Rapid screening of commercial forestry species to *Uredo rangelii* (myrtle rust) and distinguishing *U. rangelii* from *Puccinia psidii* (guava rust)
Rapid screening of commercial forestry species to *Uredo rangelii* (myrtle rust) and distinguishing *U. rangelii* from *Puccinia psidii* (guava rust)

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Executive Summary

A fungal disease was detected on 23 April 2010 on Agonis flexuosa (willow myrtle) grown for cut flowers at a property on the central coast of NSW. The pathogen causing the disease was identified by its spore morphology as myrtle rust (Uredo rangelii), a fungal species first described in 2006 as belonging to a complex of fungal species similar to guava rust (Puccinia psidii).

Guava or eucalypt rust caused by Puccinia psidii is one of the most serious disease threats to Australian native flora. Of most significance to commercial forestry, guava rust is a serious disease in species of Eucalyptus, Corymbia and Melaleuca.

Myrtle rust is described from a very small number of specimens with a limited host range and does not have any associated biological information. Guava rust has received significant attention overseas and has a reputation as a damaging pathogen.

The identification of the incursion pathogen as an unknown quantity - myrtle rust - was one factor that contributed towards a confused and slow response to the pathogen. The pathogen should have been immediately eradicated.

This project therefore was aimed at bringing clarity to the situation, to provide information which would highlight the need for speedy eradication measures. The objectives were;

- To see if molecular analyses could discriminate between myrtle rust and guava rust
- To test the susceptibility of key commercial eucalypt species to myrtle rust

The outcomes of the project are clear and indisputable.

- None of the gene regions sequenced support the distinction of Uredo rangelii from Puccinia psidii, or provide any evidence that a “species complex” exists.
- Key commercial forestry species are susceptible to “myrtle” rust, including those used in plantations and native forestry in NSW and Queensland.

These knowledge outcomes benefit the industry by providing the correct information about the pathogen detected in NSW, its biology and the level of biosecurity threat posed
to commercial forestry. Without this knowledge adequate pressure may not be exerted to eradicate/contain the pathogen. Forestry can consider the full spectrum of possible outcomes of this incursion including trade implications, germplasm movement embargos and the cost of management strategies if the pathogen becomes widespread.

To avoid confusion myrtle rust should now be referred to as guava rust (*Puccinia psidii*) although the guava rust in NSW may represent a variety of guava rust. It is evident that the rust in NSW does not have a restricted host range (as was originally claimed for myrtle rust) and at least 15 species in 11 genera are susceptible to this rust that has entered NSW. In the fight against any pathogen of damaging importance “Know your enemy” is a concept of paramount importance.

Given the high biosecurity threat posed by guava rust to the industry, it should be vigilant about the progress of the eradication campaign in NSW and the decisions that may drive the stand down of this campaign.

The industry should be proactive in planning for the worst case scenario that this pathogen is not eradicated or contained around the initial infection sites in NSW.
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Introduction

Native to South and Central America, *Puccinia psidii* infects a broad range of hosts in the Myrtaceae, including introduced species such as *Syzygium jambos* and *Eucalyptus* spp. It is recognised as a threat to Australia’s environment and economy if it were to become established in Australia (Glen et al. 2007a). Simpson et al. (2006) described a new species, *Uredo rangelii*, distinguished from *Uredo psidii*, the anamorph of *P. psidii*, based on the presence of a tonsure on the urediniospore, though this character is considered to be a variable characteristic of *P. psidii* by Brazilian plant pathologists. The description was based on two old herbarium specimens and no new collections were included. No DNA sequences were provided to support the species separation.

*Uredo rangelii* (myrtle rust) was detected in Australia in April 2010 on the Central Coast of NSW (Carnegie et al. 2010). *U. rangelii* is a taxon within the *Puccinia psidii* s.l. (guava rust) complex; it is morphologically distinct from *P. psidii* s.s. (Simpson et al. 2006), though the ribosomal DNA (rDNA) internal transcribed spacer (ITS) sequence was indistinguishable from that of *P. psidii* (Carnegie et al. 2010). This gene region is known to discriminate species of *Puccinia*, including in species complexes (Szabo 2006).

Known hosts of *U. rangelii* in Australia are *Agonis flexuosa*, *Callistemon viminalis* and *Syncarpia glomulifera* (Carnegie et al. 2010), while overseas *U. rangelii* is known from *Myrtus communis* and *Syzygium jambos* (Simpson et al. 2006). The five known hosts of *U. rangelii* are from five separate “tribes” within Myrtaceae (Wilson et al. 2005), and as such the host range is expected to be larger (Carnegie et al. 2010). *P. psidii* s.l. has a large host range, being reported on over 75 species from 16 genera within Myrtaceae (Coutinho et al. 1998; Simpson et al. 2006; Office of the Chief Plant Protection Officer 2007; Zauza et al. 2010). As *U. rangelii* has only recently been separated from *P. psidii* s.l. (Simpson et al. 2006), and is not considered a valid species by Brazilian pathologists (Alfena, pers. comm.), it is likely that literature pertaining to *P. psidii* s.l., such as host range and impact, includes reference to disease caused by *U. rangelii* (Carnegie et al. 2010).

Numerous authors have conducted host susceptibility tests for *P. psidii* s.l. with a range of myrtaceous species, including Dianese et al. (1984, 1986), Rayachhetry et al. (2001), Alfenas et al. (2003), Tommerup et al. (2003) and Zauza et al. (2010). Several studies have determined conditions for spore germination and infection (e.g., Ruiz et al. 1989; Piza and Ribeiro 1988), and although there is some variation amongst studies, it is generally agreed that optimum conditions are 15–25°C, high humidity or leaf wetness for up to 8 h, low light or darkness, with infection only occurring on immature foliage. Continued ambient temperatures and high humidity are then generally required, with uredinial pustules and sporulation observed within 10–12 days (Marlatt & Kimbrough 1979; Rayachhetry et al. 1997; Alfenas et al. 2003).

The current work has two aims. Due in part to the ambiguity between the historical host range of *U. rangelii* and *P. psidii* s.l., the first aim of this study was to test a range of myrtaceous species for susceptibility to *U. rangelii* to ascertain whether myrtle rust could be a threat to forestry in Australia. The second aim was to determine whether other gene regions support the distinction of *U. rangelii* from *P. psidii*. Van der Merwe et al. (2007) reconstructed a phylogeny for species of *Puccinia* and *Uromyces* based on sequences from transcription elongation factor 1 (tef-1) and β-tubulin genes. Both of these gene regions were able to discriminate closely related species, and indicated the possible presence of cryptic species in some lineages. Several DNA samples of *P. psidii* were available in Australia from previous studies (Langrell et al. 2008) and also from Hawaii. The only DNA sample linked to a herbarium specimen is the Hawaiian sample, and its urediniospores lack the tonsure that has
been used to separate *U. rangelii*. The Brazilian samples came from a range of different hosts and geographic locations.
Methodology

Rapid screening of commercial forestry species to *U. rangelii*

Test plants and inoculation

The species we selected were key commercial eucalypt species in Australia: *Eucalyptus agglomerata*, *E. cloeziana*, *E. globulus*, *E. grandis*, *E. pilularis* and *Corymbia maculata*. We were, however, restricted by availability, so not all key species were tested (e.g., *E. dunnii*, *E. nitens*, *E. regnans*, *C. variegata*). *Melaleuca quinquenervia* was also tested, as it is a known highly susceptible host of *P. psidii* s.l. (Rayachhetry et al. 2001). *Agonis flexuosa* cv. ‘Afterdark’ was used as a control, as it is known to be highly susceptible to *U. rangelii* in Australia (Carnegie et al. 2010). Eucalypt seedlings were potted into 150 mm pots using regular native potting mix, and kept in a glass house until used, with watering twice a day. *A. flexuosa* cv. ‘Afterdark’ plants were sourced as either 150 mm pots or 200 mm pots. Two 200 mm pots of *Melaleuca quinquenervia* were also used. Plants to be tested were selected to ensure they had fresh new growth (immature leaves).

The eight species were tested in four experiments. Due to biosecurity concerns, three experiments were conducted *in situ* at IP 1 [Infected Premise 1 – the first property where myrtle rust was detected in Australia, on the Central Coast of NSW] and the fourth in a Biological Safety Cabinet (Class II) at the Industry & Investment NSW (forestry) laboratories at West Pennant Hills. Diseased *A. flexuosa* cv. ‘Afterdark’ and/or *Syncarpia glomulifera* leaves and shoots, with fresh uredinial pustules containing urediniospores, were collected from IP 1 for inoculations. In most cases, material was either collected the same day as inoculations or a day or two previously and kept in a fridge prior to inoculations. Urediniospores were scraped from uredinial pustules using a clean scalpel such that they “dusted” onto immature leaves; in some cases scraped urediniospores were gently wiped onto immature leaves with the scalpel. In most cases, yellow urediniospores could be seen on inoculated leaves either with the naked eye or a 10× hand lens.

**Experiment I**

This experiment relied on natural infection from diseased trees at IP 1, and began in May 2010. Nine potted plants each of *E. agglomerata*, *E. cloeziana*, *E. grandis*, *E. pilularis*, *C. maculata* and *A. flexuosa* cv. ‘Afterdark’ were placed under infected *S. glomulifera* and misted with water a single time (Fig. 1). Although fungicide spraying had occurred at IP 1 prior to this experiment, the western side of the *S. glomulifera* windbreak had not been sprayed, and so plants were located under diseased branches – with freshly sporulating uredinial pustules present on leaves – on the western side of trees. Test plants were moved after 48 h into a shade house (due to an impending fungicide spray operation), where they were misted twice-daily for 30 mins in the morning and early evening. Plants were inspected weekly for evidence of myrtle rust infection.

**Experiment II**

In this experiment we attempted to increase the chance of infection by artificially inoculating plants with urediniospores freshly collected from diseased *S. glomulifera* and by maintaining leaf wetness for up to 48h. It was again conducted at IP 1, beginning in May 2010. Three potted plants each of *E. agglomerata*, *E. cloeziana*, *E. grandis*, *E. pilularis*, *C. maculata* and *A. flexuosa* cv. ‘Afterdark’ were misted with water to ensure leaf wetness on immature leaves and inoculated as described above. Inoculated plants were then covered with clear plastic
bags, which were misted with water inside to increase humidity, and left outside adjacent A. flexuosa cv. ‘Afterdark’ plants (Fig. 2). After 48h, most plants (and plastic bags) were still moist, and plants were moved into a shade house, where they were misted twice-daily for 30 mins in the morning and early evening. Plants were inspected weekly for evidence of myrtle rust infection.

**Experiment III**

This was again performed at IP 1, beginning in late May 2010. A temporary inoculation chamber was constructed out of black plastic and a wooden frame (Fig. 3) and housed in a greenhouse (Fig. 4) located at IP 1. Three plants each of E. agglomerata, E. cloeziana, E. globulus, E. grandis, E. pilularis, C. maculata, A. flexuosa cv. ‘Afterdark’ and a single plant of Melaleuca quinquenervia were misted with water to ensure leaf wetness on immature leaves and inoculated as above. Plants were then placed inside the temporary inoculation chamber, the walls of the chamber misted with water to increase humidity, and the door sealed. Temperature within the temporary inoculation chamber during this time ranged from 7–25° C. This method allowed optimum temperatures for infection (which only occur during the daytime in May) to be matched with darkness and leaf wetness. After 36 h, most plants still had moist leaves and were moved from the temporary inoculation chamber to a bench within the greenhouse and misted for 5 seconds every hour. Plants were inspected weekly for evidence of myrtle rust infection. Temperatures within the greenhouse during early June ranged from 5–25° C and in late June from 0–29° C.

**Experiment IV**

In this experiment, conducted in June 2010, we tried what we believe is a new method: using cuttings in tissue culture jars. Young shoots from actively growing seedlings were cut and immediately inserted into 1.5% or 2% water agar in 500 ml polycarbonate jars (tissue culture jars) (Fig. 5). Six species were used for this experiment: E. globulus, E. cloeziana, E. grandis, C. maculata, M. quinquenervia and A. flexuosa cv. ‘Afterdark’. Initially we used “old” inoculum of U. rangelii from both A. flexuosa cv. ‘Afterdark’ and S. glomulifera leaves collected a month or so previously from IP 1 and air dried and stored in herbarium envelopes. We then repeated the experiment using “fresh” inoculum (actively sporulating pustules) collected from potted plants of A. flexuosa cv. ‘Afterdark’ at IP 1 and kept in a paper bag in the fridge overnight. Viability of inoculum was tested by brushing urediniospores onto 2% water agar and observing germination at ~25° C after 24 h.

Cuttings were first misted with sterilised water (with Tween 20 added) to moisten actively growing leaves and inoculated as above. Jars were sealed with Parafilm®, and placed into plastic zip-lock bags which were misted with water then sealed (Fig. 5–6). Due to limited inoculum, only two cuttings of each species were inoculated, with three cuttings of the A. flexuosa cv. ‘Afterdark’ inoculated. To enhance biosecurity, inoculations were conducted in a Biological Safety Cabinet Class II (Fig. 6) at the Forest Science Centre, Industry & Investment NSW, West Pennant Hills. Inoculated cuttings were then left in the Biological Safety Cabinet, with the glass covered to ensure darkness, for 24 h. The temperature within the Biological Safety Cabinet over this period ranged from 17–26° C, with the first 8 hrs being 21–26° C. After 24 h, leaves on most cuttings were still moist. The sealed jars (still within zip-lock bags) were then placed on a laboratory bench in a secure room (i.e. restricted access) with natural light and a temperature range of 14–26° C. Cuttings were inspected regularly for presence of rust pustules for two weeks.
Morphological confirmation of infection by *U. rangelii*

Suspect positive samples were initially inspected under a binocular microscope, and then sent to Dr Michael Priest at the NSW Plant Pathology Herbarium (DAR) in Orange, NSW for confirmation. Positive samples are lodged at DAR.

**Distinguishing *U. rangelii* from *P. psidii***

**DNA amplification and phylogenetic analysis**

DNA samples, with plant hosts and geographic origin, are provided (Table 1). DNA was amplified by PCR targeting genes for transcription elongation factor 1, beta-tubulin and intergenic spacers. Additional sequences from GenBank were included in phylogenetic analyses. Sequences were aligned with ClustalX (Thompson et al. 1997) and maximum likelihood phylogenetic analysis was carried out using DNAML (Felsenstein 1989).

**Table 1. DNA samples used in the phylogenetic study.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Host</th>
<th>Geographic Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAR80678</td>
<td><em>Agonis flexuosa</em> cv. Afterdark</td>
<td>NSW (IP1)</td>
</tr>
<tr>
<td>UFV1</td>
<td>Biotype 00*</td>
<td>Brazil</td>
</tr>
<tr>
<td>UFV3</td>
<td>Biotype 02*</td>
<td>Brazil</td>
</tr>
<tr>
<td>UFV8</td>
<td><em>Psidium guajava</em> (guava)</td>
<td>Viçosa, Brazil</td>
</tr>
<tr>
<td>UFV9</td>
<td><em>Syzygium jambos</em></td>
<td>Viçosa, Brazil</td>
</tr>
<tr>
<td>UFV26</td>
<td><em>Psidium guajava</em></td>
<td>Campos, Brazil</td>
</tr>
<tr>
<td>SZ1</td>
<td><em>Syzygium jambos</em></td>
<td>Hawai‘i</td>
</tr>
<tr>
<td>SZ2</td>
<td><em>Melaleuca quinquenervia</em></td>
<td>Hawai‘i</td>
</tr>
<tr>
<td>SZ3</td>
<td><em>Metrosideros polymorpha</em></td>
<td>Hawai‘i</td>
</tr>
</tbody>
</table>

*Indicates single urediniospore isolates from the collection of Prof. Acelino Alfenas, Universidade Federale Viçosa (Langrell et al. 2008).
Results

Host Testing

Experiment I & Experiment II
No evidence of infection (purpling or uredinial pustules) was observed on any plant, including the A. flexuosa cv. ‘Afterdark’, at one, two, three or four weeks, or at eight weeks after inoculation. The fact that the A. flexuosa cv. ‘Afterdark’ was not infected, and that there was evidence of infection on planted A. flexuosa cv. ‘Afterdark’ at IP 1 during this time, indicates that neither of these techniques worked, suggesting the optimum conditions for infection were not obtained. All plant material used in these two experiments was sprayed with the fungicide Bayfidan® after 8 weeks and then destroyed, following biosecurity procedures.

Experiment III
After two weeks there was no evidence of infection on the A. flexuosa cv. ‘Afterdark’, but chlorotic to purple spots and blotches were observed on leaves of one plant each of E. pilularis and E. cloeziana. No rust pustules were associated with this discoloration though at this time. No change had occurred after three weeks; but at four weeks, uredinial pustules were observed on the three inoculated A. flexuosa cv. ‘Afterdark’. There was still no evidence (using a 10× hand lens) of uredinial pustules on the now purple spots and blotches on the E. pilularis and E. cloeziana at four weeks. Plants were not inspected in week five; but after six weeks uredinial pustules were observed on the purple spots and blotches of the E. pilularis (Figs 7–8) and E. cloeziana (Figs 9–10). On both hosts sporulation was more prevalent on the lower (abaxial) leaf surface. Also at six weeks, purple spots were now observed on two plants of E. agglomerata (Figs 11–12) and a single plant of E. grandis, but there was no evidence (using a 10× hand lens) of uredinial pustules on these plants. Later examination under a dissecting then compound microscope identified these spots on E. agglomerata and E. grandis as being associated with U. rangelii urediniospores, and rust on the A. flexuosa cv. ‘Afterdark’, E. cloeziana and E. pilularis was confirmed as U. rangelii. No other species inoculated showed any evidence of infection (Table 2). Samples were collected from E. pilularis, E. cloeziana, E. agglomerata, E. grandis and A. flexuosa cv. ‘Afterdark’ for confirmation, then all plants were sprayed with Bayfidan® and then destroyed, following biosecurity procedures.

Experiment IV
In most instances the cuttings remained “healthy” and “alive” for the duration of the experiment. However, some plants lost a few leaves prematurely, and some plants became infected with fungi that were likely inhabiting plants prior to cutting and placing in the tissue culture jars. After 24 h on water agar, ~5% of urediniospores from the “old”, air-dried inoculum had germinated, while ~25% had germinated from the “fresh” inoculum. No evidence of infection (purpling or uredinial pustules) was observed after two weeks using the “old” inoculum. However, using the “fresh” inoculum, uredinial pustules were observed after two weeks on two of the three cuttings of A. flexuosa cv. ‘Afterdark’ (Figs 13–14), one cutting of E. cloeziana (Figs 15–16) and one of the M. quinquenervia cuttings (Figs 17–18). No evidence of infection (purpling or uredinial pustules) was observed on any other host tested (Table 2). Rust pustules on A. flexuosa cv. ‘Afterdark’, E. cloeziana and M. quinquenervia were confirmed as U. rangelii.
Distinguishing *U. rangelii* from *P. psidii*

Sequences from the β-tubulin and *tef-1* genes were successfully obtained for nine samples. Phylogenetic analyses were conducted separately as the sequences from each gene region came from different collections and the other rust species (out-groups) clustered differently for each gene region (Figs 19 and 20). ITS phylogenies are also included from previous work (Figs 21 and 22, Glen unpublished). Given the lack of sequence variation in these three gene regions, sequencing of the rDNA LSU was not pursued as this region is more conserved (Maier *et al.* 2003) and so is highly unlikely to provide any additional information.
Table 2. Results of inoculation experiments (Experiment III & IV) with *Uredo rangelii* on a range of hosts

<table>
<thead>
<tr>
<th>Species</th>
<th>Provenance/Cultivar</th>
<th>Inoculation Chamber at IP 1 (Experiment III)</th>
<th>Cuttings in tissue culture jars(^2) (Experiment IV)</th>
<th>Susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agonis flexuosa</em></td>
<td>‘Afterdark’</td>
<td>3/3(^1)</td>
<td>2/3</td>
<td>+</td>
</tr>
<tr>
<td><em>Eucalyptus agglomerata</em></td>
<td>Olney State Forest, NSW</td>
<td>2/3</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>E. cloeziana</em></td>
<td>Gympie, Qld</td>
<td>1/3</td>
<td>1/2</td>
<td>+</td>
</tr>
<tr>
<td><em>E. globulus</em></td>
<td>unknown</td>
<td>0/3</td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td><em>E. grandis</em></td>
<td>Buladelah, NSW</td>
<td>1/3</td>
<td>0/2</td>
<td>+</td>
</tr>
<tr>
<td><em>E. pilularis</em></td>
<td>Newry State Forest, NSW</td>
<td>1/3</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Corymbia maculata</em></td>
<td>unknown</td>
<td>0/3</td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td><em>Melaleuca quinquenervia</em></td>
<td>unknown</td>
<td>0/1</td>
<td>1/2</td>
<td>+</td>
</tr>
</tbody>
</table>

\(^1\) Number of plants infected per plants inoculated
\(^2\) Using “fresh” inoculum
Figures 7–12. Infection on various hosts following artificial inoculation with *Uredo rangelii* during Experiment III. **7–8.** Purple spots and blotches and uredinial pustules on *Eucalyptus pilularis* (adaxial and abaxial leaf surface, respectively). **9–10.** Purple spots and blotches and uredinial pustules on *E. cloeziana* (adaxial and abaxial leaf surface, respectively). **11–12.** Purple spots on *E. agglomerata* (two separate plants).
Figure 19. Maximum Likelihood tree based on partial translation elongation factor 1 sequences, bar represents 10% expected nucleotide variation.
Figure 20. Maximum Likelihood tree based on partial beta tubulin 1 sequences, bar represents 10% expected nucleotide variation.
Figure 21. Maximum Likelihood tree based on rDNA internal transcribed spacer sequences, bar represents 10% expected nucleotide variation. Abbreviations: P = Puccinia, U = Uromyces.
Figure 22: Maximum Likelihood tree based on rDNA internal transcribed spacer sequences, including all *P. psidii* sequences from GenBank. Bar represents 10% expected nucleotide variation. Abbreviations: Br. = Brazil, E. = Eucalyptus, Hi. = Hawaii, HNR = Host not recorded (in GenBank), M. = Melaleuca, P. = Puccinia, S. = Syzygium.
Discussion

This work expands the host range of *U. rangelii* to include seven genera: *Myrtus, Syzigium, Agonis, Callistemon, Syncarpia* and now *Eucalyptus* and *Melaleuca*. Another five hosts have recently been identified during surveys for myrtle rust on the Central Coast of NSW (Carnegie, Lidbetter & Priest, unpublished), with species in *Leptospermum, Tristania, Syzygium, Metrosideros* and *Gossia (=Austromyrtus)*. Discussions to date related to the current incursion of *U. rangelii* in Australia have emphasised the “restricted” host range (Office of the Chief Plant Protection Officer 2010). The host range now includes 15 species in eight of the 17 “tribes” within Myrtaceae (Wilson *et al.* 2005): Eucalypteae, Leptospermeae, Melaleuceae, Metrosiderae, Myrteae, Syncarpieae, Syzigieae and Tristaniae (Table 3). This rapidly expanding host range is likely to increase, with further host testing underway at CSIRO in Canberra (L. Morin, unpublished). As such, the host range for *U. rangelii* (myrtle rust) is likely to more closely resemble that for *P. psidii* (guava rust).

Experiments *in situ* at IP 1 revealed that during the colder winter months the infection period increases significantly. Under optimum conditions for guava rust (15–25°C, Ruiz *et al.* 1989; Piza and Ribeiro 1988) infection occurs within two weeks (Marlatt & Kimbrough 1979; Rayachhetry *et al.* 1997; Alfenas *et al.* 2003). Likewise, we observed infection within two weeks in Experiment IV in the laboratory, where temperatures were within the optimum range. However, in Experiment III (*in situ* at IP 1), where temperatures were much colder, the infection period was four weeks on *A. flexuosa* and five weeks on the *Eucalyptus* spp. This has implications for myrtle rust surveillance and domestic quarantine, as the time from infection to observation of symptoms is significantly greater during cooler temperatures.

Although there is no doubt that the species identified here are susceptible to *U. rangelii*, results need to be used with caution. The conditions under which infection occurred in these experiments are less likely to occur on a regular basis in the native environment. We have not observed myrtle rust on species of *Eucalyptus* or *M. quinquenervia* in the field as yet. However, artificial inoculation studies are commonly used to identify the risk of introduction of exotic pests into countries (e.g. Office of the Chief Plant Protection Officer 2007; Matsuki *et al.* 2001).

None of the three gene regions sequenced support the distinction of *U. rangelii* from *P. psidii*, or provide any evidence that a “species complex” exists. As these regions have all been shown to be discriminatory at species level for rusts, including the genus *Puccinia*, we conclude that the morphological distinction of *U. rangelii* does not represent a genetically distinct species. Discussion of these results with rust taxonomists indicate that *U. rangelii* may be better placed as a variety of *P. psidii* (*Puccinia psidii* var. *rangelii*).
Table 3. Current known hosts of *Uredo rangelii* based on literature, surveys and host testing

<table>
<thead>
<tr>
<th>Tribes</th>
<th>Host of <em>Uredo rangelii</em></th>
<th>Cultivars/Varieties</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUCALYPTAEAE</td>
<td><em>Eucalyptus agglomerata</em></td>
<td>Present Study</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td><em>E. cloeziana</em></td>
<td>Present Study</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td><em>E. grandis</em></td>
<td>Present Study</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td><em>E. pilularis</em></td>
<td>Present Study</td>
<td></td>
</tr>
<tr>
<td>LEPTOSPERMEAE</td>
<td><em>Agonis flexuosa</em></td>
<td>'Afterdark'</td>
<td>Carnegie et al. (2010)</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>A. flexuosa</em></td>
<td>'Burgundy'</td>
<td>Carnegie et al. (2010)</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>A. flexuosa</em></td>
<td>'Jedda’s Dream'</td>
<td>Carnegie et al. unpublished</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>Leptospermum rotundifolium</em></td>
<td></td>
<td>Carnegie et al. unpublished</td>
</tr>
<tr>
<td>MELALEUCEAE</td>
<td><em>Callistemon viminalis</em></td>
<td>'Hannah Ray'</td>
<td>Carnegie et al. (2010)</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>Callistemon sp.</em></td>
<td>'Kings Park Special’</td>
<td>Carnegie et al. unpublished</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>Melaleuca quinquenervia</em></td>
<td></td>
<td>Present Study</td>
</tr>
<tr>
<td>MESTROSIDERAE</td>
<td><em>Metrosideros collina</em></td>
<td>'Dwarf'</td>
<td>Carnegie et al. unpublished</td>
</tr>
<tr>
<td>MYRTEAE</td>
<td><em>Gossia (=Austromyrtus) inophloia</em></td>
<td>'Aurora’</td>
<td>Carnegie et al. unpublished</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>G. inophloia</em></td>
<td>'Blushing Beauty’</td>
<td>Carnegie et al. unpublished</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>Myrtus communis</em></td>
<td></td>
<td>Simpson et al. (2006)</td>
</tr>
<tr>
<td>SYNCARPIEAE</td>
<td><em>Syncarpia glomulifera</em></td>
<td></td>
<td>Carnegie et al. (2010)</td>
</tr>
<tr>
<td>SYZYGIEAE</td>
<td><em>Syzygium jambos</em></td>
<td></td>
<td>Simpson et al. (2006)</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>S. wilsonii subsp. wilsonii × S. leuhmanii</em></td>
<td>'Cascade’</td>
<td>Carnegie et al. unpublished</td>
</tr>
<tr>
<td>TRISTANIAE</td>
<td><em>Tristania neriifolia</em></td>
<td></td>
<td>Carnegie et al. unpublished</td>
</tr>
</tbody>
</table>
Conclusions

_Uredo rangelii_ does not have a restricted host range. The present study, and observations from recent surveys, reveals at least 15 species in 11 genera are susceptible to _U. rangelii_. This supports the observation that _U. rangelii_ is part of the guava rust complex, and likely to have a similar host range.

Key commercial forestry species are susceptible to _U. rangelii_, including those used in plantations and native forestry in NSW and Queensland.

None of the three gene regions sequenced support the distinction of _Uredo rangelii_ from _Puccinia psidii_, or provide any evidence that a “species complex” exists. As these regions have all been shown to be discriminatory at species level for rusts, including the genus _Puccinia_, we conclude that the morphological distinction of _U. rangelii_ does not represent a genetically distinct species.
Recommendations

1. Forestry organisations (both commercial and environmental) to provide a prioritised list of host species for ongoing host testing. Initially this will be at a species level, but in the future would include provenances, families etc.

2. Forest production nurseries to be made aware of the implications of myrtle rust affecting the production of seedlings of susceptible species, and potential management options if/when myrtle rust expands its geographic range in Australia.

3. Awareness campaign of forest industries regarding the change in status of myrtle rust from a disease with a restricted host range to a disease with an expanding host range.
References


Ruiz RAR, Alfenas AC, & Ferreira FA (1989) Influência da temperatura, luz e origem do inóculo sobre a produção de uredosporos e teliosporos de Puccinia psidii. [Effect of
temperature, light and inoculum source on teliospore and uredospore production of *Puccinia psidii.* Fitopatologia Brasileira 14:70–73.


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