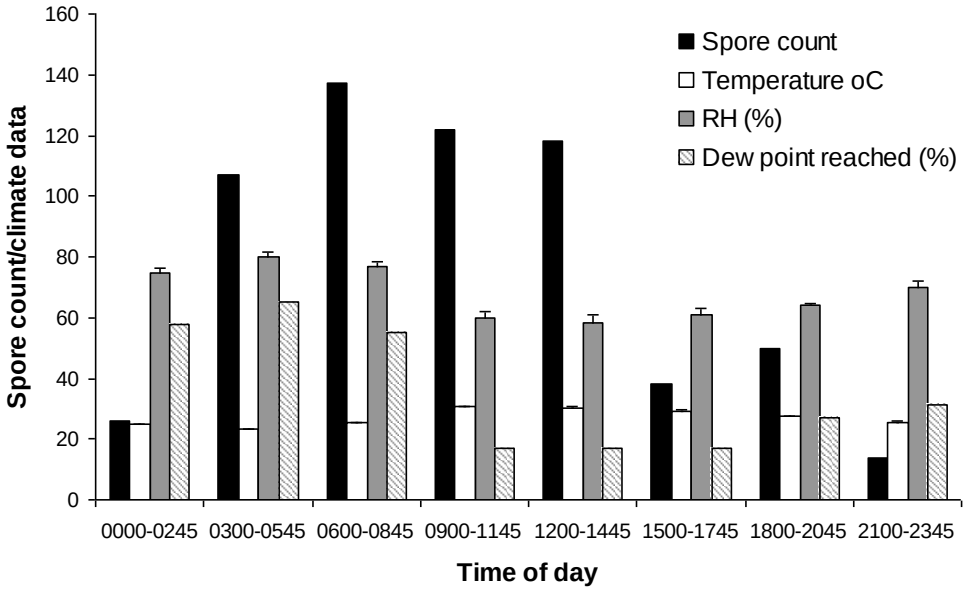

Figure 20. *Puccinia psidii* spore production in relation to daily climate conditions collected over a two week period (Pegg et al. unpublished).

3.2 Disease spread and development

A study is currently underway examining the rate of spread of myrtle rust through ecosystem regeneration plantings.

3.2.1 Methods

A site in Wacol was planted with predominantly *M. quinquenervia* seedlings in November 2011. Myrtle rust was first detected at the site in March 2012 and studies commenced to determine the distribution of *P. psidii* infections within the planting (Fig 21). Spatial patterns of disease incidence and severity within the planting have been examined and are continuing to be assessed, based on the GPS survey data collected. The patterns of spread were studied using GPS data downloaded onto digital maps using Map Source^{R} in ARC GIS.

The planting has been subdivided into 10m x 10m plots and the number of infected trees within each block will be determined over time. The distribution of infection will be determined using a goodness-of-fit to Poisson random distribution at the different times throughout the sampling period. Disease gradient data will also be examined to study spread patterns within the planting. Rates of increase in disease incidence within the planting and severity of *P. psidii* infection will be examined at the completion of the study.

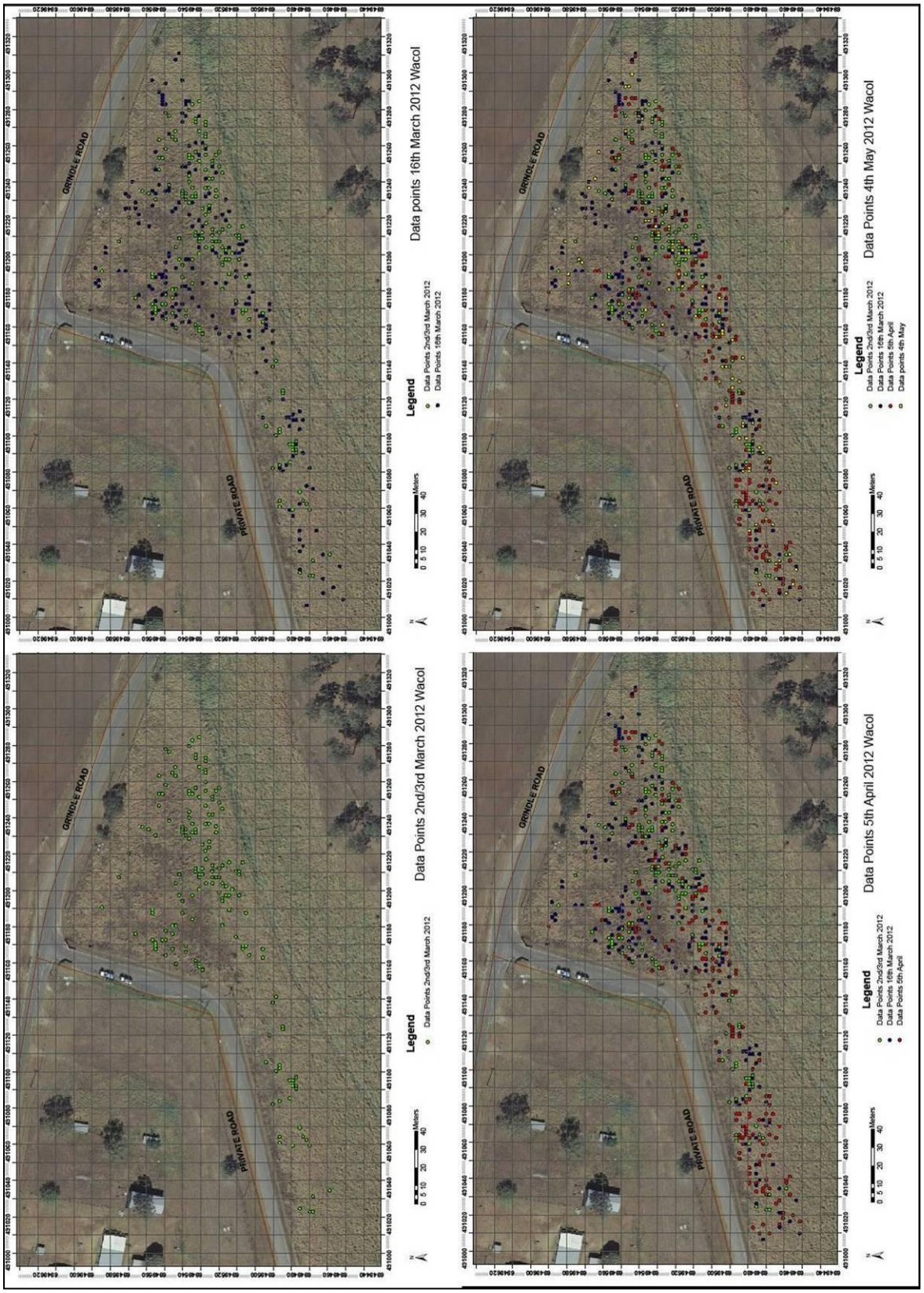

Figure 21. Spread of myrtle rust within a *Melaleuca quinquenervia* rehabilitation site at Wacol in Queensland (Pegg et al. unpublished).

3.3 Disease development - Regional

3.3.1 Methods

Survey data and a public reporting system developed by Biosecurity Queensland for myrtle rust has enabled disease data to be captured including:

- distribution data
- number of reports
- species reported
- reporting trends between regions

Climate data used has been collated from regional weather stations through the Bureau of Meteorology (BOM).

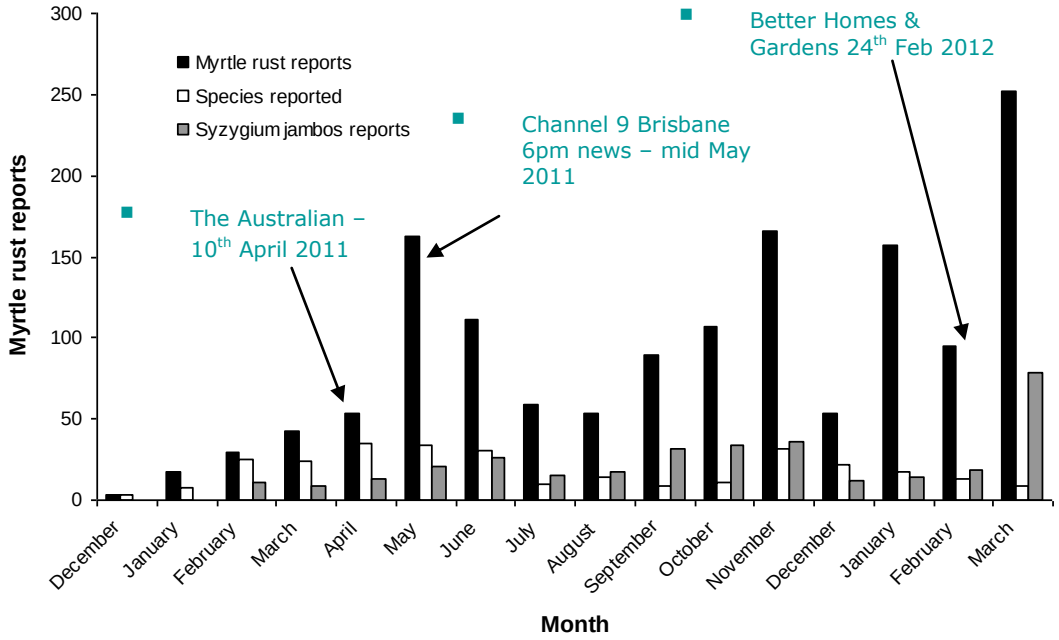

Figure 22. Reports of myrtle rust, the number of species detected and *Syzygium jambos* reports in Queensland from December 2010 when the disease was first detected through to March 2012 and associated major media releases (Pegg et al. unpublished).

3.3.2 Results to date

Based on the number of reports, peaks in myrtle rust detections occurred during the months of April, May, June of 2011 followed by a decline in the number of reports in July and August 2011. This was followed by another increase in reports beginning in August and peaking in November 2011 (Fig 22) followed by a decline in December 2011. In January 2012 a total of 157 reports of myrtle rust were received followed by a further 95 in February and a dramatic increase in reports in March 2012 with 252 positive reports of myrtle rust. In comparison only 43 reports of myrtle rust were made in March 2010, almost six times fewer.

The number of host species reported for each month increased with the increasing number of detections with the highest numbers occurring in April (35), May (34) and November (32) 2011 (Fig 22). *Syzygium jambos* was the most commonly reported host species in all months except December 2010, January 2011 and January 2012 with the highest number of reports, 79, occurring in March 2012.

The influence of media releases on the number of reports cannot be overlooked when studying climatic factors influencing disease levels. Some peaks in disease reports appear to link to major media releases including nightly news bulletins in Brisbane and a storied aired nationally on Better Homes and Gardens in late February 2012.

Reporting from the council areas in south east Queensland including Gold Coast, Brisbane and the Sunshine Coast, followed similar patterns in January 2011 through to September 2011. All areas saw a decrease in disease reports in June and July of 2011 followed by an increase in August 2011. However, in the Gold Coast council region reporting of myrtle rust remained comparatively low from September 2011 through to February 2012. However, there was a large increase in reports from the Gold Coast area in March 2012. On the Sunshine Coast reports declined in March 2012 and were significantly lower than Brisbane and Gold Coast.

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4. Implications for stakeholders

Myrtle rust is a potentially devastating disease that will impact across government, industry and community, particularly in warm temperate, subtropical and tropical areas of Australia. End-users of this research include:

- a wide range of industries which rely on myrtaceous species (e.g. nursery and garden, lifestyle horticulture including cut flower, landscaping and arboriculture, hardwood timber production, zoos/wildlife exhibitors, bush food, plant oil and bee industries) and their end-users,

- governments charged with managing natural estates (e.g. national parks and world heritage areas) and amenity parklands (e.g. local governments), and

- the community (e.g. backyarders and bushwalking public).

This is the first study in Australia investigating the susceptibility of Myrtaceae to *P. psidii* under natural conditions and the impact of the disease on native ecosystems. Data collected on the susceptibility of host species has assisted in identifying key species and ecosystems most at risk. The host list and the reported susceptibility levels have also been utilised as follows:

- in nursery management plans and as a guide for production and retail nurseries,
- by local governments in planning new peri-urban and urban plantings and replacing highly susceptible species, and
- a reference to assist in surveys during the recent myrtle rust incursion management program within Victoria.

Impact assessments have also provided information on the susceptibility of already threatened species and preliminary data on species that may become threatened as a result of myrtle rust being introduced.

Identification of factors influencing disease spread, development and severity will be essential in the developing a disease forecasting system. This information can then be used to guide chemical control programs and prevent production and retail nurseries from having to continually apply expensive chemical to control the disease. Now that the disease has established in tropical, subtropical and temperate regions of Australia there is a need to examine differences in disease development and spread under these different conditions.

Species susceptibility and data from epidemiology studies will be beneficial in future impact modelling for areas where the disease has been detected in Australia as well as other regions yet to be affected such as Tasmania, Western Australia and the Northern Territory. Data collected may also be useful for the development of impact models for other countries yet to be affected by myrtle rust including countries in Asia and Africa.

Screening host populations for resistance will be essential for the industries relying on Myrtaceae, including production nurseries. Adoption of routine screening programs coupled with continued investigation for changes in pathogen populations over time. Screening for resistance could also benefit:

- local governments when establishing peri-urban and urban landscapes,
- selection of species or resistance within species for use in land/ecosystem rehabilitation sites, and
- international forest industries relying on eucalypts for sawlog and paper production through adoption of new resistant germplasm developed from screening programs in Australia.

5. Recommendations

This project only provides preliminary data on the host range, impact and disease epidemiology of myrtle rust. There is a need to continue gathering data and information on all aspects of host specificity, disease epidemiology and impact in different climatic zones within Australia. This is required before any disease management plan or disease forecasting system can be formulated.

Given the observed impacts of myrtle rust in Australia within the first two years since the disease was initially detected, this research is critical to minimise the impacts of this disease incursion on plant industries, the environment and communities in Australia.

6. Abbreviations/glossary

ABBREVIATION	FULL TITLE
CRCNPB	Cooperative Research Centre for National Plant Biosecurity
DAFF	Department of Agriculture Fisheries and Forestry

7. Plain English website summary

CRC project no:	CRC70186
Project title:	Understanding myrtle rust epidemiology and host specificity to determine disease impact in Australia
Project leader:	Dr Suzy Perry
Project team:	Dr Geoff Pegg, Dr Angus Carnegie, Dr Kylie Ireland, Dr Fiona Giblin
Research outcomes:	This research into the epidemiology and host specificity of myrtle rust will: • Help inform the development of disease forecasting systems to assist in fungicide management systems for plant industries reliant on species of Myrtaceae. • Provide important baseline information to help gain an understanding of environments most threatened by myrtle rust allowing for development of strategic disease management programs. • Provide information on the strain of *P. psidii* present in Australia, thus improving our capabilities to detect new strains of the rust. • Provide information on host resistance mechanisms for the development of rapid resistance screening methods. • Provide crucial data for more accurate predictive modelling systems for future monitoring and impact

	studies for states and territories where the disease has yet to spread, and other countries where myrtle rust has not yet been detected. • Provide data for predicting impact levels in relation to future climate patterns within states of Australia already impacted by the disease.
Research implications:	The information and knowledge generated within this project provides the basis for other areas of research and development on this disease e.g. surveillance, population genetics, resistance breeding and monitoring impacts.
Research publications:	Research publications are in preparation, however are not yet complete given the short time frame of this project.
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