



***Puccinia psidii* in Queensland, Australia: disease symptoms, distribution and impact**

G. S. Pegg^{ab*}, F. R. Giblin^b, A. R. McTaggart^c, G. P. Guymer^d, H. Taylor^e,
K. B. Ireland^e, R. G. Shivas^f and S. Perry^e

^aDepartment of Agriculture, Fisheries and Forestry, Horticulture and Forestry Science, Agri-Science Queensland, GPO Box 267, Brisbane, Qld 4001; ^bForest Industries Research Centre, University of the Sunshine Coast, Locked Bag 4, Maroochydore DC, Qld 4558;

^cQueensland Alliance for Agriculture and Food Innovation, The University of Queensland, Ecosciences Precinct, GPO Box 267, Brisbane, Qld 4001; ^dDepartment of Science, Information Technology, Innovation and the Arts, Queensland Herbarium, Brisbane Botanic Gardens Mt Coot-tha, Mt Coot-tha Road, Toowong, Qld 4066; ^eDepartment of Agriculture, Fisheries and Forestry, Plant Biosecurity and Product Integrity, Biosecurity Queensland, GPO Box 267, Brisbane, Qld 4001; and ^fDepartment of Agriculture, Fisheries and Forestry, Plant Pathology Herbarium, Biosecurity Queensland, GPO Box 267, Brisbane, Qld 4001, Australia

Puccinia psidii has long been considered a significant threat to Australian plant industries and ecosystems. In April 2010, *P. psidii* was detected for the first time in Australia on the central coast of New South Wales (NSW). The fungus spread rapidly along the east coast and in December 2010 was found in Queensland (Qld) followed by Victoria a year later. *Puccinia psidii* was initially restricted to the southeastern part of Qld but spread as far north as Mossman. In Qld, 48 species of Myrtaceae are considered highly or extremely susceptible to the disease. The impact of *P. psidii* on individual trees and shrubs has ranged from minor leaf spots, foliage, stem and branch dieback to reduced fecundity. Tree death, as a result of repeated infection, has been recorded for *Rhodomyrtus psidioides*. Rust infection has also been recorded on flower buds, flowers and fruits of 28 host species. Morphological and molecular characteristics were used to confirm the identification of *P. psidii* from a range of Myrtaceae in Qld and compared with isolates from NSW and overseas. A reconstructed phylogeny based on the LSU and SSU regions of rDNA did not resolve the familial placement of *P. psidii*, but indicated that it does not belong to the Pucciniaceae. *Uredo rangelii* was found to be con-specific with all isolates of *P. psidii* in morphology, ITS and LSU sequence data, and host range.

Keywords: eucalyptus rust, guava rust, Myrtaceae, myrtle rust, *Puccinia psidii*, systematics

Introduction

Puccinia psidii was first described from *Psidium guajava* (guava) in Brazil in 1884 (Coutinho *et al.*, 1998), from which its common name guava rust was derived. The disease has since been reported from a range of plant species in the Myrtaceae in South and Central America as well as the United States (Florida and California; Coutinho *et al.*, 1998). More recently, *P. psidii* has been reported outside of the Americas, with detections in Hawaii (Uchida *et al.*, 2006), Japan (Kawanishi *et al.*, 2009), China (Zhuang & Wei, 2011) and South Africa (Roux *et al.*, 2013).

Historically, *P. psidii* has had a significant impact on industries reliant on Myrtaceae, including the all-spice (*Pimenta dioica*) industry in Jamaica (MacLachlan, 1938) and the eucalypt plantation industry in Brazil (Ferreira, 1983; Glen *et al.*, 2007). In the 1970s, the disease earned a new common name of eucalyptus rust, because of the severe damage caused to eucalypt

plantations grown for paper and pulp production in Brazil (Coutinho *et al.*, 1998).

For many years, *P. psidii* has been considered a significant threat to Australian plant industries and ecosystems (Grgurinovic *et al.*, 2006; Glen *et al.*, 2007), and strict biosecurity measures were implemented to prevent its introduction. In April 2010, *P. psidii* was identified for the first time in Australia on the central coast of New South Wales (NSW) (Carnegie *et al.*, 2010). Originally detected on *Agonis flexuosa*, *Melaleuca viminalis* (*Callistemon viminalis*) and *Syncarpia glomulifera* (Carnegie *et al.*, 2010), the host range rapidly increased as the rust fungus spread within Australia. Carnegie & Lidbetter (2012) reported the host range of *P. psidii*, from natural infections in Australia, as 107 species from 30 genera of Myrtaceae.

The geographic distribution of *P. psidii* has expanded rapidly since it was first detected in December 2010 at a retail nursery in Brisbane, Queensland (Qld). This paper discusses the spread and the impact of *P. psidii* on host species in Qld and implications for natural ecosystems and commercial operations. In addition, new host records are identified and the systematics of *P. psidii* is discussed in the light of morphological and molecular data.

*E-mail: geoff.pegg@daff.qld.gov.au

Materials and methods

Distribution and spread

To determine the distribution and spread of *P. psidii* following its initial detection in Qld, surveillance was conducted in nurseries (retail, production and wholesale), parks, gardens and natural bushland areas. Initial surveys focused in and around infected premises but were extended as the number of detections and host records increased. During the initial stages of the incursion, samples were collected for all suspect reports for disease confirmation in the laboratory. As the disease became more widespread, samples were only collected from new host species and/or new geographical locations.

To track disease spread and identify new hosts, a public reporting system for *P. psidii* was implemented. Samples from reports of new hosts or locations were collected for botanical confirmation before infected specimens were deposited in the Qld Plant Pathology Herbarium (BRIP). The location of infected plants in native vegetation and home gardens was recorded using a Global Positioning System (GPS; Garmin 76 Series). All data were mapped by GIS mapping systems (ARCGIS v. 10.0; ESRI). Maps were generated monthly to show changes in disease distribution. The database system BioSIRT (Biosecurity Surveillance, Incident Response and Tracing) was used as the primary repository for data relating to *P. psidii* detections in Qld. Surveys and public reports were recorded and located spatially in BioSIRT.

Host range and diagnostics

To determine the host range of *P. psidii* following the initial detection of the disease in Qld, inspections of retail, wholesale and production nurseries were conducted in addition to surveillance in parks, gardens and natural bushland areas. Samples were pressed and dried prior to examination by a botanist to confirm the host species. Host range data were also captured through the public reporting system, including information on the host species, as well as severity of rust symptoms, assessed from digital photographs. Samples were deposited in BRIP after *P. psidii* was confirmed. Reports without photographs of disease symptoms and host were recorded as suspect but were not included as a confirmed report.

Identification of *P. psidii* was through a combined morphological and molecular barcoding approach with the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA; Schoch *et al.*, 2012). Specimens of *P. psidii* in Qld were compared to those collected in NSW and from overseas. All samples were examined under the light microscope for sori and characteristic spores. Slides of sori and spores were examined at $\times 400$ for the presence of urediniospores, teliospores and basidiospores. Urediniospores were examined under oil immersion at $\times 1000$ and by scanning electron microscopy as described by Pegg *et al.* (2008).

Uredinia and telia were selectively removed from fresh leaf material with a vacuum pump and stored in DNA extraction buffer. DNA was extracted according to the protocol outlined by Aime (2006) using the UltraClean Plant DNA Isolation kit (MoBio Laboratories). The ITS region was amplified with ITS1F/ITS4B (Gardes & Bruns, 1993). The ITS2-large subunit (LSU) region was amplified with Rust2inv (Aime, 2006)/LR7 (Vilgalys & Hester, 1990) and nested with LROR/LR6 (Vilgalys & Hester, 1990) according to the protocol by Aime (2006). The small subunit (SSU) region was amplified with NS1 (White *et al.*, 1990)/Rust 18S-R (Aime, 2006). Amplification of the LSU by nested PCR required an initial denaturation of 3 min at

94°C; 42 cycles of 30 s at 94°C, 1 min at 58°C and 1.5 min at 72°C; with a final extension for 7 min at 72°C. The nested SSU protocol was identical, except for annealing at 63°C for 1 min.

The LSU and SSU sequences for specimens of *P. psidii* were added to the data set of Minnis *et al.* (2012). *Prosopidium tuberculatum* was included in the data set to increase sampling of the Uropyxidaceae. Maximum likelihood was implemented as a search criterion in RAxML (Stamatakis, 2006) and PHYML v. 3.0 (Guindon *et al.*, 2010). GTRGAMMA was specified as the model of evolution in both programs. The RAxML analyses were run with a rapid bootstrap analysis (command -f a) using a random starting tree and 1000 maximum likelihood bootstrap replicates. The PHYML analyses were implemented using the ATGC bioinformatics platform (available at: <http://www.atgcmontpellier.fr/phyml/>), with SPR tree improvement, and support obtained from an approximate likelihood ratio test (Anisimova *et al.*, 2011). MRBAYES was used for a Markov chain Monte Carlo (MCMC) search in a Bayesian analysis (Ronquist & Huelsenbeck, 2003). A user-defined tree obtained from the maximum likelihood analyses was used as a starting point. Four runs, each consisting of four chains, were implemented for 5 000 000 generations. The cold chain was heated at a temperature of 0.25. Substitution model parameters were sampled every 5000 generations and trees were saved every 5000 generations. Convergence of the Bayesian analysis was confirmed using AWTY (available at: ceb.csit.fsu.edu/awty/; Nylander *et al.*, 2008) and used to calculate a burn-in.

Symptoms and impact

Targeted surveys were conducted to determine susceptibility of host species to *P. psidii*. These surveys were conducted in public parks and surrounding bushland, private gardens and arboreta in Tallebudgera Valley and Cooroy on the Sunshine Coast, natural bushland in Brisbane, the Gold and Sunshine Coasts and surrounding suburbs, and botanical gardens in Mackay, the Gold and Sunshine Coasts and Brisbane (Mt Coot-tha). National parks surveyed included Lamington (Green Mountain) and Springbrook in the Gold Coast hinterland, Kondalilla in the Sunshine Coast hinterland and Kuranda, Herberton Range and Crater Lakes (Lake Eacham) in the Wet Tropics of far north Qld.

A disease rating system was developed to record species susceptibility. Host plants, including seedlings, saplings and mature trees, showing evidence of infection by *P. psidii*, were rated for susceptibility with the following scale (Fig. 1):

Relatively tolerant: minor leaf spots with rust sori on <10% of expanding leaves and shoots, limited sori per infected leaf;
Moderately susceptible: rust sori present on 10–50% of expanding leaves and shoots, limited–multiple sori per infected leaf;

Highly susceptible: rust sori present on 50–80% of expanding leaves and shoots, evidence of rust on juvenile stems and older leaves, leaf and stem blighting and distortion, multiple sori per leaf/stem;

Extremely susceptible: rust sori present on all expanding leaves, shoots and juvenile stems; foliage dieback; evidence of stem and shoot dieback.

Results

Distribution and spread

In December 2010, following the first detection of *P. psidii* on *Gossia inophloia* in a retail nursery in southeast Qld, three additional nurseries were found to have infected



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

plants. At that time there was no evidence of infection in peri-urban landscapes or natural bushland. By the end of January 2011, *P. psidii* had been found at a further 19 locations in southeast Qld, including urban landscapes and natural bushland (Figs 2 & 3). The first detection of *P. psidii* on the Gold Coast occurred in February 2011. By June 2011, *P. psidii* had been found as far west as Toowoomba (127 km west of Brisbane, 27°58'S, 151°93'E) and by September 2011, north to Maryborough (260 km north of Brisbane, 25°32'S, 152°42'E; Fig. 2). By January 2012, *P. psidii* was detected in Bundaberg and Rockhampton, 370 km (24°51'S, 152°21'E) and 650 km (23°23'S, 150°30'E) north of Brisbane, respectively. Surveys in far north Qld failed to detect the disease until May 2012, when *P. psidii* was found in natural bushland near Cairns. By August 2012, additional detections extended from Townsville to Daintree National Park, c. 100 km north of Cairns (Fig. 3).

By the end of August 2012 there were more than 1000 public reports and detections of *P. psidii* in Qld. To date, *P. psidii* has been detected in coastal areas as far north as the Wet Tropics World Heritage Area (including Daintree, Kuranda, Barron Gorge, Crater Lakes and Hypipamee National Parks) as well as Herberton

Ranges. In far north Qld, *P. psidii* has also been detected in the drier regions on the Atherton Tablelands (including Tolga, Yungaburra and Mareeba). Apart from detections in plant nurseries, *P. psidii* has not been identified in areas west of the Great Dividing Range.

Based on data collected from southeast Qld, the number of reports of *P. psidii* peaked during April, May and June 2011 followed by a decline in July and August 2011. Reports began to increase again towards the end of August, peaking in November 2011 (Fig. 4), followed by another decline in December 2011. In January 2012, a total of 157 reports of *P. psidii* were received followed by a further 95 in February and a dramatic increase in reports in March 2012 with 252 reports. Comparatively fewer reports (43) were made in April 2012. Some of these report peaks coincided with media releases (Dayton & Higgins, 2011).

The number of host species reported each month increased as the number of detections increased. The highest diversity of species reported occurred in April (35), May (34) and November (32) of 2011 (Fig. 4). *Syzygium jambos* was the most commonly reported species in all months, with the highest number of reports for this species (79) occurring in March 2012. There were 82 new hosts

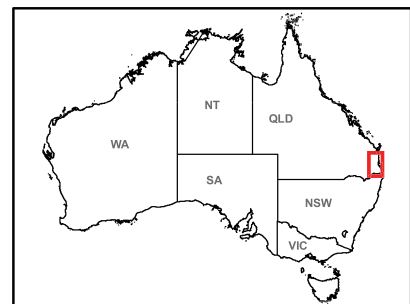
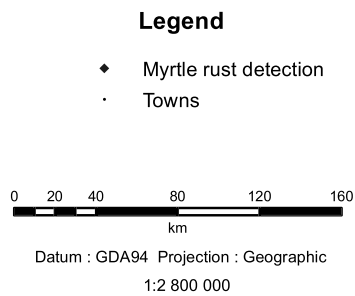
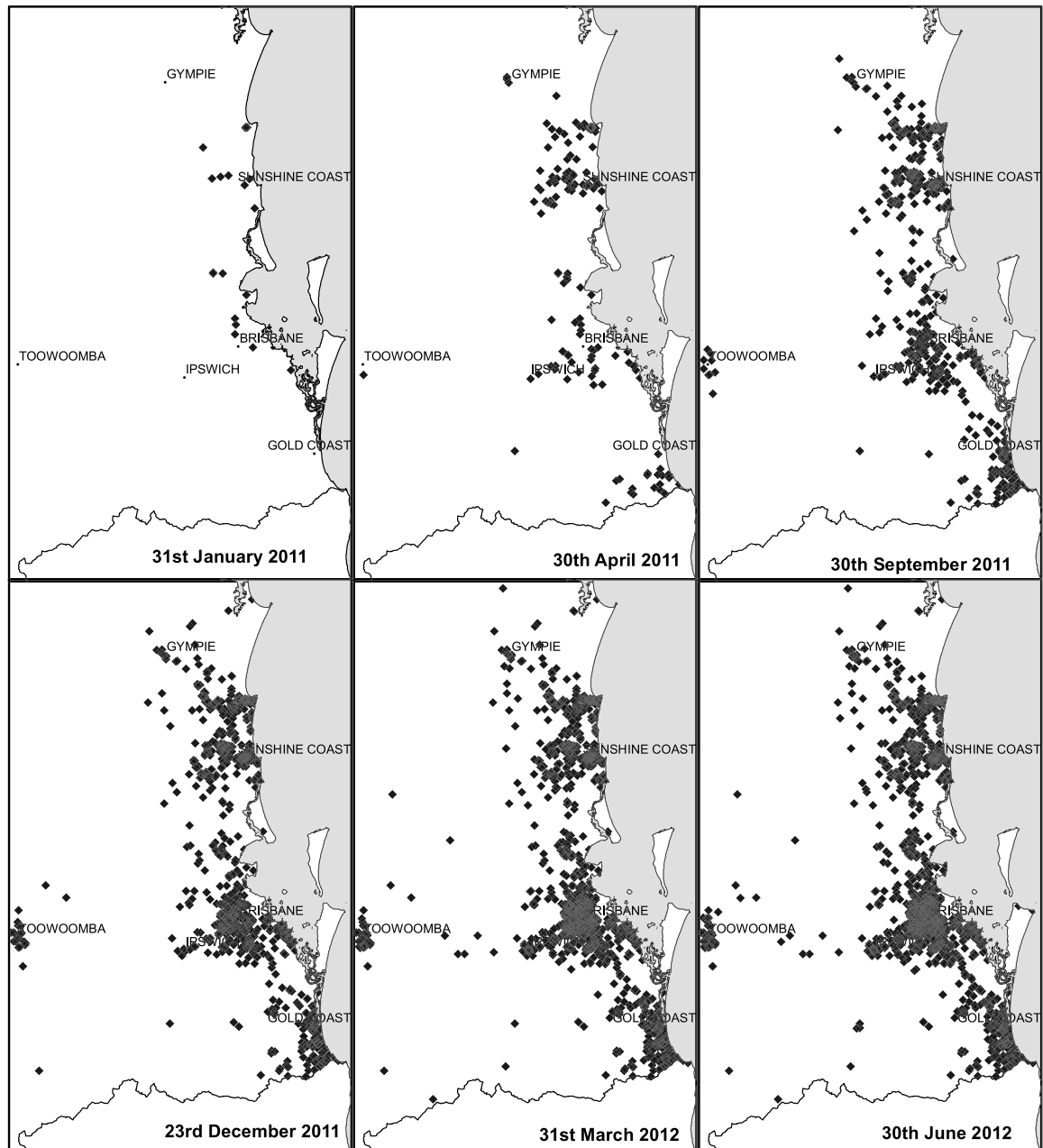


Figure 2 Map of southeast Queensland plotting the number of detections and public reports of *Puccinia psidii* within the first 12 months of initial detection.

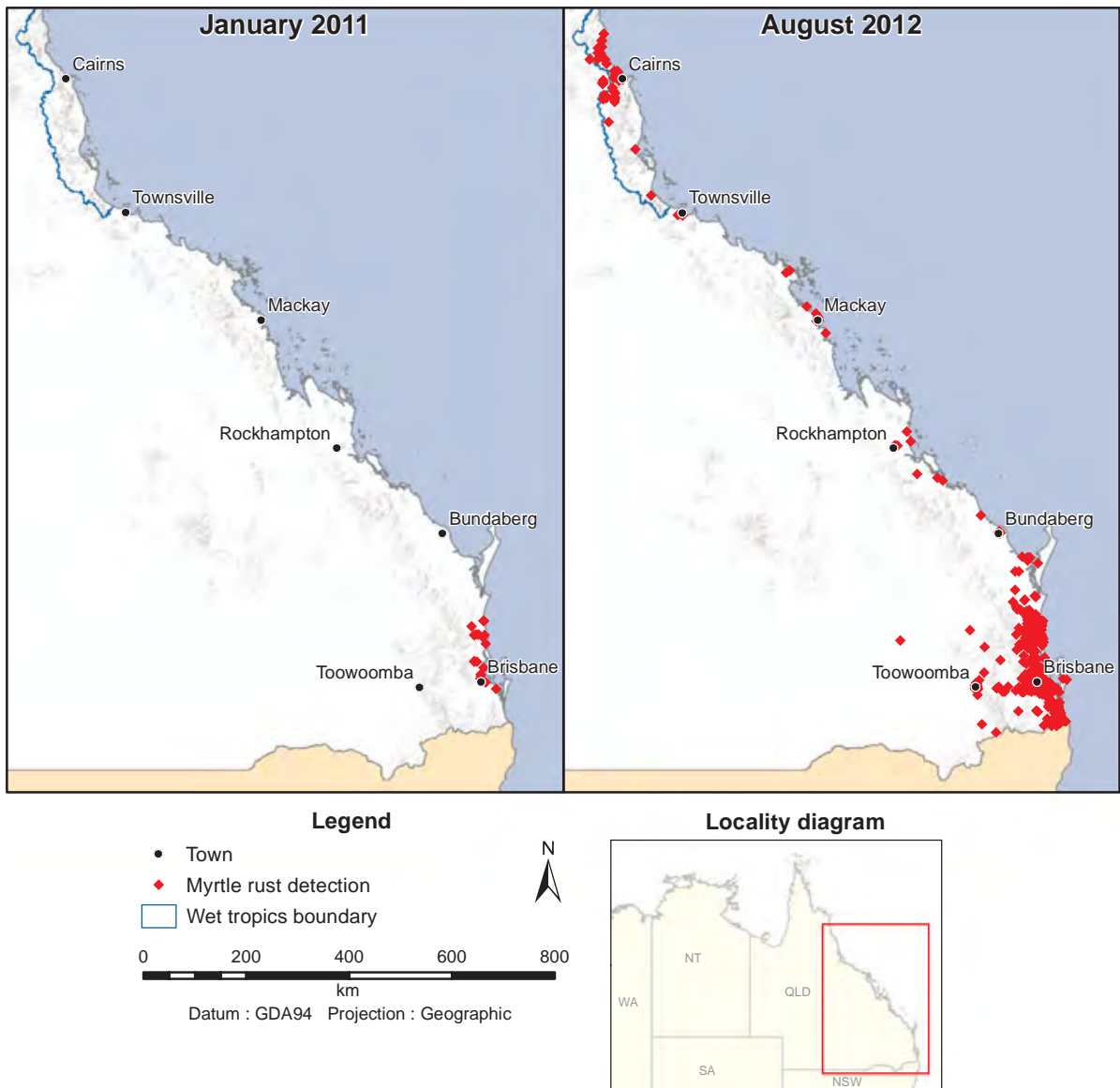


Figure 3 Changes in distribution of *Puccinia psidii* in Queensland from January 2011 to August 2012.

recorded within the first 6 months following the initial detection of *P. psidii* in December 2010 (Fig. 5). Only six new hosts were identified in the following 4-month period of July to October 2011. From November 2011 to February 2012 there was an increase in the number of species identified, with 37 new hosts recorded and a further 13 new hosts between March 2012 and July 2012. The majority (77%) of host species rated as highly or extremely susceptible were identified within 6 months of *P. psidii* being first detected. Since then, only a further eight species have been added to this category.

Host range

Since *P. psidii* was first detected in Qld, 165 species from 38 different genera have been identified as hosts

based on natural infections, the majority from the tribes Myrteae (31%) and Syzygieae (28%) (Table 1; Wilson *et al.*, 2005). New host records of *P. psidii* were found for 61 species in 22 genera from 11 tribes (Table 1), including *Acmena* (1 species), *Austromyrtus* (1), *Backhousia* (4), *Corymbia* (1), *Decaspermum* (1), *Eucalyptus* (3), *Eugenia* (2), *Gossia* (3), *Homoranthus* (3), *Hypocalymma* (1), *Leptospermum* (3), *Lophostemon* (1), *Melaleuca* (5), *Metrosideros* (2), *Pilidiostigma* (1), *Rhodamnia* (3), *Rhodomyrtus* (5), *Syzygium* (17), *Thryptomene* (1) and *Waterhousea* (1). These records also included two previously unreported host genera, *Mitrantia* (*Mitrantia bilocularis*) and *Sphaerantia* (*Sphaerantia discolor*). *Puccinia psidii* has not been recorded from common guava (*Psidium* spp.) in Qld despite its wide distribution and weed status. *Puccinia psidii* was

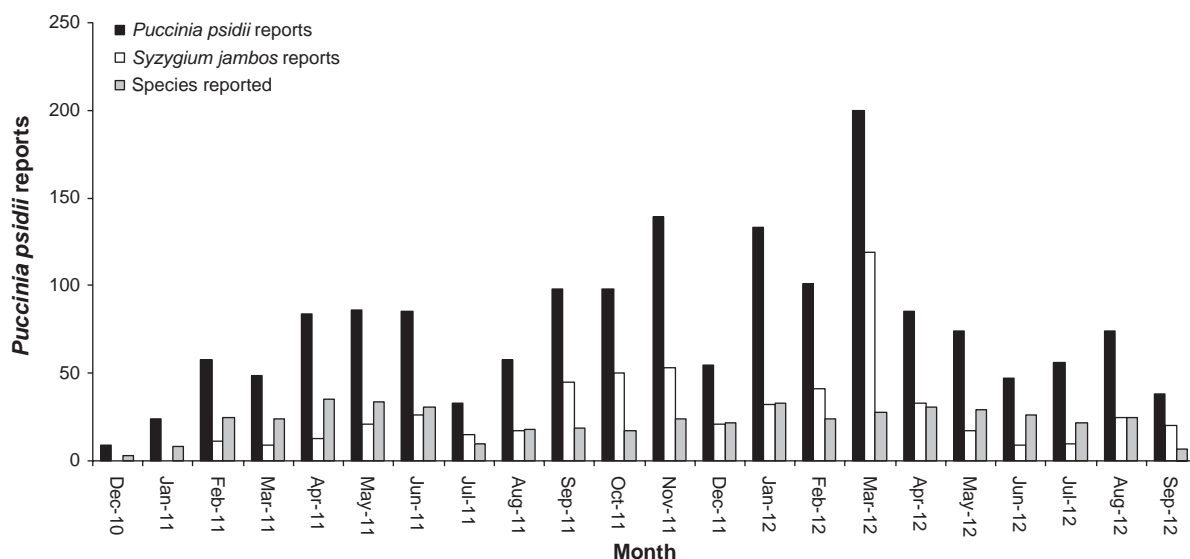


Figure 4 Number of new reports of *Puccinia psidii* in Queensland and the number of host species per month in comparison to the number of reports of infected *Syzygium jambos* from first detection in December 2010 to September 2012 when public reporting ceased.

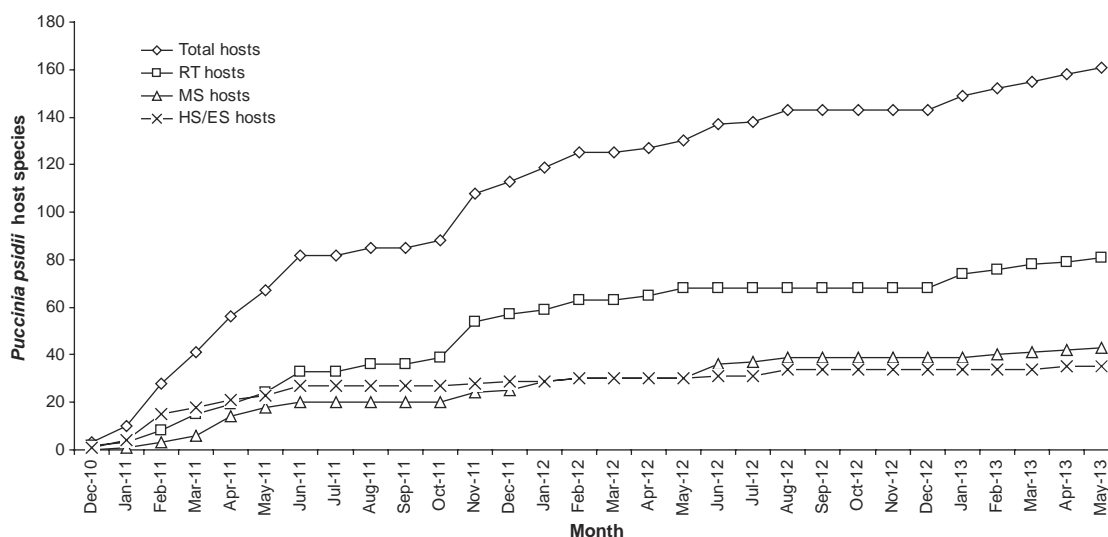


Figure 5 Cumulative number of host species since detection of *Puccinia psidii* in Queensland in December 2010.

confirmed from a single sample of *Psidium* sp. collected in northern NSW (*P. Entwistle*, NSW, Australia & G. S. Pegg, unpublished). Several genera and species were found free of disease symptoms at sites where *P. psidii* was detected (Table 2).

Sequence data

The ITS region was identical for isolates collected on 12 host genera (Table 3). A high identity was returned in a BLAST search to other sequences of *P. psidii* on GenBank from eight other genera within the Myrtaceae. Sequence trace chromatograms had three sites with single nucleotide polymorphisms. These sites were variable in sequences obtained from GenBank. The LSU and SSU

regions were identical for 16 and four isolates, respectively. The specimen collected on *Myrtus communis* (BRIP 58517), the type host of *Uredo rangelii*, was molecularly identical to all other isolates of *P. psidii* in the ITS and LSU regions.

Phylogenetic analysis

Puccinia psidii was recovered as a rogue taxon in three separate phylogenetic analyses on the combined LSU-SSU data set. It did not have a well-supported relationship with any rust family. It was sister to the Pucciniaceae in RAXML and Bayesian inference, or in a clade with members from the Pileolariaceae and Uropyxidaceae reconstructed in PHYML (Fig. 6).

Table 1 Current known host list of *Puccinia psidii* in Queensland, Australia and susceptibility level

Host name	New host record	Tribe ^a	Disease susceptibility rating ^b	Flower/fruit infection	Rust spore type ^c
<i>Acmena hemilampra</i>		Syzygieae	RT		II
<i>Acmena ingens</i>	x	Syzygieae	RT		II
<i>Acmena smithii</i>		Syzygieae	RT-MS	x	II
<i>Acmenosperma claviflorum</i>		Syzygieae	MS		II III
<i>Agonis flexuosa</i>		Leptospermeae	ES		II III
<i>Anetholea (Backhousia) anisata</i>		Backhousieae	RT-HS		II III
<i>Asteromyrtus brassii</i>		Leptospermeae	RT		II
<i>Austromyrtus dulcis</i>		Myrteae	RT-HS	x	II
<i>Austromyrtus</i> sp. (Lockerbie Scrub)	x	Myrteae	RT		II
<i>Austromyrtus tenuifolia</i>		Myrteae	RT		II
<i>Backhousia angustifolia</i>		Backhousieae	RT		II
<i>Backhousia bancroftii</i>	x	Backhousieae	RT		II
<i>Backhousia citriodora</i>		Backhousieae	MS-HS	x	II III
<i>Backhousia gundara</i> (Prince Regent)	x	Backhousieae	RT		II
<i>Backhousia hughesii</i>	x	Backhousieae	MS		II
<i>Backhousia leptopetala</i>		Backhousieae	HS		II III
<i>Backhousia myrtifolia</i>		Backhousieae	MS		II
<i>Backhousia oligantha</i>	x	Backhousieae	HS		II
<i>Backhousia sciadophora</i>		Backhousieae	RT		II
<i>Backhousia subargentea</i>		Backhousieae	RT		II
<i>Chamelaucium uncinatum</i>		Chamelaucieae	ES	x	II
<i>Corymbia citriodora</i> subsp. <i>variegata</i> ^d		Eucalypteae	RT		II
<i>Corymbia ficifolia</i> × <i>C. ptychocarpa</i> ^d	x	Eucalypteae	RT		II
<i>Corymbia henryi</i> ^d		Eucalypteae	RT		II
<i>Corymbia torelliana</i> ^d		Eucalypteae	RT		II
<i>Darwinia citriodora</i>		Chamelaucieae	MS		II III
<i>Decaspermum humile</i>		Myrteae	ES		II III
<i>Decaspermum humile</i> (North Qld form)	x	Myrteae	RT		II
<i>Eucalyptus carnea</i>	x	Eucalypteae	RT-HS		II
<i>Eucalyptus cloeziana</i> ^d		Eucalypteae	RT		II
<i>Eucalyptus curtisii</i>	x	Eucalypteae	RT-HS		II
<i>Eucalyptus grandis</i>		Eucalypteae	RT-MS		II
<i>Eucalyptus planchoniana</i> ^d	x	Eucalypteae	RT-MS		II
<i>Eucalyptus tereticornis</i> ^d		Eucalypteae	RT		II
<i>Eucalyptus tindaliae</i> ^d		Eucalypteae	MS		II
<i>Eugenia natalitia</i>	x	Myrteae	MS		II
<i>Eugenia reinwardtiana</i>		Myrteae	ES	x	II III
<i>Eugenia uniflora</i>		Myrteae	MS	x	II
<i>Eugenia zeyheri</i> ^d	x	Myrteae	MS		II
<i>Gossia acmenoides</i>		Myrteae	HS		II III
<i>Gossia bamagensis</i>	x	Myrteae	RT		II
<i>Gossia bidwillii</i>		Myrteae	RT		II
<i>Gossia floribunda</i>		Myrteae	RT		II
<i>Gossia fragrantissima</i>		Myrteae	MS		II
<i>Gossia gonoclada</i>		Myrteae	HS		II
<i>Gossia hillii</i>		Myrteae	HS-ES		II III
<i>Gossia inophloia</i>		Myrteae	ES		II III
<i>Gossia lewisensis</i>	x	Myrteae	MS-HS		II
<i>Gossia macilwraithensis</i>		Myrteae	MS		II III
<i>Gossia myrsinocarpa</i>	x	Myrteae	MS-HS	x	II
<i>Gossia punctata</i>		Myrteae	MS		II III
<i>Homoranthus melanostictus</i>	x	Chamelaucieae	MS		II
<i>Homoranthus papillatus</i>	x	Chamelaucieae	MS		II
<i>Homoranthus virgatus</i>	x	Chamelaucieae	MS	x	II
<i>Hypocalymma angustifolium</i>	x	Chamelaucieae	RT		II
<i>Lenwebbia lasioclada</i>		Myrteae	RT		II
<i>Lenwebbia prominens</i>		Myrteae	HS	x	II III
<i>Lenwebbia</i> sp. Blackall Range		Myrteae	RT		II

(continued)

Table 1 (continued)

Host name	New host record	Tribe ^a	Disease susceptibility rating ^b	Flower/fruit infection	Rust spore type ^c
<i>Leptospermum liversidgei</i>	x	Leptospermeae	MS		II
<i>Leptospermum luehmannii</i>		Leptospermeae	RT		II
<i>Leptospermum madidum</i>	x	Leptospermeae	MS		II
<i>Leptospermum petersonii</i>		Leptospermeae	RT		II III
<i>Leptospermum semibaccatum</i> ^d	x	Leptospermeae	RT		II
<i>Leptospermum trinervium</i>		Leptospermeae	MS		II
<i>Lindsayomyrtus racemoides</i>		Lindsayomyrteae	RT		II III
<i>Lophostemon suaveolens</i>	x	Lophostemoneae	RT		II
<i>Melaleuca fluviatilis</i>		Melaleuceae	HS		II
<i>Melaleuca formosa</i>	x	Melaleuceae	RT		II
<i>Melaleuca leucadendra</i>		Melaleuceae	RT-HS	x	II
<i>Melaleuca linariifolia</i>		Melaleuceae	RT		II
<i>Melaleuca nervosa</i>	x	Melaleuceae	HS		II
<i>Melaleuca nesophila</i>		Melaleuceae	RT		II
<i>Melaleuca nodosa</i>		Melaleuceae	HS-ES		II
<i>Melaleuca pachyphylla</i>		Melaleuceae	RT		II
<i>Melaleuca paludicola</i>	x	Melaleuceae	HS		II
<i>Melaleuca polandii</i>		Melaleuceae	HS		II
<i>Melaleuca quinquenervia</i>		Melaleuceae	RT-ES	x	II III
<i>Melaleuca salicina</i>	x	Melaleuceae	RT		II III
<i>Melaleuca saligna</i>		Melaleuceae	MS		II
<i>Melaleuca viminalis</i>		Melaleuceae	MS-HS		II III
<i>Melaleuca viridiflora</i>	x	Melaleuceae	HS		II III
<i>Metrosideros collina</i>		Metrosidereae	RT		II III
<i>Metrosideros collina</i> × <i>villosa</i>	x	Metrosidereae	RT		II
<i>Metrosideros kermadecensis</i>		Metrosidereae	RT		II
<i>Metrosideros thomasi</i>	x	Metrosidereae	RT		II III
<i>Mitranthia bilocularis</i>	x	Kanieae	MS		II
<i>Myrciaria cauliflora</i>		Myrteae	RT		II
<i>Myrtus communis</i>		Myrteae	MS-HS	x	II III
<i>Piliodiostigma glabrum</i>		Myrteae	RT-MS	x	II III
<i>Piliodiostigma tetramerum</i>	x	Myrteae	MS		II III
<i>Rhodamnia acuminata</i>	x	Myrteae	RT		II
<i>Rhodamnia angustifolia</i>		Myrteae	ES	x	II
<i>Rhodamnia arenaria</i>		Myrteae	MS	x	II III
<i>Rhodamnia argentea</i>		Myrteae	MS-HS		II
<i>Rhodamnia australis</i>	x	Myrteae	HS	x	II
<i>Rhodamnia blairiana</i>	x	Myrteae	RT-MS		II
<i>Rhodamnia costata</i>		Myrteae	HS		II
<i>Rhodamnia dunicola</i>		Myrteae	HS		II
<i>Rhodamnia glabrescens</i>		Myrteae	MS		II
<i>Rhodamnia maideniana</i>		Myrteae	ES	x	II III
<i>Rhodamnia pauciovulata</i>		Myrteae	MS		II III
<i>Rhodamnia rubescens</i>		Myrteae	HS-ES	x	II III
<i>Rhodamnia sessiliflora</i>		Myrteae	MS-ES	x	II III
<i>Rhodamnia spongiosa</i>		Myrteae	HS	x	II III
<i>Rhodomyrtus canescens</i>	x	Myrteae	HS	x	II III
<i>Rhodomyrtus effusa</i>	x	Myrteae	MS		II
<i>Rhodomyrtus macrocarpa</i>	x	Myrteae	MS		II III
<i>Rhodomyrtus pervagata</i>	x	Myrteae	MS-HS	x	II III
<i>Rhodomyrtus psidioides</i>		Myrteae	ES	x	II III
<i>Rhodomyrtus sericea</i>	x	Myrteae	MS		II
<i>Rhodomyrtus tomentosa</i>		Myrteae	MS-HS	x	II
<i>Rhodomyrtus trineura</i> subsp. <i>capensis</i>		Myrteae	MS		II III
<i>Ristantia waterhousei</i>		Kanieae	RT		II
<i>Sphaerantia discolor</i>	x	Kanieae	MS		II
<i>Stockwellia quadrifida</i>		Eucalypteae	HS		II

(continued)

Table 1 (continued)

Host name	New host record	Tribe ^a	Disease susceptibility rating ^b	Flower/fruit infection	Rust spore type ^c
<i>Syzygium angophoroides</i>	×	Syzygieae	MS		II
<i>Syzygium apodophyllum</i>	×	Syzygieae	RT		II
<i>Syzygium aqueum</i>	×	Syzygieae	RT		II
<i>Syzygium argyropedicum</i>		Syzygieae	RT		II
<i>Syzygium armstrongii</i>		Syzygieae	RT		II III
<i>Syzygium australe</i>		Syzygieae	RT-MS	×	II III
<i>Syzygium bamagense</i>		Syzygieae	MS		II III
<i>Syzygium banksii</i>	×	Syzygieae	MS		II
<i>Syzygium boonjee</i>	×	Syzygieae	RT		II
<i>Syzygium canicortex</i>		Syzygieae	RT		II III
<i>Syzygium cormiflorum</i>		Syzygieae	RT		II III
<i>Syzygium corynanthum</i>		Syzygieae	RT		II
<i>Syzygium cryptophlebium</i>	×	Syzygieae	MS		II
<i>Syzygium cumini</i>		Syzygieae	MS		II
<i>Syzygium dansiei</i>	×	Syzygieae	RT		II
<i>Syzygium endophloium</i>	×	Syzygieae	RT		II
<i>Syzygium erythrocalyx</i>	×	Syzygieae	RT		II
<i>Syzygium eucalyptoides</i>		Syzygieae	HS		II
<i>Syzygium eucalyptoides</i> subsp. <i>eucalyptoides</i>	×	Syzygieae	MS		II III
<i>Syzygium forte</i> subsp. <i>forte</i>	×	Syzygieae	RT		II
<i>Syzygium forte</i> subsp. <i>potamophilum</i>	×	Syzygieae	RT		II III
<i>Syzygium jambos</i>		Syzygieae	ES	×	II III
<i>Syzygium kuranda</i>	×	Syzygieae	MS		II
<i>Syzygium luehmannii</i>		Syzygieae	MS		II III
<i>Syzygium luehmannii</i> × <i>S. wilsonii</i>		Syzygieae	RT		II III
<i>Syzygium macilwraithianum</i>	×	Syzygieae	RT		II
<i>Syzygium minutiflorum</i>	×	Syzygieae	RT		II
<i>Syzygium moorei</i>		Syzygieae	RT		II
<i>Syzygium nervosum</i>	×	Syzygieae	HS	×	II
<i>Syzygium oleosum</i>		Syzygieae	HS		II
<i>Syzygium paniculatum</i>		Syzygieae	RT		II
<i>Syzygium pseudofastigiatum</i>		Syzygieae	RT		II
<i>Syzygium puberulum</i>		Syzygieae	MS		II III
<i>Syzygium rubrimolle</i>		Syzygieae	RT		II
<i>Syzygium suborbiculare</i>	×	Syzygieae	MS		II
<i>Syzygium tierneyanum</i>		Syzygieae	RT		II
<i>Syzygium wilsonii</i>		Syzygieae	RT		II
<i>Syzygium xerampelinum</i>		Syzygieae	MS		II
<i>Thryptomene saxicola</i>	×	Chamelauceae	RT-MS	×	II III
<i>Tristania neriifolia</i>		Tristanieae	MS		II
<i>Tristaniopsis exilliflora</i>		Kanieae	HS		II
<i>Tristaniopsis laurina</i>		Kanieae	RT		II
<i>Uromyrtus tenella</i>		Myrteae	RT		II
<i>Waterhousea floribunda</i>		Syzygieae	RT		II
<i>Waterhousea hedraiophylla</i>		Syzygieae	RT		II
<i>Waterhousea mulgraveana</i>		Syzygieae	RT		II III
<i>Waterhousea unipunctata</i>	×	Syzygieae	MS		II
<i>Xanthostemon chrysanthus</i>		Xanthostemoneae	RT-MS		II
<i>Xanthostemon oppositifolius</i>		Xanthostemoneae	HS		II
<i>Xanthostemon youngii</i>		Xanthostemoneae	MS	×	II III

^aTribes according to Wilson *et al.*, 2005.

^bRT, relatively tolerant, restricted leaf spot or spots only; MS, moderate susceptibility, blight symptoms on new shoots and expanding foliage; HS, high susceptibility, blight symptoms on new shoots and expanding foliage and juvenile stems; ES, extreme susceptibility, death of new shoots and severe blighting on all foliage types, shoot and stem dieback. Susceptibility ratings are based on observations to date.

^cII, urediniospore; III, teliospore.

^d*Puccinia psidii* identified from seedlings only.

Table 2 Host species assessed and identified as free of disease at sites where *Puccinia psidii* was detected

<i>Acmena resa</i>
<i>Acmena</i> Normanby River
<i>Allosyncarpia ternata</i>
<i>Archirhodomyrtus beckleri</i>
<i>Eugenia aggregata</i>
<i>Eugenia luschnathiana</i>
<i>Lophostemon confertus</i>
<i>Lophostemon grandiflorus</i> subsp. <i>riparius</i>
<i>Melaleuca cheelii</i>
<i>Myrciaria edulis</i>
<i>Myrciaria glomerata</i>
<i>Psidium guajava</i>
<i>Psidium littorale</i> Raddi var. <i>littorale</i>
<i>Syncarpia glomulifera</i> subsp. <i>glomulifera</i>
<i>Syzygium alatoramulum</i>
<i>Syzygium alliiignum</i>
<i>Syzygium branderhorstii</i>
<i>Syzygium johnsonii</i>
<i>Syzygium malaccense</i>
<i>Syzygium papyraceum</i>
<i>Syzygium sayeri</i>
<i>Syzygium velae</i>
<i>Syzygium wilsonii</i> subsp. <i>cryptophlebium</i>
<i>Thaleropia queenslandica</i>

Taxonomy

Isolates of *P. psidii* collected in Qld were identical morphologically and in DNA sequence data to those collected from NSW. Urediniospores were found to be morphologically plastic, ranging from globose to obpyriform in shape and with a broader size range than previously described (Table 3). The presence of a tonsure (smooth patch) on urediniospores was often observed, but its presence or absence was not consistent even in the same sorus. Teliospores were produced on a sample of *Myrtus communis* (BRIP 58517). A composite morphological description of *P. psidii* based on host samples from 11 genera collected in Qld follows:

Uredinia on chlorotic, red-purple or greyish leaf spots with a darker margin up to 1 mm diameter, amphigenous, mostly abaxial, subepidermal, erumpent, round, up to 500 μm , yellowish brown (Fig. 7).

Urediniospores globose, subglobose, ellipsoidal to ovoid, obpyriform, yellowish brown, 14–22 \times 15–26 μm ; wall 1.0–3.0 μm thick, finely echinulate, germ pore absent or inconspicuous (Fig. 7).

Telia on fruit, leaves or stems, up to 0.5 mm diameter, abaxial, erumpent, pulvinate, yellow to yellowish brown (Fig. 7).

Teliospores cylindrical to ellipsoidal, apex rounded, pale yellowish brown, 23–50 \times 14–28 μm ; wall 1.0–2.0 μm thick, smooth, 2-celled, remnant of pedicel remains attached up to 15 μm long (Fig. 7).

Basidia cylindrical, up to 110 μm long \times 6–8 μm wide, hyaline, 4-celled, produced from each cell of the teliospores, apically in upper cell and laterally in lower cell.

Basidiospores globose to pyriform, 8–11 μm diameter, hyaline, smooth, germinate *in situ* without dormancy from an apical germ pore.

Teliospores were identified from 98 samples (20% of total *P. psidii* samples), from 50 different host species (Table 1). Teliospores were commonly found in the autumn months of March, April and May in both 2011 and 2012 and June of 2012. In both years, this was followed by a decline in detections in July and August (winter months) with only urediniospores identified on samples collected during these months in 2011 and 2012.

Symptoms and impact

Symptoms of infection by *P. psidii* ranged from minor leaf spots to severe foliage and stem blight as well as infection on flowers and fruit of some species. Based on observations to date, 67 host species have been rated as having low susceptibility to *P. psidii*, with only a small percentage of leaves with 1–2 sori per leaf recorded (Table 1). A further 50 host species were considered moderately susceptible with higher numbers of sori per leaf and a greater percentage of expanding foliage and shoots infected.

Forty-eight species were rated as highly or extremely susceptible, with infection occurring on a high percentage of expanding leaves and shoots and evidence of shoot and stem dieback (Table 1). Highly or extremely susceptible species include the environmentally significant *Melaleuca quinquenervia*, and the rare and endangered species *Backhousia oligantha*, *Gossia gonoclada* and *Rhodammia angustifolia*.

To date in Qld *P. psidii* has been identified from 11 species of eucalypts (includes both *Eucalyptus* and *Corymbia*), mostly on seedlings and at low incidence and severity. On mature trees of *Eucalyptus curtisii*, which generally do not exceed 7 m in height, significant infection has been observed causing shoot and stem dieback and death of coppice growth from cut stems. Infection of leaves and stems of coppice from the base of a mature *Eucalyptus carnea* tree was recorded. Stem dieback, leaf blight and shoot death was recorded on *Eucalyptus planchoniana* seedlings, and leaf and shoot blight on *Eucalyptus grandis* saplings.

Variability in disease severity was identified within 26 host species (Table 1), indicating potential variation in susceptibility to infection by *P. psidii* infection. For *Melaleuca quinquenervia*, ratings on individuals ranged from resistant to low susceptibility, with no evidence of sori, to severe stem and shoot dieback as a result of repeat infection of growing tips. For some species, such as *Rhodomyrtus psidioides*, *Rhodammia angustifolia* and *Rhodammia maideniana*, all plants assessed across a range of sites were rated as extremely susceptible, with severe dieback on older trees as well as saplings and seedlings.

Changes in susceptibility on individual trees have also been observed over time, with increased disease susceptibility on *Acmena smithii* and *Syzygium nervosum*. Both

Table 3 Hosts, GenBank accession numbers and spore measurements of isolates used in this study. GenBank accession numbers in bold were obtained in this study

Isolate	Host	LSU	SSU	ITS	Urediniospore measurements
BRIP 58332	<i>Agonis flexuosa</i>	NA	NA	HM448900	16–21 × 18–25 μm, wall 1–2 μm, globose to obpyriform
	<i>Backhousia oligantha</i>	KF318436	NA	KF318421	
BRIP 58330	<i>Chamelaucium uncinatum</i>	KF318437	NA	KF318422	15–20 × 19–25 μm, wall 1–2 μm, globose to obpyriform
BRIP 57997	<i>Eucalyptus</i>	NA	NA	FJ710803–FJ710808	15–18 × 19–24 μm, wall 1–2 μm, globose to obpyriform
	<i>Eugenia reinwardtiana</i>	KF318438	NA	NA	
BRIP 58331	<i>Eugenia reinwardtiana</i>	KF318439	NA	KF318423	15–18 × 19–24 μm, wall 1–2 μm, globose to obpyriform
BRIP 58329	<i>Gossia myrsinocarpa</i>	KF318440	NA	KF318424	14–18 × 15–19 μm, wall 1–3 μm, globose to obpyriform
BRIP 58319	<i>Lenwebbia lasioclada</i>	KF318441	NA	KF318425	16–20 × 17–22 μm, wall 1–2 μm, globose to obpyriform
BRIP 58333	<i>Lenwebbia prominens</i>	KF318442	NA	KF318426	
BRIP 57991	<i>Melaleuca leucadendra</i>	KF318443	KF318455	KF318427	19–22 × 21–26 μm, wall 1–2 μm, globose to obpyriform
BRIP 57922	<i>Melaleuca quinquenervia</i>	KF318444	KF318456	NA	
BRIP 58164	<i>Metrosideros</i>	NA	NA	EU711421	15–20 × 17–21 μm, wall 1–2 μm
	<i>Mitranthia bilocularis</i>	KF318445	NA	KF318428	
BRIP 58328	<i>Mitranthia bilocularis</i>	KF318446	NA	KF318429	17–19 × 19–25 μm, wall 1–2 μm
BRIP 58517	<i>Myrciaria cauliflora</i>	NA	NA	KC543299–KC543317	15–21 × 16–23 μm, wall 1–2 μm, globose to obpyriform, teliospores present
	<i>Myrtus communis</i>	KF318447	NA	KF318430	
BRIP 58317	<i>Pilidiostigma glabrum</i>	KF318448	NA	KF318431	17–19 × 19–25 μm, wall 1–2 μm
BRIP 57793	<i>Rhodamnia angustifolia</i>	KF318449	KF318457	NA	15–20 × 17–21 μm, wall 1–2 μm
BRIP 58000	<i>Rhodamnia rubescens</i>	KF318450	NA	NA	
BRIP 58334	<i>Rhodomyrtus psidioides</i>	KF318451	NA	KF318432	15–20 × 18–23 μm, wall 1–2 μm, globose to obpyriform
BRIP 58315	<i>Sphaerantia discolor</i>	KF318452	NA	KF318433	
BRIP 57985	<i>Syzygium jambos</i>	NA	NA	KC543318–KC543330	15–20 × 18–23 μm, wall 1–2 μm, globose to obpyriform
	<i>Syzygium jambos</i>	KF318453	KF318458	KF318434	
BRIP 58335	<i>Syzygium nervosum</i>	KF318454	NA	KF318435	

species were originally rated as being of low susceptibility when *P. psidii* was first detected, but were later recorded as moderately or highly susceptible, respectively. There has not been any evidence of individual hosts showing lower disease susceptibility levels over time apart from trees recovering during extended periods of low or no rainfall, which do not favour disease development.

The impact of infection by *P. psidii* on individual trees and shrubs ranged from minor leaf spots through to reduced fecundity from loss of flowers and fruit, and even tree death. Foliage, stem and branch dieback has been observed on a range of hosts including *Chamelaucium uncinatum*, *Eugenia reinwardtiana*, *Gossia billii*, *Melaleuca quinquenervia*, *Rhodamnia dumicola*, *Rhodamnia maideniana* and *Rhodomyrtus psidioides*. Repeated infection of new shoots and young foliage of *Rhodamnia angustifolia* resulted in tree dieback and significant reduction in canopy density over time (Fig. 8). Branch death and dieback, as well as reduced shoot development on the entire tree, became evident 15 months after *P. psidii* was first detected. Tree death as a result of repeated infection has been recorded for *Rhodomyrtus psidioides*, with

regenerating seedlings of the same species killed by *P. psidii* at the cotyledon stage (Fig. 9).

Puccinia psidii was recorded on flower buds, flowers and fruits of 28 host species (Table 1; Fig. 10). Sori of *P. psidii* were observed on various parts of the flower including the peduncle, receptacle, sepals (calyx) and petals. On some species, e.g. *Chamelaucium uncinatum*, sori formed on the inside of the flower bud, affecting the anthers, filaments, styles, stigmas and ovaries (Fig. 10). Infection of fruit was common on *Austromyrtus dulcis*, *Eugenia reinwardtiana*, *Rhodamnia rubescens* and *Rhodamnia sessiliflora*. *Puccinia psidii* was also recorded on flowers and fruit of introduced species, e.g. *Syzygium jambos*, some of which are significant weed species, including *Eugenia uniflora* and *Rhodomyrtus tomentosa*.

Discussion

This study reports the dramatic increase in geographic distribution and host range of *P. psidii* following its initial detection in Qld in December 2010. The disease now extends from subtropical coastal and drier inland areas east of the Great Dividing Range to tropical coastal and

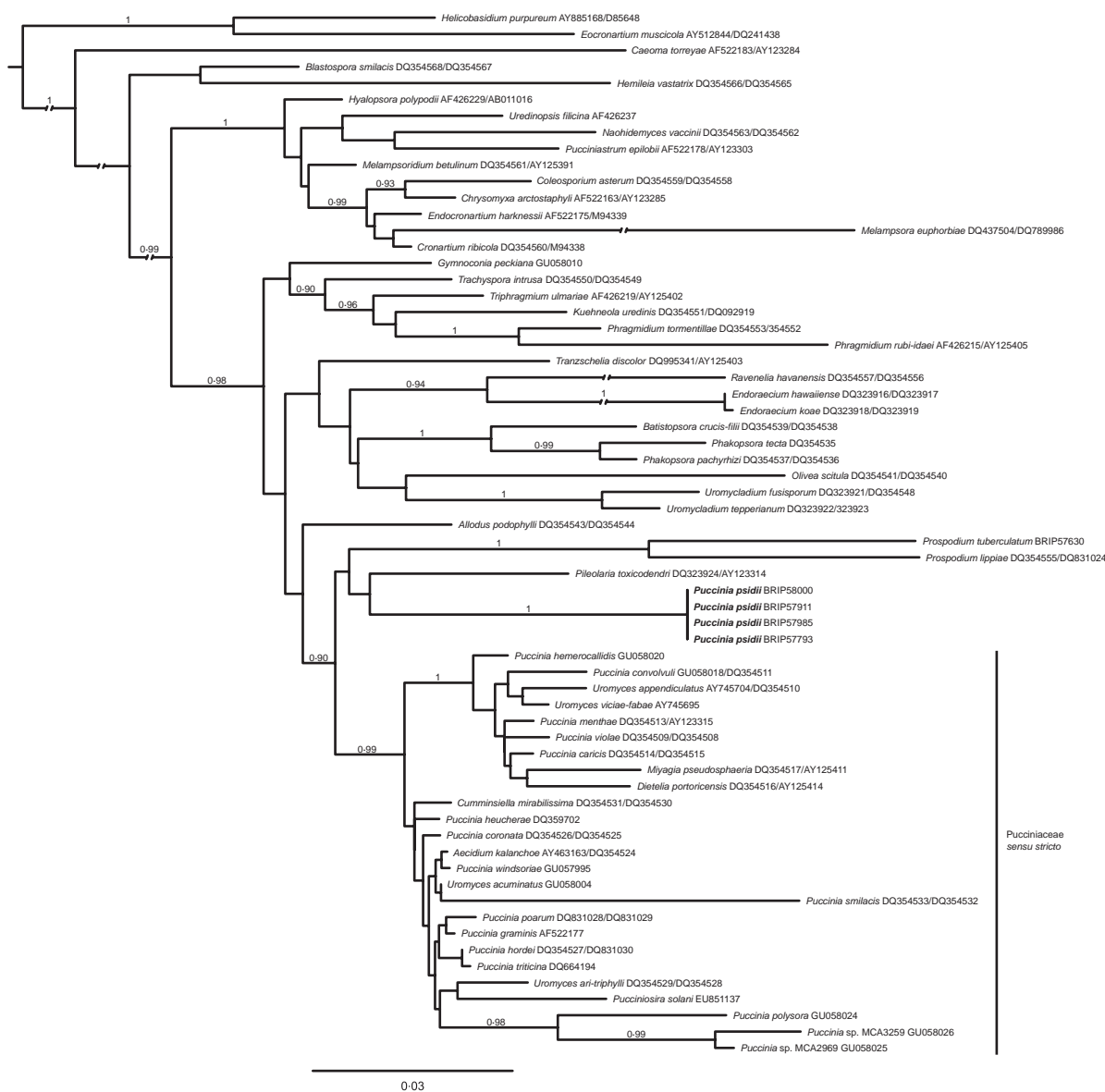


Figure 6 Phylogram obtained from PHYLML in a maximum likelihood search on a combined dataset of the LSU and SSU regions. aRLT support values (>0.90) above nodes.

tableland vegetation. Despite an abundance of potential host species west of the Great Dividing Range, *P. psidii* has not so far established in that region, although it has been reported from plant nurseries. Climate modelling by Glen *et al.* (2007) and Booth & Jovanovic (2012) predicted the likelihood of rust epidemics in these regions as possible and dependent on short-term variations in climatic conditions.

Van Der Merwe *et al.* (2008) first identified the ambiguous systematic position of *P. psidii* based on an analysis of protein coding loci from 80 species in the Pucciniaceae. The phylogenetic analysis in this study based on combined LSU and SSU data has not resolved the familial placement of *P. psidii* within the Pucciniales. The systematics of several rust families, such as the

Uropyxidaceae, which often have puccinoid teliospores, will need to be resolved before *P. psidii* can be confidently placed at the family level.

Simpson *et al.* (2006) reviewed the rust taxa that infected Myrtaceae. They introduced *Uredo psidii*, a superfluous name for the uredinial stage of *P. psidii*, which already had several validly published anamorphic names, for example *Uredo subneurophila* and *Uredo neurophila*. They also introduced the name *Uredo rangellii* for two specimens on *Myrtus communis* and *Syzygium jambos*. The basis for this new taxon was the presence of a tonsure on the lower half of the urediniospores, the shape and wall thickness of the urediniospores and symptoms such as a lack of infection on stems or petioles and the size of uredinia, all of which were considered by

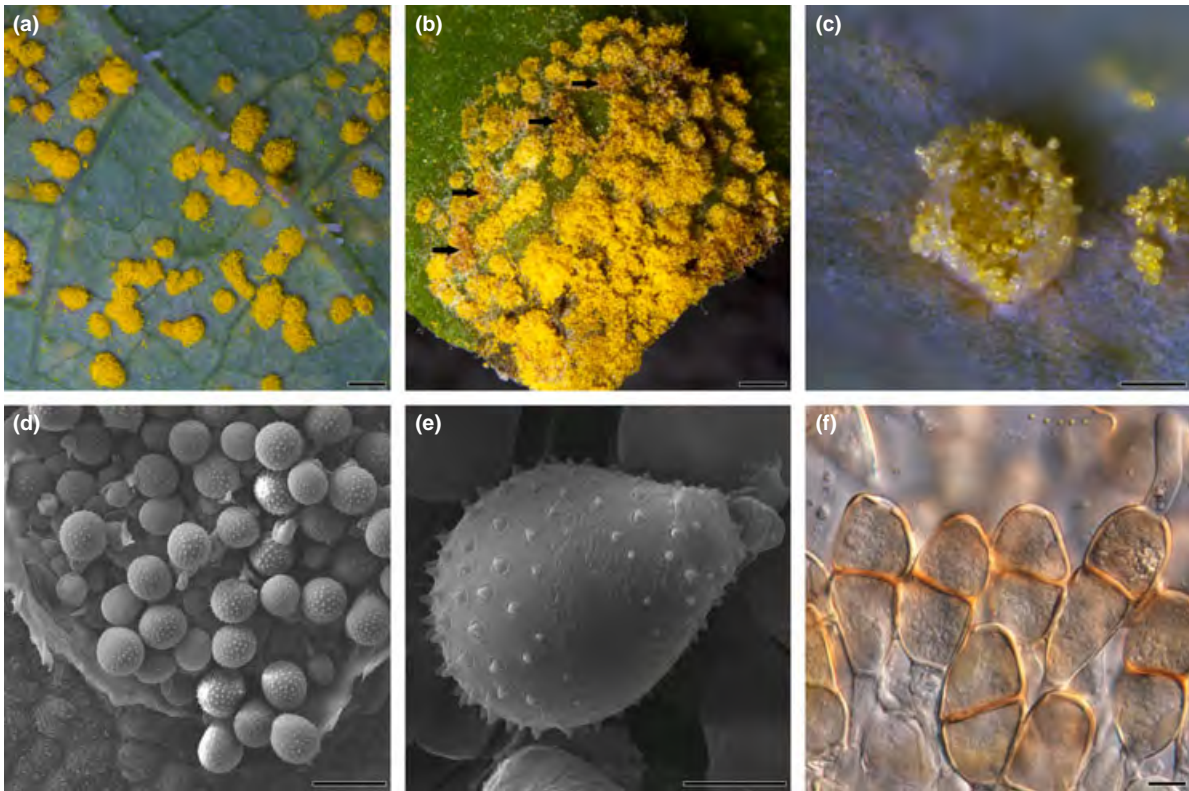


Figure 7 *Puccinia psidii*. (a) Uredinia on abaxial surface (scale bar = 500 μm), (b) uredinia and telia (arrowed; scale bar = 500 μm), (c) erumpent uredinium (scale bar = 125 μm), (d) erumpent uredinium (scale bar = 20 μm), (e) single urediniospore with tonsure (scale bar = 5 μm), (f) teliospores (scale bar = 10 μm).



Figure 8 Photographic sequence showing the impact of *Puccinia psidii* over time on *Rhodamnia angustifolia*, a rare and endangered Queensland species. (a) Initial detection of rust on new shoots and expanding foliage, March 2011; (b) high level of *P. psidii* infection on new shoots and expanding leaves, December 2011; (c) severe defoliation following repeated infection by *P. psidii*, January 2012; (d) foliage and branch dieback 15 months after initial infection was detected, June 2012. Photographs are of cultivated plants, Brisbane.

the authors (Simpson *et al.*, 2006) as different from *P. psidii*. When this rust first appeared in Australia it was referred to as *Uredo rangelii* (Carnegie *et al.*, 2010). Molecular sequence data from the ITS and LSU regions, host studies and morphological data from two life cycle stages support the premise that one taxon, *P. psidii*, is responsible for widespread infection of Myrtaceae in Australia.

Puccinia psidii is now identified from a range of native forest ecosystems including coastal heath (*Austromyrtus dulcis*, *Homoranthus* spp.), coastal and river wetlands (*Melaleuca quinquenervia*, *Melaleuca viridiflora*), sand island ecosystems of Moreton, Stradbroke and Fraser Islands, and littoral, montane, subtropical and tropical rainforests (*Syzygium* spp., *Rhodamnia* spp., *Rhodomyrtus* spp.). The disease is prevalent in urban and



Figure 9 Branch dieback and infection of shoots on a mature *Rhodomyrtus psidioides* tree (a, b) and infection and dieback of regenerating seedlings under adult trees (c, d). Photographs are of cultivated plants in the Brisbane Botanic Gardens, Mt Coot-tha.

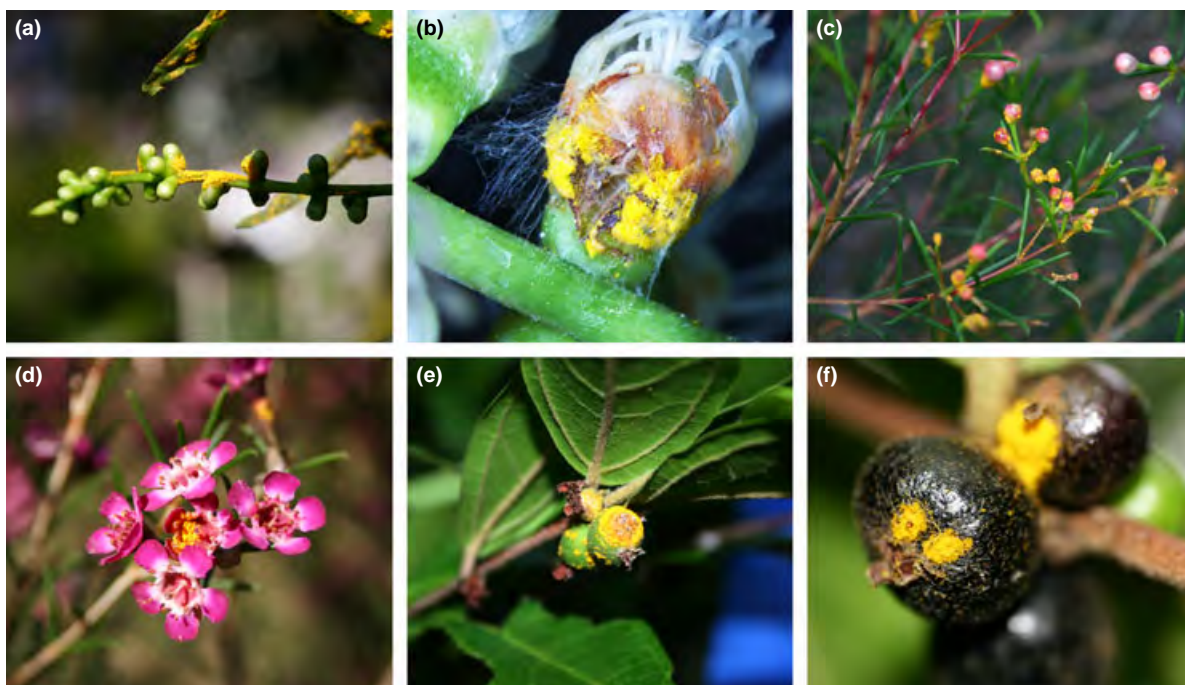


Figure 10 *Puccinia psidii* infection on inflorescences of *Melaleuca leucadendra* (a, b), inflorescences and flowers of *Chamelaucium uncinatum* (c, d), and immature fruit of *Rhodamnia sessiliflora* (e), and mature fruit of *Rhodamnia rubescens* (f).

peri-urban environments around major cities and towns, commonly reported from botanic gardens and nature reserves, with disease impacts ranging from minor leaf spots to severe dieback and infection, and premature senescence of flowers and fruits. In comparison, *P. psidii* is rarely severe on native vegetation in Brazil, even though it has been identified from a range of native Myrtaceae and causes occasional epidemics in native guava plantations (Ribeiro & Pommer, 2004).

The spread of *P. psidii* via movement of infected nursery stock, and other human assisted mechanisms,

played a significant role in the initial distribution and establishment of the disease in different regions of Qld. Now that the disease is established and widespread in Qld, further spread of *P. psidii* into new regions is likely to result from wind and rain dispersal of spores. Short distance dispersal is facilitated by animals and insects (Coutinho *et al.*, 1998). Dominant southeasterly winds and the presence of susceptible species, e.g. *Melaleuca quinquenervia* and *Melaleuca leucadendra*, that provide a near-contiguous corridor along the east coast of Australia (Carnegie & Lidbetter, 2012), are

significant factors for the dispersal of this rust in Australia.

Following the initial increase in the number of reported detections of *P. psidii* in Qld, report numbers have fluctuated. Peaks in reporting were often followed by declines, a pattern repeated several times over the duration of this study. Factors influencing these patterns have not yet been studied in detail in Australia. However, Tessmann *et al.* (2001) identified disease outbreaks of *P. psidii* on *Syzygium jambos* in Brazil as being closely linked to duration of leaf wetness and relative humidity (RH), combined with nocturnal temperatures ranging from 18 to 22°C. A high correlation between progression of *P. psidii* on *Eucalyptus grandis* and days with 90% RH or higher for 8 h, combined with temperatures between 18 and 25°C has been demonstrated (Glen *et al.*, 2007). Data collected as part of the current study indicate that temperature is not the main factor influencing disease development, with reports of new infections throughout the year. The influence of host physiology and changes under different climatic conditions is also likely to influence disease development. This requires further study.

Climatic conditions since *P. psidii* was first detected in Qld have favoured spread and disease development with above average rainfall and associated periods of high relative humidity occurring across most of coastal Qld (www.bom.gov.au). This has undoubtedly led to optimal plant growth conditions, providing repeated growth flushes and high numbers of new shoots and young leaves, which are most susceptible to infection (Coutinho *et al.*, 1998). Interestingly, a decline in reporting of *P. psidii* coincided with consecutive days of heavy rainfall. Previous studies (Lana *et al.*, 2012) have also observed lower levels of *P. psidii* with increased rainfall levels in areas of Brazil. A reduction in spore levels due to high rainfall over a short period of time is a possible explanation. Reduced human activity outdoors during rainfall periods may also have reduced rust observations and reporting.

The host range of *P. psidii* in Qld has expanded rapidly from the five species initially detected in January 2011 to more than 160 species in July 2012. As reported by Carnegie & Lidbetter (2012), the host range recorded in Australia is significantly greater than the known host range for this disease internationally. This study alone has identified a further 56 host species and two genera not previously reported in Australia or internationally (Carnegie & Lidbetter, 2012). The first new genus and species was *Mitrantia bilocularis*, a rare rainforest species endemic to north Qld, which appears moderately susceptible to *P. psidii* and is considered a vulnerable species (Atlas of Living Australia; www.ala.org.au). The second was *Sphaerantia discolor*, also endemic to north Qld rainforest ecosystems and also listed as vulnerable (Atlas of Living Australia; www.ala.org.au). The host range of *P. psidii* is likely to continue expanding as the fungus becomes established in new geographic regions and where new host species exist.

Teliospores were identified from a range of host species with different levels of susceptibility to *P. psidii*. The detection of teliospores did not appear to be limited to season, with detections made during warmer wetter months of summer and the drier winter months. Ruiz (1988) reported that teliospores occur under natural conditions in Brazil on *Eucalyptus cloeziana* during the warmer months of the year (December to March). Other studies indicate that temperature plays a role in spore development, with the ideal temperature for germination of urediniospores being 20°C and subsequent maintenance of infected plants at 25°C or above likely to produce telia rather than uredinia (Coutinho *et al.*, 1998). Urediniospores have been detected on a range of host plants in Qld at all times of the year.

Symptoms of infection by *P. psidii* range from minor leaf spots to severe foliage and stem blight, as well as infection of flowers and fruit of some species. Of the highly or extremely susceptible species, several have importance economically, e.g. *Backhousia citriodora* and *Chamaelucium uncinatum*, and environmentally, e.g. *Melaleuca quinquenervia*. The level of natural resistance within species populations in Australia is unknown. Field observations indicate variability in susceptibility to the disease within some species. It is unclear at this point in time if this is a true reflection of resistance or variation in host phenology and/or localized microclimatic and edaphic conditions. Variations in inoculum levels may also be important.

The impacts that *P. psidii* will have on fragile and threatened ecosystems in Australia, e.g. *Melaleuca* wetlands, are unknown and difficult to predict. The disease has been recorded on 15 species of *Melaleuca* with half considered highly or extremely susceptible based on survey data from this study, including *Melaleuca viridiflora*, which occurs predominantly in higher rainfall areas of northern Australia (Boland *et al.*, 1992). This species is an integral component of diverse tropical lowland environments in northern Qld (Skull & Congdon, 2008) and is regarded as an endangered ecological community (EPBC, 2012).

Melaleuca quinquenervia is considered highly susceptible to *P. psidii*, with infection causing seedling and tree dieback, reduced flower production and flower death. Similar observations were made in Florida (Rayamajhi *et al.*, 2006), where *M. quinquenervia* is a weed and *P. psidii* has been used as a biocontrol agent. *Melaleuca quinquenervia* habitats are threatened in Australia, with large areas cleared for housing, road development and agriculture (Catterall & Kingston, 1994). Impact on growth and regeneration of *M. quinquenervia* by *P. psidii* may impact on ecosystems crucial to maintaining biodiversity as well as the quality of coastal waterways.

The known impact of *P. psidii* on eucalypts in Australia is limited and restricted to seedlings, apart from *Eucalyptus curtisii* where infection has been identified on new shoots of mature trees and coppice. In Brazil, heavy infection of juvenile leaves and meristems of eucalypts causes plants to become stunted and multibranched, with highly susceptible individuals grossly malformed, and

some dying as a result of infection by *P. psidii*. Infection levels have been reported as 20–30% of trees, impacting significantly enough to affect growth rates and subsequent profitability (Booth *et al.*, 2000). Many eucalypt plantations in Qld are subcoastal and located in areas where *P. psidii* has not been detected outside of nurseries. The majority of plantations are also more than 2 years old and less likely to be affected by *P. psidii* based on observations in Brazil (Glen *et al.*, 2007).

Some plant species are at risk of disappearing altogether from their natural ecosystems because of infection by *P. psidii*, especially species that are already rare and endangered, e.g. *Rhodamnia angustifolia*, *Rhodamnia maideniana*, *Gossia gonoclada* and *Backhousia oligantha*. Only 11 *R. angustifolia* trees remain in their natural habitat in central Qld (Snow & Guymmer, 1999). Given this restricted gene pool, the likelihood of identifying any resistance in this population is limited. Similarly, only 12 *G. gonoclada* trees exist naturally and indications are that this species is highly susceptible to *P. psidii*.

Some host range studies had investigated the susceptibility of Australian native Myrtaceae to *P. psidii*, before it entered Australia. Zauza *et al.* (2010) identified 60.5% of *Rhodamnia rubescens* seedlings as being resistant. Similarly, they found 80% resistance in *Eugenia reinwardtiana*. Both species are considered extremely susceptible in Australia with no evidence of resistance, and often the first host to be recorded as the disease extended its geographic range in Qld. This raises the important issue of pathogen variability within *P. psidii* and the need to maintain strict border controls, preventing additional strains entering Australia. In addition, the requirement for more investigation into potential resistance within host populations should be considered.

Loss or significant impact on common, dominant and keystone species such as *Melaleuca quinquenervia*, *M. viridiflora* and *M. leucadendra* are likely to have far more devastating effects on ecosystem health than the loss of minor ecosystem species, even species that are listed as threatened. Loss of biodiversity through impact on a wide range of species will occur as *P. psidii* spreads. This is particularly salient given that Qld's terrestrial ecosystems are dominated by native Myrtaceae (REDD, 2012). Booth *et al.* (2000) highlight the potential impact the disease may have on ecosystems and tourism, focusing on the environmental attractions of coastal Qld. The full impact of this disease in Qld and Australia may not be realized for some years.

Acknowledgements

The authors acknowledge the facilities, and the scientific and technical assistance, of the Australian Microscopy & Microanalysis Research Facility at the Centre for Microscopy and Microanalysis, The University of Queensland. Funding for the work provided by the Queensland government and The CRC for Plant Biosecurity is greatly appreciated. A. R. M. acknowledges the Australian Biological Resources Study for funding (grant number RFL212-33).

The authors also wish to thank Tony Bean and Stephen McKenna for assistance in the identification and collection of plant species as well as staff from Botanical Gardens in Queensland. They would also like to thank Charlie Booth for allowing access to his collection of Myrtaceae.

References

- Aime MC, 2006. Toward resolving family-level relationships in rust fungi (Uredinales). *Mycoscience* 47, 112–22.
- Anisimova M, Gil M, Dufayard JF, Dessimoz C, Gascuel O, 2011. Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation schemes. *Systematic Biology* 60, 685–99.
- Boland DJ, Brooker MIH, Chippendale GM *et al.*, 1992. *Forest Trees of Australia*. Victoria, Australia: CSIRO Publishing.
- Booth TH, Jovanovic T, 2012. Assessing vulnerable areas for *Puccinia psidii* (eucalyptus rust) in Australia. *Australasian Plant Pathology* 41, 425–9.
- Booth TH, Old KM, Jovanovic T, 2000. A preliminary assessment of high risk areas for *Puccinia psidii* (*Eucalyptus* rust) in the Neotropics and Australia. *Agriculture Ecosystems & Environment* 82, 295–301.
- Carnegie AJ, Lidbetter JR, 2012. Rapidly expanding host range of *Puccinia psidii sensu lato* in Australia. *Australasian Plant Pathology* 41, 13–29.
- Carnegie AJ, Lidbetter JR, Walker J *et al.*, 2010. *Uredo rangellii*, a taxon in the guava rust complex, newly recorded on Myrtaceae in Australia. *Australasian Plant Pathology* 39, 463–6.
- Catterall C, Kingston M, 1994. *Remnant Bushland of South East Queensland in the 1990's: Its Distribution, Loss, Ecological Consequences, and Future Prospects*. Brisbane, Australia: Institute of Applied Environmental Research, Griffith University.
- Coutinho TA, Wingfield MJ, Alfenas AC, Crous PW, 1998. Eucalyptus rust: a disease with the potential for serious international implications. *Plant Disease* 82, 819–25.
- Dayton L, Higgins E, 2011. Myrtle rust “biggest threat to ecosystem”. The Australian, 9 April 2011. [<http://www.theaustralian.com.au/news/health-science/myrtle-rust-biggest-threat-to-ecosystem/story-e6frg8y6-1226036247221>]. Accessed 9 April 2011.
- EPBC, 2012. Broad leaf tea-tree (*Melaleuca viridiflora*) woodlands in high rainfall coastal north Queensland. [<http://www.environment.gov.au/cgi-bin/sprat/public/publicshowcommunity.pl?id=122>]. Accessed July 2013.
- Ferreira FA, 1983. Eucalyptus rust. *Revista Arvore* 7, 91–109.
- Gardes M, Bruns TD, 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2, 113–8.
- Glen M, Alfenas AC, Zauza EAV, Wingfield MJ, Mohammed C, 2007. *Puccinia psidii*: a threat to the Australian environment and economy – a review. *Australasian Plant Pathology* 36, 1–16.
- Grgurinovic CA, Walsh D, Macbeth F, 2006. Eucalyptus rust caused by *Puccinia psidii* and the threat it poses to Australia. *EPPO Bulletin* 36, 486–9.
- Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O, 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PHyML 3.0. *Systematic Biology* 59, 307–21.
- Kawanishi T, Uemastu S, Kakishima M *et al.*, 2009. First report of rust disease on ohia and the causal fungus, *Puccinia psidii*, in Japan. *Journal of General Plant Pathology* 75, 428–31.
- Lana VM, Mafia RG, Ferreira MA *et al.*, 2012. Survival and dispersal of *Puccinia psidii* spores in eucalypt wood products. *Australasian Plant Pathology* 41, 229–38.
- MacLachlan JD, 1938. A rust of the pimento tree in Jamaica, BWI. *Phytopathology* 28, 157–70.
- Minnis D, McTaggart A, Rossman A, Aime MC, 2012. Taxonomy of mayapple rust: the genus *Allodus* resurrected. *Mycologia* 104, 942–50.

- Nylander JAA, Olsson U, Alström P, Sanmartín I, 2008. Accounting for phylogenetic uncertainty in biogeography: a Bayesian approach to dispersal–vicariance analysis of the thrushes (Aves: Turdus). *Systematic Biology* 57, 257–68.
- Pegg GS, O'Dwyer C, Carnegie AJ, Burgess TI, Wingfield MJ, Drenth A, 2008. *Quambalaria* species associated with plantation and native eucalypts in Australia. *Plant Pathology* 57, 702–14.
- Rayamajhi MB, Van TK, Pratt PD, Center TD, 2006. Interactive association between *Puccinia psidii* and *Oxyops vitiosa*, two introduced natural enemies of *Melaleuca quinquenervia* in Florida. *Biological Control* 37, 56–67.
- REDD, 2012. Regional Ecosystem Description Database. [http://www.ehp.qld.gov.au/ecosystems/biodiversity/regional-ecosystems/index.php]. Accessed July 2013.
- Ribeiro IJA, Pommer CV, 2004. Breeding guava (*Psidium guajava*) for resistance to rust caused by *Puccinia psidii*. *Acta Horticulturae* 632, 75–8.
- Ronquist F, Huelsenbeck JP, 2003. MrBAYES version 3.0: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–4.
- Roux J, Greyling I, Coutinho TA, Verleur M, Wingfield MJ, 2013. The Myrtle rust pathogen, *Puccinia psidii*, discovered in Africa. *IMA Fungus* 4, 155–9.
- Ruiz RAR, 1988. *Epidemiologia e Controle Químico da Ferrugem (Puccinia psidii Winter) do Eucalipto*. Viçosa, Brazil: Universidade Federal de Viçosa, MS thesis.
- Schoch CL, Seifert KA, Huhndorf S *et al.*, 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Cunninghamia* 109, 6241–6.
- Simpson JA, Thomas K, Grgurinovic CA, 2006. Uredinales species pathogenic on species of Myrtaceae. *Australasian Plant Pathology* 35, 549–62.
- Skull SD, Congdon RA, 2008. Floristics, structure and site characteristics of *Melaleuca viridiflora* (Myrtaceae) dominated open woodlands of the wet tropics lowlands. *Cunninghamia* 10, 423–38.
- Snow N, Guymer GP, 1999. *Rhodamnia angustifolia* (Myrtaceae), a new and endangered species from south-eastern Queensland. *Austrobaileya* 5, 421–6.
- Stamatakis A, 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–90.
- Tessmann DJ, Dianese JC, Miranda AC, Castro LHR, 2001. Epidemiology of a neotropical rust (*Puccinia psidii*): periodical analysis of the temporal progress in a perennial host (*Syzygium jambos*). *Plant Pathology* 50, 725–31.
- Uchida J, Zhong S, Killgore E, 2006. First report of a rust disease on ohia caused by *Puccinia psidii* in Hawaii. *Plant Disease* 90, 524.
- Van Der Merwe MM, Walker J, Ericson L, Burdon JJ, 2008. Coevolution with higher taxonomic host groups within the *Puccinia Uromyces* rust lineage obscured by host jumps. *Mycological Research* 112, 1387–408.
- Vilgalys R, Hester M, 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172, 4238–46.
- White TJ, Bruns T, Lee S, Taylor JW, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR Protocols: a Guide to Methods and Applications*. San Diego, USA: Academic Press, 315–22.
- Wilson PG, O'Brien MM, Heselwood MM, Quinn CJ, 2005. Relationships within Myrtaceae *sensu lato* based on *matK* phylogeny. *Plant Systematics and Evolution* 251, 3–19.
- Zauza EAV, Alfenas AC, Old KM, Couto MMF, Graça RN, Maffia LA, 2010. Myrtaceae species resistance to rust caused by *Puccinia psidii*. *Australasian Plant Pathology* 39, 405–11.
- Zhuang JY, Wei SX, 2011. Additional materials for the rust flora of Hainan Province, China. *Mycosystema* 30, 853–60.