INDUSTRY BIOSECURITY PLAN

FOR THE NURSERY & GARDEN INDUSTRY

Threat Specific Contingency Plan

Guava (eucalyptus) rust

Puccinia psidii

Plant Health Australia

March 2009

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1 Purpose and background of this contingency plan

This contingency plan provides background information on the pest biology and available control measures to assist with preparedness for an incursion into Australia of guava rust (*Puccinia psidii*). It provides guidelines and options for steps to be undertaken and considered when developing a Response Plan to this pest. Any Response Plan developed using information in whole or in part from this Contingency Plan must follow procedures as set out in PLANTPLAN and be endorsed by the National Management Group prior to implementation.

This contingency plan was developed for the Nursery and Garden Industry Australia (NGIA), and therefore is focussed on production nurseries covered by this association. In the event of an incursion, operations not covered by the NGIA (e.g. retail nurseries) will not be eligible for Owner Reimbursement Costs, as defined in the Emergency Plant Pest Response Deed, if affected by actions carried out under the Response Plan.

The information for this plan has been primarily obtained from the "Forestry, rural and urban biosecurity plan – pest specific contingency plan for *Puccinia psidii* (draft)", prepared by the Office of the Chief Plant Protection Officer (OCPPO) in May 2007. Modifications and additions to the plan have been completed to make the information relevant to an incursion of *P. psidii* in the nursery and garden industry. The use of the OCPPO contingency plan in this manner has been authorised by OCPPO.

2 Australian nursery industry

The Australian nursery industry is a significant horticultural sector with a combined supply chain (production to retail/grower) valued at more than $6 billion dollars annually. The industry employs approximately 45,000 people spread over more than 20,000 small to medium sized businesses including production nurseries and retail outlets. The industry is located predominantly along the Australian coastline and in major inland regions servicing urban and production horticulture.

Nursery production adds value to Australia‟s primary industry‟s sector and in 2008/2009 is forecast to contribute more than $2 billion to the national economy. Nursery production is a highly diverse primary industry servicing the broader $14 billion horticultural sector within Australia (Table 1).

Table 1. *Nursery production supply sectors within Australian horticulture*

Production Nursery	Horticultural markets	Economic value
Container stock[1]	Ornamental/urban horticulture	$2 billion retail value
Foliage plants[1]	Interior-scapes	$87 million industry
Seedling stock[2]	Vegetable growers	$3.3 billion industry
Forestry stock[3]	Plantation timber	$1.7 billion industry
Fruit and nut tree stock[2]	Orchardists (citrus, mango, etc)	$5.2 billion industry
Landscape stock[1]	Domestic & commercial projects	$2 billion industry
Plug and tube stock[4]	Cut flower	$319 million industry
Revegetation stock[1]	Farmers, government, landcare	$109 million industry
Mine revegetation	Mine site rehabilitation	Value unknown
	Total horticultural market value	**$14.5 billion**

3 Eradication or containment decision matrix

Eradication of *P. psidii* will only be technically feasible if the rust is detected while still contained within a very small area and the spore load is quite light. Determination of the extent of the incursion should be completed quickly and commence as soon as there is a reasonable suspicion of the presence of *P. psidii*, without waiting for confirmation, as any delay may be critical in allowing further spread. If the initial detection is contained within an area small enough and/or isolated enough that eradication is considered feasible, eradication procedures should also be implemented immediately, without waiting for the results of delimiting surveys, as any delay will allow further spore production and dissemination, reducing the likelihood of successful eradication.

The limits on technical feasibility will also limit eradication costs. As *P. psidii* would potentially have a high environmental impact, the decision should be based solely on technical feasibility. In addition to direct economic costs, eradication may cause environmental damage as all the fungicides that have been shown to be effective in control of *P. psidii* are toxic to aquatic organisms, and some are also toxic to insects or to mammals. However, any damage caused by an eradication attempt will be confined to a much smaller area than that which is likely to sustain environmental damage from the introduction of *P. psidii*. The greatest cost of an eradication attempt is likely to be in follow-up surveys, which will be needed to verify the success of the eradication. Two years with suitable weather conditions and no detections of the disease will be necessary before rust-free status can be declared.

The decision matrix to aid in the decision between eradication or containment is shown in Figure 1.

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[1] Data sourced from Market Monitor

[2] Data sourced from Horticultural Handbook 2004

[3] Data sourced from ABARE 2005

[4] Data sourced from industry

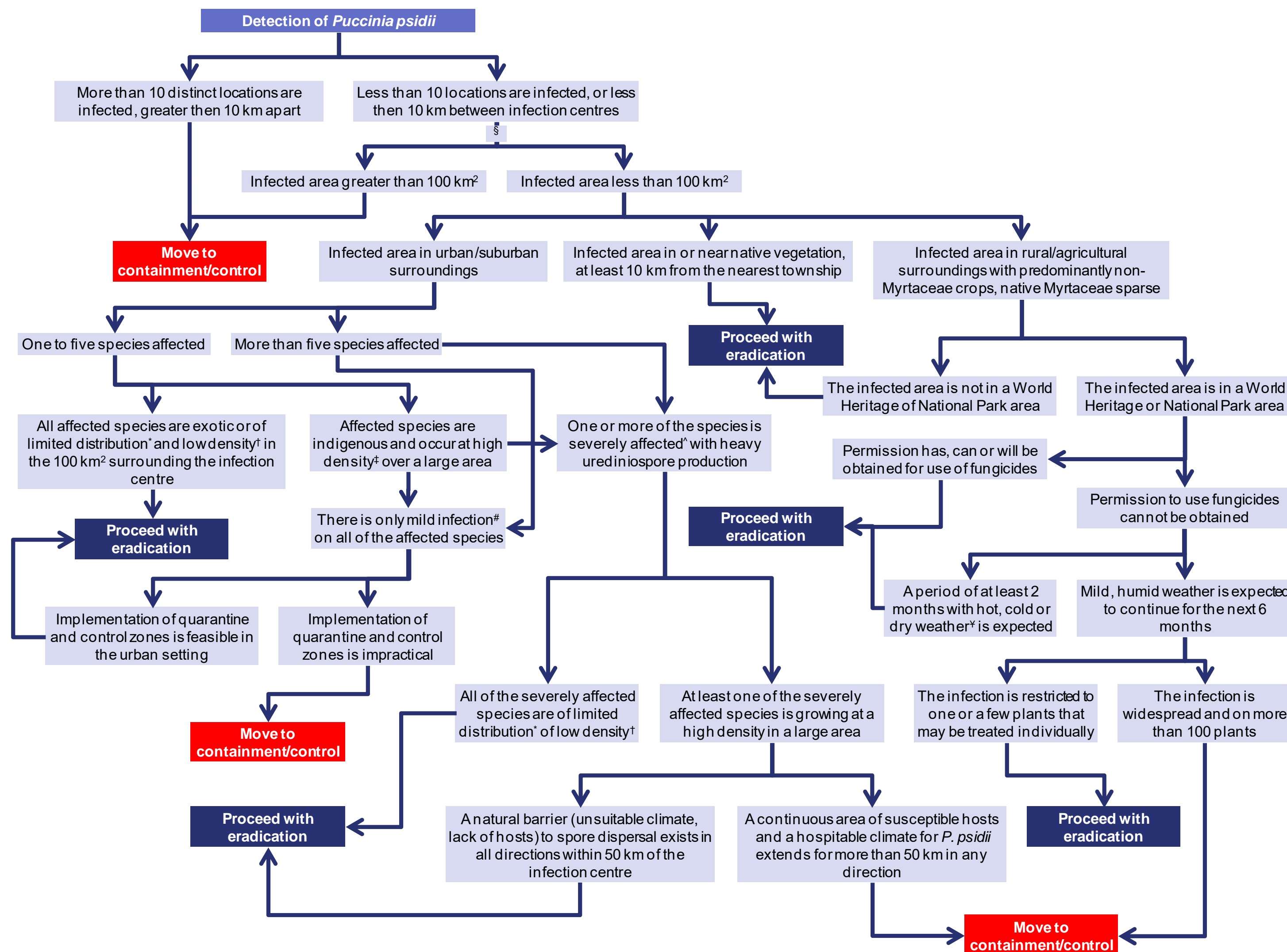

Figure 1. *Decision matrix for the eradication or control of P. psidii should it be detected in Australia*

Notes/Clarifications

§ Complete the questionnaire for each infection centre. If the outcome for any one of these centres is a „move to containment/control", the procedure applies to all infection centres, so there is no need to complete the questionnaire for the remaining centres.

* Limited distribution: The host range extends over an area of less than 100 km^2.

† Low density: The mean distance between individual plants of the same species, or between clumps of the same species is greater than 1 km.

‡ High density: plants are distributed throughout the affected area and the mean distance between individuals or clumps of the same species is less than 500 m.

\# Mild infection: Affected leaves are not distorted and lesions are small (less than 1 mm diameter) and covering less than 10% of the area of any leaf. No defoliation. If fruit or flower buds are affected, lesions are small, covering less than 10% of the surface area of the fruit.

^ Severe infection: Leaves are distorted, tips may be defoliated. Affected fruit rots and drops before maturing.

¥ Hot, cold or dry weather: Temperatures not lower than 24˚C or not above 15˚C. Relative humidity above 80% for less than 6 h per day.

4 Pest information/status

4.1 Pest details

Common names: Guava rust, eucalyptus rust

Scientific name: *Puccinia psidii* (anamorph *Uredo psidii*)

Synonyms: Many species have been listed as synonyms of *P. psidii* (Table 2). Walker (1983) also listed *U. subneurophila* Speg. as a probable synonym, though Hennen *et al*. (2005) have established that this is a distinct species and the host is not a Myrtaceae.

Table 2. *Rust species recorded on Myrtaceae in south/central America that are most likely P. psidii (Walker, 1983; Satão* et al., *2001; Hennen* et al., *2005; Simpson* et al., *2006).*

Name	Recorded hosts
Aecidium glaziovii	Myrtaceae, indeterminate
Bullaraia psidii	
Caeoma actinostemonis	
Caeoma eugeniarum	
Puccinia actinostemonis	Myrtaceae, indeterminate, erroneously reported as *Actinostemon* sp.
P. barbacensis	Myrtaceae, indeterminate
P. brittoi	*Campomanesia maschalantha*
P. camargoi	*Melaleuca leucodendron*
P. cambucae	*Eugenia* sp., *Marlierea edulis*
P. eugeniae	*Eugenia grandis*
P. grumixamae	*Eugenia brasiliense*
P. jambolani	*Eugenia jambolana*
P. jambosae	*Syzygium jambos*
P. jambulana	*Syzygium jambos*
P. neurophila	Myrtaceae, species not identified
P. rochaei	*Marlierea edulis, Myrcia jaboticaba, Myrciaria* sp.
Uredo cambucae	*Eugenia edulis*
U. eugeniarum	*Eugenia* sp., *E. uvalha*
U. goeldiana	*Eugenia* sp., *Marlierea edulis*
U. myrciae	*Myrcia acuminata*
U. myrtacearum	*Eugenia grandis, E. pungens, E.* sp.
U. neurophila	Myrtaceae, species not identified
U. pitanga	Eugenia pitanga

Name	Recorded hosts
U. puttemansii	
U. rochaei	*Marlierea edulis, Myrcia jaboticaba, Myrciaria cauliflora*
U. seclusa	Myrtaceae, species not identified
U. flavidula	Species of *Eugenia, Marlierea, Myrcia, Psidium, Syzygium*
U. rangelii	*Myrtus communis, Syzygium jambos*

4.1.1 Background

Puccinia psidii causes a rust disease on a broad range of hosts in the Myrtaceae and Heteropyxidaceae families. It is native to South America where it can cause severe disease in eucalypt plantations and other introduced Myrtaceae. The pathogen has recently expanded its geographical range to Hawaii, increasing concerns about the potential for an incursion in Australia. *P. psidii* originated in South and Central America, and all its indigenous hosts are Myrtaceae. One species from another family in the Myrtales is also susceptible to *P. psidii* infection (Alfenas *et al*., 2005). This is *Heteropyxis natalensis*, a native of South Africa and one of three *Heteropyxis* species in the single genus family Heteropyxidaceae. It is possible that genera in other Myrtales family may also be susceptible, but the probability is low.

Brazil, where *P. psidii* is widespread, has approximately 1000 species in 23 genera of native Myrtaceae (Landrum & Kawasaki, 1997) and *P. psidii* has been reported on at least 10 of these genera (Marlatt & Kimbrough, 1979; Hennen *et al.,* 1982; Walker, 1983; Rayachhetry *et al.,* 2001). Australia has 70 genera and 1646 species of native Myrtaceae, approximately half of the world"s 147 genera and 3,000 species (Department of Environment and Heritage, 2004a). Eighty-three native Australian Myrtaceae species from 19 genera have been tested for susceptibility to *P. psidii*, with 73 species from 16 genera showing some degree of susceptibility (for more details refer to Section 4.2; Castro *et al.,* 1983; Rayachhetry *et al.,* 2001; Alfenas *et al.,* 2003; Tommerup *et al.,* 2003; Dankers *et al*., 2004; Alfenas, Tommerup & Zauza, unpublished results). More Australian native plant species belong to the Myrtaceae than to any other family. Myrtaceae are dominant species in many of the major Australian ecosystems, from tall forests to swamps and wetlands.

In testing, susceptibility to *P. psidii* varied within Australian host species depending on the origin of seedlots (Tommerup *et al.,* 2003), and rust biotypes also show variation in host range (Aparecido *et al.,* 2003a). Therefore species which have so far tested as resistant to *P. psidii* may prove susceptible with further challenges. However, if the current ratio of susceptible to resistant species is maintained, it is projected that 1447 of Australia"s native plants are potential hosts. The disease is not usually severe on hosts that have had a long association with the pathogen. However, if the pathogen becomes established in Australia, its effects on naive hosts may be far more unpredictable. In addition, species with low levels of susceptibility may also play a significant role in maintaining the pathogen and assisting its spread while not being greatly threatened themselves.

A guava rust incursion into Australia would impact the native biodiversity, commercial operations that utilise Myrtaceae such as nursery operations, forestry and wildflower production, as well as adversely impacting on urban streetscapes and gardens. Host plants are generally more susceptible at the seedling stage, making plant propagation and forest regeneration highly vulnerable. Mature eucalypts are generally resistant to the disease (Junghans *et al.,* 2003), although hosts such as allspice and guava are susceptible to the pathogen in their young tissue even in older trees. There is also a strong suggestion from Florida and South America that *P. psidii* is likely to have switched hosts on more than one occasion resulting in a devastating impact on the new host.

Unlike most other rusts, *Puccinia psidii* can complete its entire life cycle on a single host plant. High humidity and moderate temperatures (15-25°C) favour spore germination. The urediniospores can survive for weeks (Aparecido *et al.,* 2003a) and are readily wind-dispersed. The urediniospore cycle can be as short as 10 days, allowing a rapid build-up of inoculum levels and the capacity to cause epidemics when climatic conditions are suitable. Therefore unless an incursion is detected at an early stage in a relatively geographically isolated location, it is a disease that will be very difficult to eradicate. It is also highly unlikely that containment will be possible. Eradication of a rust disease has been attempted in only a few situations, with only two known success cases.

P. psidii arrived in Hawaii in 2005 and within a few months of its first detection had spread through all but one of the Hawaiian islands (Killgore & Heu, 2005). When first reported in Florida, the rust had already spread to several locations up to 35 km apart (Marlatt & Kimbrough, 1979). When the pathogen developed a capacity to infect allspice in the 1930s, the allspice-infecting strain had already spread over a wide area before concerns were raised (McLachlan, 1936).

If the rust becomes established in Australia, all neighbouring, climatically suitable areas are likely to be colonised within a year or two of its establishment, due to rapid wind dispersal and the abundance of known and potential host species throughout the country. For example, past incursions of new strains and species of rusts pathogenic to wheat have spread quickly throughout the wheat-growing regions of Australia (NSW Department of Primary Industries, 2003). However, if the rust establishes first in eastern Australia, there may be a delay before it establishes in the west, as climatic conditions are expected to be less favourable in Western Australia.

In the event of an incursion, early detection and rapid response will be critical to the success of any control program.

4.1.2 Life cycle

Puccinia psidii has an autoecious, but incomplete, life cycle (Figure 2; Coutinho *et al.,* 1998). Urediniospore germination leads to appressorium formation and haustorium development (for images of these structures refer to Section 9.4.1) that may result in formation of either urediniosori (inner cycle in Figure 2) or teliosori (outer cycle in Figure 2). High humidity and moderate temperatures (15-25°C) favour spore germination. The urediniospores can survive for weeks (Aparecido *et al.,* 2003a) and are readily wind-dispersed. The urediniospore cycle can be as short as 10 days, allowing a rapid build-up of inoculum levels and the capacity to cause epidemics when climatic conditions are suitable.

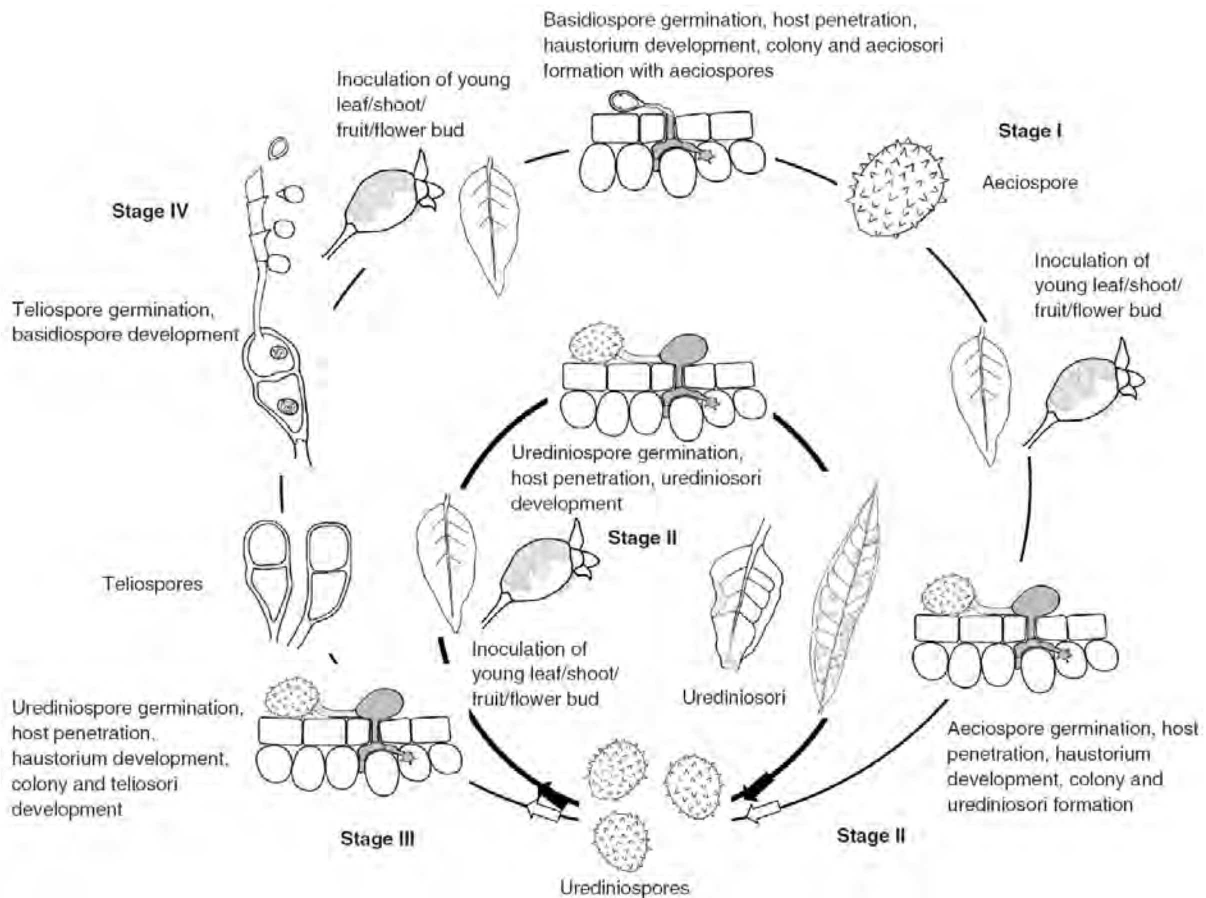

Figure 2. *Schematic of the P. psidii life cycle. Figure taken from Glen* et al. *2007.*

4.2 Affected hosts

4.2.1 Host range

P. psidii infects new leaves and shoots in a large number of genera and species in the Myrtaceae and Heteropyxidaceae families (Table 3), with an estimated 1447 species of possible hosts present in Australia. Rust infecting any plants in these families should be investigated thoroughly to rule out the possibility of *P. psidii* infection. Only two other rusts have been recorded on Myrtaceae in Australia. Both are limited in host range and geographic distribution and are morphologically distinct from *P. psidii* (refer to Section 4.3). In South and Central America, where *P. psidii* is present, many rusts recorded on Myrtaceae may be synonymous with *P. psidii* (Table 2).

The major host species of guava rust worldwide include:

- *Callistemon speciosus* (grey bottlebrush)
- *Eucalytptus* species, including *E. camaldulensis* (red gum), *E. citriodora* (lemon-scented gum), *E. cloeziana* (Gympie messmate), *E. globulus* (Tasmanian blue gum), *E. grandis* (saligna gum), *E. maculata* (spotted gum), *E. microcorys* (tallowwood), *E. paniculata* (grey ironbark), *E. pellita* (red mahogany), *E. phaeotricha*, *E. punctata*, *E. saligna* (Sydney blue gum), *E. tereticornis* (forest red gum), *E. urophylla* (Timor mountain gum)
- *Eugenia, E. dombeyi* (brazil cherry), *E. pyriformis* var. *uvalha*, *E. uniflora* (surinam cherry)

- *Marlierea edulis*
- *Melaleuca, M. quinquenervia* (melaleuca)
- *Pimenta, P. dioica* (Allspice), *P. racemosa* var. *racemosa* (bay-rum-tree)
- *Psidium guajava* (guava), *P. guineense* (Guinea guava)
- *Syzygium cumini* (black plum), *S. jambos* (rose apple), *S. malaccense* (malay-apple)

Table 3. *Genera of plants recorded as hosts of P. psidii*[5]

Genus[6]	Number of susceptible species	Genus	Number of susceptible species
Angophora	1	*Myrcia*[7]	2
Astartea	2	*Mycianthes*	2
Callistemon	5	*Myrciaria*[7]	2
Campomanesia	3	*Mytus*	1
Corymbia	2	*Pericalymma*	1
Eremaea	2	*Pimmenta*	4
Eucalyptus	29	*Phyllocalyx*	1
Eugina[7]	14	*Pseudomyrianthes*	1
Heteropyxis	1	*Psidium*	4
Hypocalymma	1	*Regalia*	1
Kunzea	2	*Syncarpia*	1
Marlierea	1	*Siphoneugena*	1
Melaleuca	7	*Syzygium*[7]	4
Metrosideros	1	*Thryptomene*	1

For additional information relating to *P. psidii* host range refer to Appendix 2 (Section 9.2).

4.2.2 Geographic distribution

Important climatic factors for propagation of the pathogen include daily minimum and maximum temperatures and leaf wetness period. Low light intensity (overcast conditions) also favours rust development.

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[5] Note that this is not a comprehensive host range as many species in the Myrtaceae are yet to be tested. This list is included as an indication of the unusually broad host range of *P. psidii* in comparison with other *Puccinia* species

[6] All genus are members of the Myrtaceae family with the exception of *Heteropyxis*

[7] These species are listed as recorded by the authors of the original reports. Since the time of reporting some species have been transferred between *Eugenia* and *Syzygium*, and between *Myrcia* and *Myrciaria*.

4.2.2.1 CURRENT DISTRIBUTION

Guava rust is native to South America, where it is wide spread and causes severe disease in eucalypt plantations. Currently, guava rust is found:

- **South America:** Argentina, Brazil, Colombia, Ecuador, Paraguay, Uruguay, Venezuela
- **Central America:** Costa Rica, Cuba, Dominica, Dominican Republic, El Salvador, Guatemala, Jamaica, Puerto Rico, Trinidad and Tobago
- **North America:** Mexico, USA (Florida, Hawaii)
- **Asia:** China (Taiwan[8]), India

4.2.2.2 POTENTIAL DISTRIBUTION IN AUSTRALIA

A bioclimatic analysis of locations where the disease had occurred on *Eucalyptus* species has been used as the basis for predicting the risk of *P. psidii* establishment in different regions of Australia if the pathogen were introduced (Booth *et al.*, 2000). A preliminary risk map for Australia highlights the areas most likely to foster epidemic disease in eucalypts if the pathogen is introduced, however more detailed data are required to accurately predict the likelihood of *P. psidii* establishment. A subsequent analysis partly based on results from controlled environment experiments (Ruiz *et al.,* 1989) produced a map that expands the previously highlighted area and provides five categories of risk (Booth & Jovanovic, pers. comm.; Figure 3). A third risk map, developed using APHIS NAPPFAST software (Figure 4) highlights a larger area that is predicted to be conducive to rust establishment in at least 1 year out of 10. This map provides 9 risk categories and expands even further the favourable area in southern Queensland and northern New South Wales. It also highlights parts of South Australia, Western Australia and Victoria as likely to be conducive to rust infection for 3-4 years each decade. The parameters used to develop this map included an average maximum temperature of less than 34°C and average minimum of at least 13°C, with 5-25 rain days per month. Both of these maps have been developed using mean monthly data and therefore cannot be expected to provide assurance that an area not highlighted on the map would be free of risk from *P. psidii*.

[8] Only a single report (Wang, 1992), and the pathogen was never definitively identified and has not become established

Figure 3. *Guava rust risk areas in Australia based on Eucalyptus distribution and climatic conditions. Figure taken from Glen* et al., *2007.*

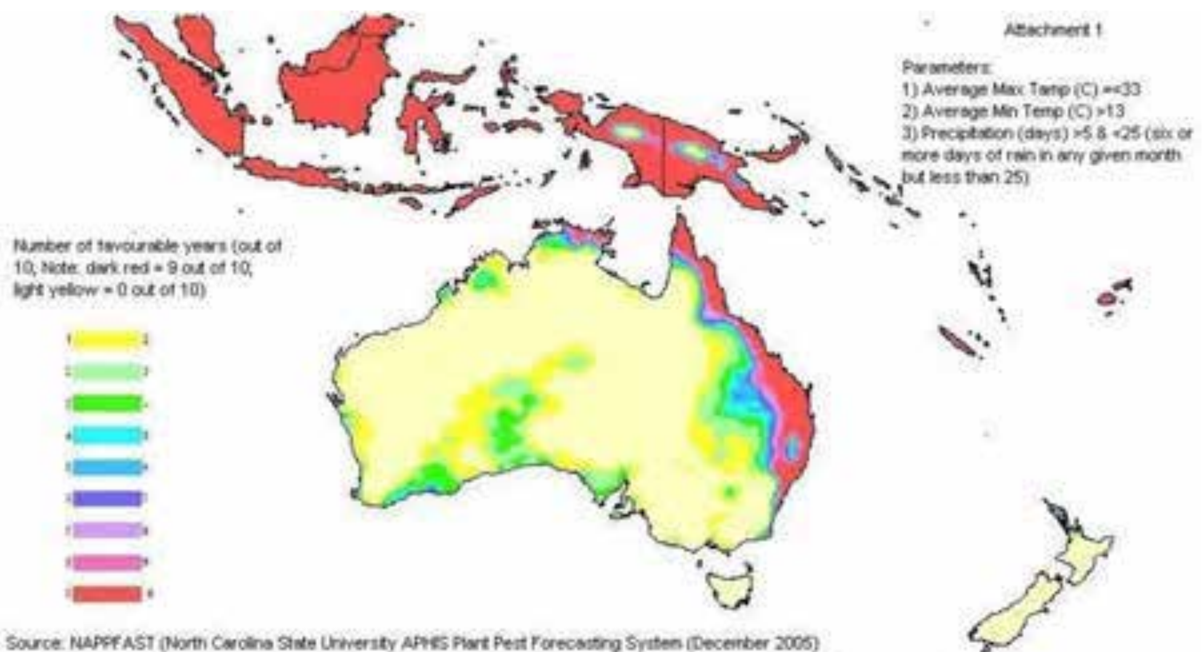

Figure 4. *An alternative rust risk map for Australia developed using NAPPFAST software (Borchett & Magarey, 2004)*

Another caveat on the current risk mapping is that the presence of a highly susceptible host is important in defining geographical range of rust. For example, in Brazil, *P. psidii* occurs commonly on *S. jambos*, but only infects *Eucalyptus* spp. in more climatically favourable regions. Therefore the

selection of data based on a limited host range does not accurately predict the risk of epidemics in the diverse native Australian Myrtaceae. Susceptibility is also affected by the genetics of the host and pathogen. There appear to be patterns of host specificity among biotypes of the rust, and seedlots from different provenances of some plant species vary widely in their degree of susceptibility/resistance (Tommerup *et al*., 2003). It is rare to achieve 100% susceptibility or 100% resistance in inoculation trials, however some Australian species have shown 100% susceptibility in glass-house tests (Zauza & Alfenas, unpublished).

Once established in one area of Australia, *P. psidii* is expected to spread very quickly over the climatically suitable regions, like other *Puccinia* species (Park *et al*., 2002), as the urediniospores have a short generation time of approximately 10 to 23 days and are wind-dispersed over large distances (Viljanen-Rollinson & Cromey, 2002). Insect-vectored spread may also contribute. The strain attacking allspice in Jamaica covered an area of 5,000 km^2 one year after it was first reported (Smith, 1935). The likelihood of eradicating a wind-dispersed pathogen with a short reproductive cycle from an area of this size is very low.

The risk of an incursion will also be greatly increased if *P. psidii* becomes established in countries to the north of Australia (e.g. Indonesia and Papua New Guinea, that both have extremely suitable climates) as these are within wind-borne travelling range for rust spores. Cereal and poplar rusts have travelled from Australia to New Zealand on wind currents (Wilkinson & Spiers, 1976; Viljanen-Rollinson & Cromey, 2002). An incursion from this pathway is likely to establish in a remote region, delaying detection and increasing the risk of substantial spread before detection and therefore decreasing the probability of successful eradication.

P. psidii is unlikely to establish in arid regions of Australia, due to its requirement for a long period of leaf wetness for germination of the urediniospores (Ruiz *et al.,* 1989). The theoretically more durable teliospores are produced at less favourable temperatures (Alfenas *et al.,* 2003; Aparecido *et al.,* 2003b). These teliospores could be an important inoculum source in regions where climatic conditions are less frequently favourable for *P. psidii*.

4.2.3 Symptoms

4.2.3.1 ON SUSCEPTIBLE HOSTS

Lesions are produced on young, actively growing leaves and shoots (Figure 5), as well as on fruits (Figure 6) and sepals. Lesions are brown to grey with masses of bright yellow or orange-yellow urediniospores (Figure 7). Occasionally, lesions have dark brown teliospores or a mixture of the two spore types (Figure 5H and Figure 7). Older lesions show a purpling of their margins on leaves and shoots of many *Eucalyptus*, *Melaleuca* and *Callistemon* hosts. Lesions on fleshy fruits of *Eugenia, Psidium* and *Syzygium* may not have obvious margins; due to being covered with heavy spore masses when young and rot caused by secondary pathogens as the fruits ripen. Severe rust disease in young trees may kill shoot tips, causing loss of leaders and a bushy habit. Prolific branching and galling in eucalypts (Figure 6F) is a sign of previous rust disease. Persistent localized lesions and stem swellings on *M. quinquenervia* have also been reported and illustrated (Rayachhetry, 2001). Similar symptoms may occur in other species but have not been recorded as many host species have only been tested at seedling stage.

Additional images of host symptoms can be found in Appendix 3 (Section 9.3).

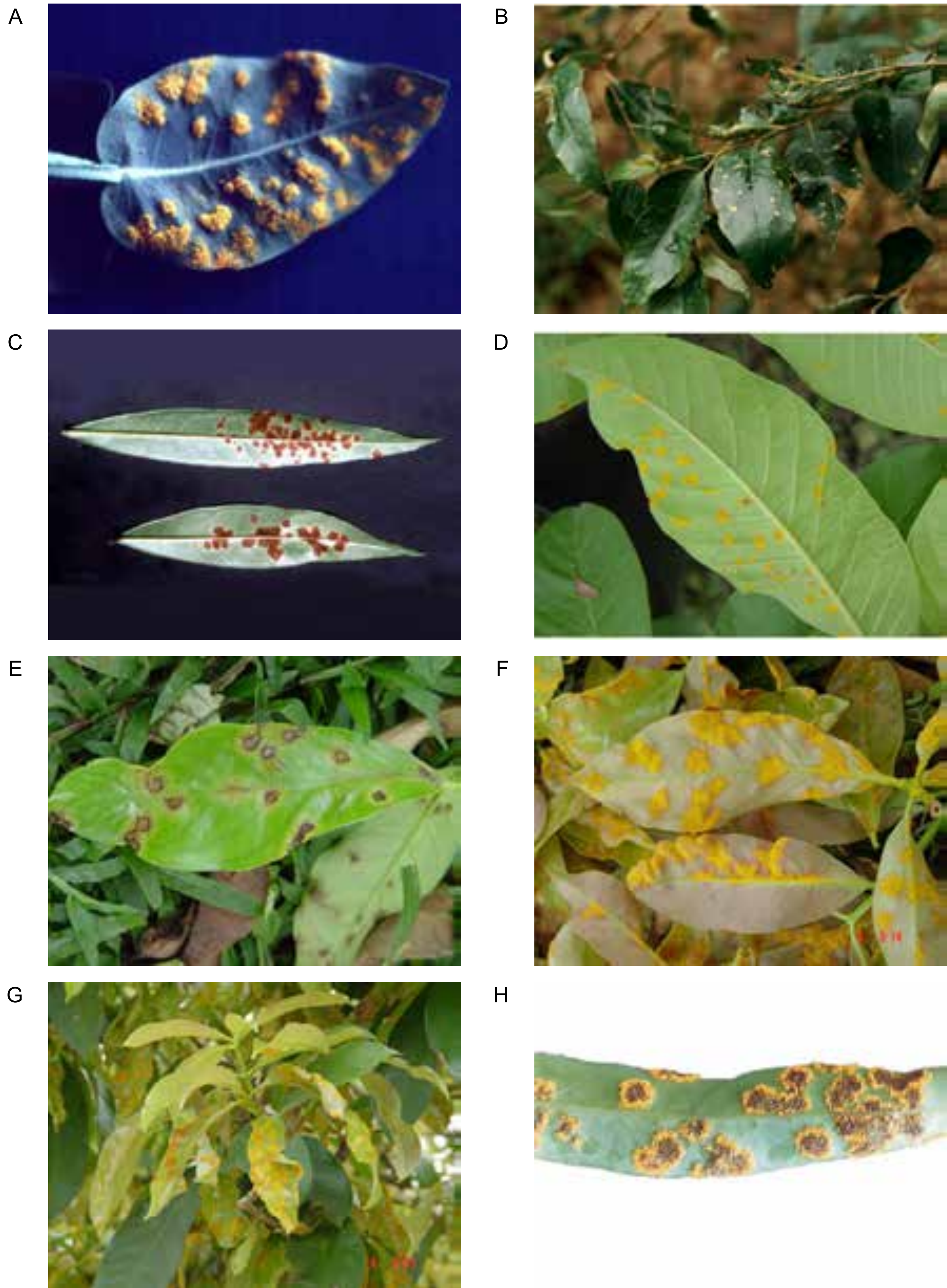

Figure 5. *Examples of leaf symptoms displayed on host plants infected with P. psidii. (A-B) Eucalyptus sp., (C) Syzygium sp., (D) guava, (E) Malabar plum, (F-G) Malaysian apple and (H) Syzygium jambos (urediniospores – yellow, teliospores – brown). Images taken from PaDIL (**www.padil.gov.au**).*

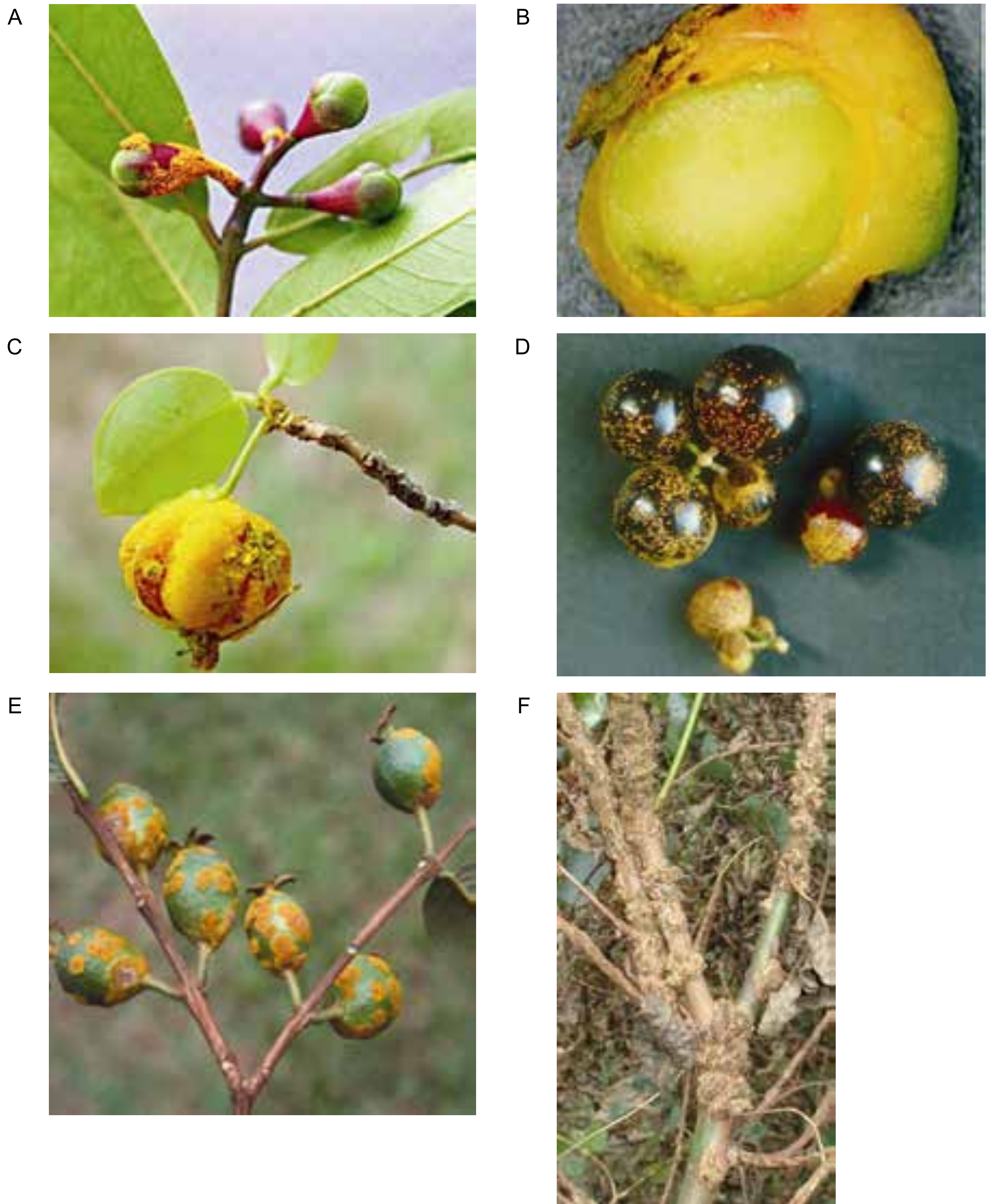

Figure 6. *Examples of P. psidii on fruit, flower bud and stems. (A) Flower buds of S. jambos, fruit of Eugenia uniflora (B-C), Myrciaria cauliflora (D) and Psidium guajava (E), and stem galls produced on E. grandis (F). Images © Forest Pathology and Genetics of Plant Pathogen Interactions Laboratory, Federal University of Viçosa (A-E) or CSIRO (F).*

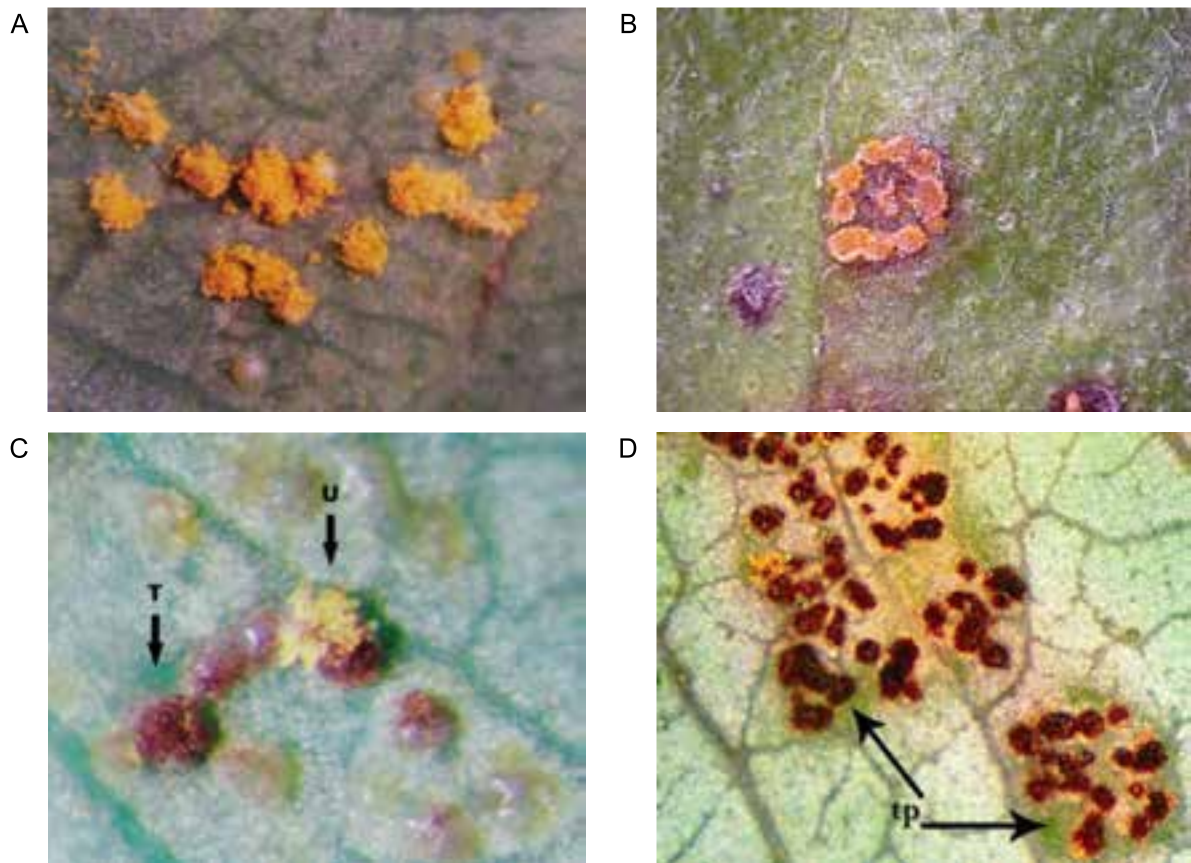

Figure 7. *P. psidii pustules on leaves of susceptible hosts. Images show urediniosori (A-C) and teliosori (C-D). Images taken from PaDIL (A & C;* ***www.padil.gov.au****) or © Hank Dankers, Plant Diagnostic Clinic, University of Florida (B) or Forest Pathology and Genetics of Plant Pathogen Interactions Laboratory, Federal University of Viçosa (D).*

4.2.3.2 ON RESISTANT HOSTS

On resistant plants the pathogen may induce a hyposensitive reaction (HR), which produces chlorotic halos or brown spots but no urediniospores or teliospores (Figure 12).

4.3 Diagnostic information

An expert in Myrtaceae leaf and shoot pathogens and preferably with a good knowledge of *P. psidii* or of rust pathogens in non-Myrtaceae hosts should investigate lesions for rust spores using appropriate magnification without destroying the lesions or contaminating them with histological fluids. Urediniospores and teliospores of *P. psidii* are readily distinguishable using high power transmission microscopy.

Diagnosis of *P. psidii* can be achieved through microscopic examination of spores or through molecular (nested PCR) approaches. The Nested PCR approach is the most sensitive, being able to detect a single urediniospore (Langrell *et al.,* 2008). For detailed diagnostic information for *P. psidii* refer to Appendix 4 (Section 9.4)

4.4 Pathogen risk ratings and potential impacts

A pest risk analysis has been carried out on this pathogen, taking into account the entry, establishment and spread potentials, together with the economic and environmental impact of establishment. A summary of these ratings are shown in Table 4. Based on this information, *P. psidii* is considered a high to extreme overall risk to Australia.

Table 4. *Pest risk ratings for guava rust as determined in the Industry Biosecurity Plan for the Nursery and Garden Industry*

Potential or impact	Rating
Entry potential	High
Establishment potential	High
Spread potential	High-Extreme
Economic impact	High-Extreme
Environmental impact	High
Overall risk	**High-Extreme**

4.4.1 Damage

P. psidii infects young shoots, and depending on the host, floral buds and young fruits. It is unable to initiate infection in mature foliage. Infection of young, growing shoots and leaves can cause shoot death, defoliation and death of young trees. In Brazil, this has resulted in reduced growth and poor form in plantation seedlings, and loss of almost entire plantings, even of clones that had been classed as resistant based on greenhouse trials (Alfenas & Zauza, unpublished). In Australia, it may result in reduced rate of regeneration of dominant species in natural forests, altering the biodiversity and ecology. There will be greater impact of fire on habitat regeneration, as coppice regrowth is highly susceptible, and this will impact on forest management practices using fire as a vegetation management tool. Secondary rotting of fruits (e.g. in *Syzygium* and *Psidium* trees), and reduced aesthetic value in native forests and amenity plantings will also occur. In some areas the physical environment could be affected as a result of canopy decline with consequential erosion and water quality loss.

While it is unlikely that entire species would be eliminated, unless they are already critically endangered, genetic diversity in highly susceptible species could be greatly reduced. Plant community composition may be altered and the delicate balance of certain ecosystems may be adversely affected.

4.4.2 Impact

Table 14 (see Appendix 2) lists the known susceptible species in Australia (both endemic and introduced) and Table 5 indicates the different genera of Myrtaceae present in native vegetation, amenity plantings and commercial use. As 95% of Australian Myrtaceae species have not been exposed to *P. psidii*, many additional native Australian species are also likely to be hosts of *P. psidii*. To achieve a balance between exaggeration and understatement of the possible impact, the following discussion focuses mainly on the areas determined by NAPPFAST modelling to be conducive to rust

in 5 or more years out of 10 (blue, purple and red areas in Figure 4, here-after referred to as the „rust risk zone"), with some attention paid to the areas indicated to be conducive to rust in 3 or more years out of 10 (green and blue-green areas in Figure 4). This „rust risk zone" includes the areas predicted by Booth *et al.* (2000) to be highly favourable to *P. psidii* establishment, but also extends further inland, including the areas indicated by the revised model of Booth & Jovanovic, and indicating a lower risk in additional areas in southern Queensland, northern New South Wales, western South Australia and a few scattered areas of Western Australia.

Table 5. *Myrtaceae genera in the various land management categories throughout Australia*

Category	Myrtaceae genera[9]
Agriculture	***Melaleuca***, *Backhousia*
Urban plantings	*Agonis*, ***Astartea, Beaufortia***, ***Callistemon, Corymbia, Eucalyptus, Eugenia, Kunzea***, *Leptospermum*, ***Melaleuca, Myrciaria, Psidium, Regelia, Syncarpia, Syzygium, Thryptomene***
Conservation and native forests	*Acmena*, *Agonis*, ***Astartea,*** *Asteromyrtus, Austromyrtus, Backhousia, Barongia*, ***Beaufortia, Callistemon***, *Choricarpia*, ***Corymbia,*** *Decaspermum*, ***Eucalyptus, Eugenia, Kunzea***, *Leptospermum*, ***Melaleuca***, *Neofabricia, Pilidiostigma*, ***Regelia,*** *Rhodamnia, Rhodomyrtus, Ristantia, Sphaerantia, Stockwellia*, ***Syncarpia, Syzygium, Thryptomene,*** *Tristania*
Estuarine, coastal and riparian areas and swamps	*Agonis, Asteromyrtus*, ***Beaufortia, Callistemon***, *Calothamnus*, ***Eucalyptus***, *Leptospermum*, ***Melaleuca, Pericalymma***
Forest plantations	***Corymbia, Eucalyptus, Melaleuca***
Heathlands	*Asteromyrtus*, ***Beaufortia***, *Calothamnus*, ***Eremaea, Eucalyptus*** (mallee forms), ***Kunzea***, *Leptospermum*, ***Melaleuca***, *Neofabricia, Phymatocarpus*
Mangroves	*Osbornia*
Woodlands	*Agonis, Asteromyrtus, Calothamnus*, ***Corymbia***, ***Eremaea, Eucalyptus, Kunzea***, ***Melaleuca***, *Neofabricia*

4.4.2.1 ENVIRONMENTAL IMPACT

The natural occurrence of some of the known highly susceptible plant species is entirely or partially within the areas predicted to be highly favourable for rust epidemics. These include dominant species in natural ecosystems such as *Eucalyptus, Melaleuca* and *Syzygium*. As such, the environmental impact of *P. psidii* establishment would be high. The impacts in different ecosystems are outlined below, and for more details refer to OCPPO (2007).

Urban amenity and household plantings

Species of *Eucalyptus, Callistemon, Corymbia, Eugenia, Kunzea, Leptospermum, Melaleuca* and *Syzygium* are all widely grown in parks, gardens and street verges. The value of urban plantings to the community such as improving air quality, noise minimisation, city cooling, storm water velocity reduction and as wildlife corridors are hard to define in real terms. The loss of these significant urban assets will have both an environmental and community health impact that would add to any direct financial cost(s) attributed to crop losses due to *P.psidii.*

[9] Genera in bold have known susceptible species

Eucalypt forests and woodlands

Many *Eucalyptus* species are endemic to regions within the rust risk zone (Brooker & Kleinig, 1999, 2004) and three of these species are classed as threatened. Additional threatened and vulnerable species are endemic to regions in WA with a lower, but significant, rust risk. Another 203 species of *Eucalyptus* and *Corymbia* have their natural range wholly or mainly within the rust risk zone (Brooker & Kleinig, 1999, 2004), including *E. andrewsii*, which has been tested and shown to be highly susceptible to *P. psidii*. The introduction and establishment of the pathogen therefore has the potential to increase the number of vulnerable and threatened species. The natural occurrence of 33 species of *Leptospermum*, 20 *Melaleuca*, 15 *Callistemon*, 8 *Kunzea* and 3 *Neofabricia* species is also wholly or mainly within the rust risk zone (Wrigley, 1993), with 3 of these species classed as vulnerable.

Rainforest

All of Queensland‟s, and most of the Northern Territory‟s and New South Wales‟ rainforests lie within the rust risk zone. This includes at least 75% of Australia‟s 4.2 million hectares of rainforest and over half of Australia‟s World Heritage listed rainforest. Floyd (1979) lists 32 species of Myrtaceae trees in NSW rainforests, most of which also occur in Queensland‟s rainforests. A guava rust incursion would affect food sources for many species of bats, birds and mammals, as well as the tourism industry in these rainforest regions.

Melaleuca woodland

These occur mainly in Queensland and the Northern Territory in estuarine plains, seasonal swamps and beside streams and swamps (Department of Agriculture, Fisheries and Forestry, 2003), with the majority falling within the high risk zone. Many are too small to be mapped but they are ecologically important. Loss of riparian vegetation in these areas may result in erosion leading to poor water quality and impacting on aquatic organisms. In addition, wild plants of various *Melaleuca* species are harvested for essential oil production.

Heath communities

Mallee *Eucalyptus* and *Leptospermum* spp. are dominants in many of these plant communities. *Callistemon* species also occur in heath communities.

4.4.2.2 ECONOMIC IMPACT

Introduction of guava rust into Australia is likely to have the greatest economic impact on forestry operations, including at the production nursery and plantation levels. Agriculture, horticulture and tourism industries would also be highly affected, together with government agencies responsible for urban plantings. Economic impacts would arise from loss of production, eradication costs and negative effects on international trade due to phytosanitary clearance (refer to Appendix 9, Section 9.9).

Production Nurseries

Production nurseries are likely to be one of the most highly impacted industries upon the introduction of guava rust to Australia. Ideal environmental conditions for the propagation of *P. psidii* exist in production nurseries, in particular the high humidity, and a continuous supply of young, vulnerable shoots and leaves. Australian production nurseries also produce substantial numbers of susceptible host plants, particularly for use in forestry operations or urban plantings (see Table 5).

Guava rust introduction would impact through production losses (reduced quality and growth, as well as up to 90% mortality in infected plants), higher production costs (increases in chemical applications), restrictions in trade (through movement restrictions) and potential loss of markets (refer to Appendix 9, Section 9.9). If eradication or containment activities were put in place, movement restrictions would be imposed on plants from within the rust-affected areas. Additional movement controls would be applied to both host and non-host plant species, due to the possibility of spore movement on machinery and plant material during transport. As a result, all trade of plants from within the rust-affected area may be stopped for all, or some, of the eradication/containment period.

Agriculture

Small but increasing areas of *Backhousia* spp., *Leptospermum* spp. and *Melaleuca* spp. are grown for essential oil production. There are around 250 tea tree oil plantations (mainly *M. alternifolia*) in the rust risk area (Rural Industries Research and Development Corporation, 2005) covering an area of 4,500 ha and producing an export income of up to $18m. Most of the oil is exported and large drops in the price of the oil have occurred recently.

Floriculture

Species of *Baeckia*, *Callistemon, Chamelaucium, Kunzea, Leptospermum, Melaeuca* and *Thryptomene* are grown for export and plants of this sector are supplied by production nurseries. The industry is currently small but expanding and recent export income is difficult to determine. Two popular export species, *C. citrinus* and *K. baxteri* are both highly susceptible and grown within the rust risk zone.

Plantation forestry

Eucalypts are the main hardwoods grown in Australian plantations. The disease is not expected to affect acacias or softwoods. Breeding for resistance would be the main control method used, as chemical control is environmentally and economically unviable. Until resistant germplasm is available, losses in young plantations may be quite high in rust prone areas. Use of highly desirable species with very low levels of resistance (e.g. *E. cloeziana* and *E. phaeotricha*) would probably have to be discontinued in high risk areas in favour of more resistant species that may have a lower yield or less valuable timber. Detection of guava rust would impact both the direct production of new growth within plantations, as well as the ability to obtain new plant stock from affected production nurseries.

Table 6. *The most common plantation species grown in Queensland*

Species	End use	Estimated area (ha)	Susceptibility[10]
Corymbia citriodora subsp. *variegata*	Sawlog	5 000	LS, MS[11]
Eucalyptus argophloia	Sawlog	700	U
E. camaldulensis x *E. grandis*	Pulp	9 000[12]	U
E. cloeziana	Sawlog	1 500	HS
E. dunnii	Pulp	18 000	MS
E. grandis	Pulp	9 000[12]	LS, MS, HS

The economic impact of eucalypt rust on plantation forestry in Australia will to a large extent depend on its impact on *E. globulus* ssp. *globulus* (blue gum). Blue gum plantations dominate Australian hardwood plantations, accounting for well over 65% of the total area and it is a species of moderate susceptibility to eucalypt rust. *P. psidii* has been reported on *E. globulus* in Brazil (Alfenas *et al*., 2003) and Uruguay (Telechea *et al*., 2003). In Australia, blue gums are grown mainly in Tasmania, Western Australia, South Australia and Victoria.

Table 7. *The most common species in young plantations in New South Wales*

Species	End use	Estimated area (ha)	Susceptibility[13]
Corymbia citriodora subsp. *variegata*	Sawlog	7 500[14]	LS, MS[15]
Corymbia maculata	Sawlog	7 500[14]	U
E. camaldulensis x *E. grandis*	Sawlog	<200	U
E. cloeziana	Sawlog	800	HS
E. dunnii	Sawlog/Pulp	8,100/5,000	MS
E. grandis	Sawlog/Pulp	2,600/5,000	LS, MS, HS
E. nitens	Sawlog	<200	LS, MS
E. pilularis	Sawlog	4,000	LS, MS

Exports

Lack of phytosanitary certification may jeopardise exports of timber and wild-harvested and plantation grown Australian native wildflowers (including species from other plant families if they are grown in a rust-affected area). See plantation forestry (above) for export income from timber. Australian

[10] Susceptibility is reported as LS – low, MS – moderate, HS – highly susceptible or U – unknown. Susceptibility among seedlots of the same species may vary, hence more than one rating may be recorded for each species

[11] The subspecies of the *Corymbia citriodora* tested was not listed

[12] The area planted to *E. grandis* and *E. camaldulensis* x *E. grandis* is 9,000 ha in total

[13] Susceptibility is reported as LS – low, MS – moderate, HS – highly susceptible or U – unknown. Susceptibility among seedlots of the same species may vary, hence more than one rating may be recorded for each species

[14] The area planted to *Corymbia citriodora* subsp. *variegata* and *C. Maculata* was 7,500 ha in total

[15] The subspecies of the *Corymbia citriodora* tested was not listed

wildflower exports are valued at $50 million with the mid-north and north coast of NSW having seen recent rapid expansion in wildflower cultivation (NSW Department of Primary Industries, 2003). As production nurseries supply the bulk of plugs and seedlings for the cut flower industry, rust-affected production nurseries would impact the supply of new material for these enterprises.

Cost-benefit analysis

No rigorous cost benefit analysis has yet been undertaken for *P. psidii* in Australia. For estimates of these values refer to Office of the Chief Plant Protection Officer (2007).

5 Pest management

5.1 Response checklist

Guidelines for Response Checklists are still to be endorsed. The following checklist (Table 8) provides a summary of generic requirements to be identified and implemented within a Response Plan.

Table 8. *Checklist of requirements to be identified in a Response Plan*

Checklist item	Further information
Destruction methods for plant material, soil and disposable items	Sections 6.1.1 and 6.1.2
Disposal procedures	Section 6.1.5
Quarantine restrictions and movement controls	Section 6.3
Decontamination and property cleanup procedures	Section 6.5
Diagnostic protocols and laboratories	Section 4.3
Trace back and trace forward procedures	Section 6.6
Protocols for delimiting, intensive and ongoing surveillance	Section 5.2
Zoning	Section 6.4
Reporting and communication strategy	Appendix 8, Section 9.8

Additional information is provided by Merriman and McKirdy (2005)[16] in the Technical Guidelines for Development of Pest Specific Response Plans.

5.2 Surveys and epidemiology studies

Information provided in Section 5.2.1 to 5.2.3 provides a framework for the development of early detection and delimiting surveys for *P. psidii* in Australia. For additional information on these areas refer to Appendix 5 (Section 9.5).

[16] Available on the Plant Health Australia website (**www.planthealthaustralia.com.au**)

5.2.1 Technical information for planning surveys

When developing surveys for *P. psidii* presence and/or distribution, the following characteristics of the pathogen provide the basic biological knowledge that informs the survey strategy:

- Several races or biotypes of *P. psidii* are known to exist and may need to be considered
- Conditions of infection – urediniospores require high humidity and moderate temperatures for germination
- No specific vectors are known in Australia, although bees, birds, mammals and equipment/machinery can transport pollen and fruit carrying the pathogen
- *P. psidii* only infects young tissue (under 30-40 days of age)
- The pathogen can have a generation time of as little as 10 days under ideal conditions
- Urediniospores and teliospores can survive for long periods of time (up to several months)
- Spore survival in the soil in unknown
- Endemic host species numbers are substantial in Australia and widely dispersed
- Spores are readily wind dispersed over large distances
- Mechanical transmission risk is high on clothing, equipment and personal effects
- Significant proportions of Australia have favourable climatic conditions for *P. psidii* spread

5.2.2 Surveys for early detection of an incursion in a nursery

If an incursion of *P. psidii* is to be eradicated, it must be detected very early, before the spores have had the opportunity to disperse very far. It is therefore necessary to consider pathways and plan surveys and/or sentinel plantings accordingly. Important points to consider when developing early detection surveys are:

- The greatest risk currently comes from travellers and importations of host plants or other goods. Therefore surveys at importing nurseries, ports and populated areas are more critical than surveys of large areas of inaccessible rainforest.
- Sentinel plantings are recommended to be placed at air and sea ports, nurseries and other locations such as local council offices or visitor centres throughout the rust risk area for early detection of an incursion. *S. jambos*[17] is the most suitable sentinel plant species due to its high susceptibility to most biotypes, but the use of multiple species is recommended.
- Awareness information should be targeted at people who are in regular close contact with potential hosts in high risk areas or movement vectors (e.g. nursery operators, plantation forestry workers and National Park rangers and guides).
- Raised awareness of *P. psidii* in neighbouring countries, such as Papua New Guinea or Indonesia, to prevent establishment of the pest. If *P. psidii* becomes established in neighbouring countries it will be extremely difficult to prevent future establishment in Australia.

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[17] Care must be taken when using *S. jambos,* to collect fruit before maturity as this species is a potential weed in Australia

5.2.3 Delimiting surveys in the event of an incursion

- In the event of an incursion, delimiting surveys will be required to inform the decision-making process.
- The size of the survey area will depend on the size of the infected area and the severity of the infection as well as prevailing winds during the period prior to detection.
- All potential host species (i.e. all Myrtaceae; refer to Section 4.2) should be surveyed, with particular attention paid to the species in which the pathogen was initially detected.
- In addition to inspection of possible host plants, sentinel plants and spore traps should be established and the trapped material tested using the PCR diagnostic protocol (Section 9.4.2.1). Cyclone-style traps that deposit spores into a centrifuge tube rather than onto an adhesive surface are more suitable for PCR detection.
- If the incursion is in a populated area, publication and distribution of information sheets and appeals for public assistance may assist.

5.2.4 Collection and treatment of samples

Protocols of the collection, transport and diagnosis of suspect Emergency Plant Pests must follow PLANTPLAN (Plant Health Australia, 2008)

5.2.4.1 COLLECTION OF SPECIMENS

Type of specimen required for morphological and molecular diagnostic tests

Collect specimens of suspected rust and/or suspicious lesions on affected plant stems, flowers, fruits, shoots and leaves. One rust lesion with urediniospores and or teliospores is the minimum required for morphological and molecular diagnosis of *P. psidii*. With increasing numbers of lesions the chances of finding morphological features for a robust diagnosis are increased. In the absence of young material with spores, old lesions on young shoots, fruits and leaves can be used for molecular diagnostics (Langrell *et al.*, 2003a,b; Tommerup *et al.*, 2003; Langrell, Tommerup, Glen, Zauza & Alfenas, unpublished data).

Number of specimens to be collected

Where possible, collect triplicate samples from each host species, each sample comprising several rust sori covering the range of stages available (Figure 5). Also collect woody twigs and branches with swellings or galls, which are indicative of an older infection (Figure 6).

Note as many details as possible about the host identity, and collect flowers, fruits and capsules, if available, to aid in identification. For less readily identifiable seedlings, identify neighbouring trees and collect their flowers, fruits, capsules etc. Record the location, GPS co-ordinates if possible, a map reference or distance and direction from a suitable landmark. If the land is privately owned, record the owner"s details with contact telephone numbers.

What to collect

Rust sori with urediniospores or teliospores (Figure 5 and Figure 8 to Figure 10).

How to collect plant samples

Always collect the rust on affected vegetative and/or floral shoots and leaves (i.e. do not attempt to scrape spores from the plant surface). In the event of an older infection woody galls or other evidence of infection should be sampled. To avoid further spread of the rust, do not touch the specimens with bare hands. Rust spores are readily dispersed by wind, carried on clothing and other equipment, and can survive for up to 3 months. Place a paper bag around the specimen before snipping the affected part from the plant. Seal the paper bag and place inside another paper bag with additional paper to absorb moisture. Keep dry and send immediately to a diagnostic laboratory. If the infection is severe and on several plants, biohazard overalls should be worn by anyone entering the infected area and these should be autoclaved or incinerated afterwards.

Each sample should be placed in a single bag, sealed and sufficiently labelled with time, date and location (to tree level if possible) of collection, sample number, sample type (leaf, bud, etc) and name of collector. The collector should sign the label and allow space for further handling instructions to be added during transit (e.g. handed to courier). A list of samples should be included in the package sent for diagnosis, with a copy retained by the collector (or sent to Lead Agency).

Packaging

Each sealed paper bag should be placed in a second paper bag along with additional paper (or similar) to absorb excess moisture. Bagged samples should then be placed in a cardboard box or padded envelope with paper/bubble/foam to fill the remaining space and protect samples during transit.

All sample containers should be clearly labelled with the name, address and contact phone number of both the sending and receiving officers. In addition containers should be clearly labelled in accordance with the requirements of PLANTPLAN (Plant Health Australia, 2008; Appendix 3). Containers need to be carefully sealed to prevent loss, contamination or tampering of samples. The State Chief Plant Health Manager will select the preferred laboratory.

5.2.4.2 TREATMENT OF SAMPLES

How to preserve plant samples with rust

Preserve all plant material as air dried specimens in sealed paper bags. Dry the sample as quickly as possible (still in the paper bag) in circulating air at temperatures of 25-29°C.

Isolation of target organism

P. psidii, like other *Puccinia* species, cannot be cultured; therefore identification is dependent upon characteristics from infected host material, microscopic analysis and DNA characterisation (refer to Sections 4.3 and 9.4)

Transport of samples with rust

Always transport the rust on the collected host plant material. Samples in sealed paper packaging are safe for transport. Refer to PLANTPLAN (Plant Health Australia, 2008) for packing instructions under IATA 650. The State Chief Plant Health Manager will select the preferred laboratory.

Receipt

On receipt of the samples the diagnostic laboratory should follow strict quarantine and processing guidelines. In keeping with ISO 17025 refer to PLANTPLAN (Plant Health Australia, 2008).

5.2.5 Epidemiological study

The extent of infection on a nursery, property or within a region will depend on the amount of inoculum available and whether conditions have been favourable for the pathogen to spread from the initial foci. Sampling of plants within a district and beyond will be based upon the origins of the initial suspect sample(s). Factors to consider will be:

- The proximity of other susceptible plants to the initial infection source, including both current and previous crops. This will include crops on the nursery or property with the initial infection source and those on neighbouring properties
- What machinery or vehicles have been into the infected crop or in close proximity to the infection source
- The extent of human movements into and around the infected crop. A possible link to the recent importation of plant material, overseas travel or visitors from other regions should also be considered
- The source of any propagation material that was not seeds
- If any other crops have been propagated from the same source and/or distributed from the affected nurseries

5.2.6 Models of spread potential

No models of spread potential have been developed for *P. psidii*. However, an integrated aerobiology modelling system has been applied to soybean rust (*Phakospora pachyrizi*) in the USA (Isard *et al.,* 2007) that could be used as a basis for developing a model of spread potential for guava rust. Other research that should be considered in the development of a model of spread potential for guava rusts includes investigations into the aerial dispersal of pathogens across a continental scale (Aylor *et al.,* 2003) and the use of a system for weather-based mapping of plant pathogens, such as NAPPFAST (Magarey *et al.,* 2003).

5.2.7 Pest Free Area guidelines

Determination of Pest Free Areas (PFAs) should be completed in accordance with the International Standards for Phytosanitary Measures (ISPMs) 8 and 10 (FAO, 2007).

General points to consider are:

- Design of a statistical delimiting field survey for symptoms on host plants (see Section 5.2 for points to consider in the design)
- Plant sampling should be completed as described in the BioSecure *HACCP* manual (Nursery and Garden Industry Australia, 2008), including monitoring processes (summarised in Table 9 and Table 10), indicator plants and weed monitoring.

- Surveys should also consider alternative hosts (see Section 4.2.1) and not be limited to the primary infected host
- Survey around irrigation systems or waterways that may have transported spores
- Surveys should utilise both sentinel plants and spore traps
- Information (including absence of the pest) should be recorded

Table 9. *Summary of monitoring processes for protected production areas as described in* BioSecure HACCP *Guidelines*

Wear protective clothing and disposable gloves when handling suspect plants
Walk at random through the area in a zigzag pattern
Take at least 10 minutes to inspect 10-20 plants or plug trays per 100 m^2 of production area
Inspect the entire plant if it has less than 6 leaves, or from larger plants select six leaves from all parts of the plant (upper, lower, middle) and examine them individually
If any leaves show suspect disease symptoms (refer to Section 4.2.3) take a sample (refer to Section 5.2.4) to be formally diagnosed (refer to Section 4.3)
Routinely inspect growing areas and remove alternate hosts and reservoirs of the pathogen, including weeds, crop residues and old plants that will not be marketed

Table 10. *Summary of monitoring processes for field production areas as described in* BioSecure HACCP *Guidelines*

Wear protective clothing and disposable gloves when handling suspect plants
Pay particular attention to areas on the windward side, the sides bordering ditches, canals or other uncultivated areas and growing block centres
Place a flag or other marker at the entrance to the block or sampling area at the beginning of each inspection
Vary the entrance point in the sampling area (1m to 3m) for each subsequent sampling so that the same plants are not inspected each time
Walk at random through the area in a zigzag pattern
The scout should follow the same general pattern at each sampling
Make an effort to select those plants that appear less healthy for visual inspection
Take at least 10 minutes to inspect 10-20 plants or plug trays per 100 m^2 of production area
Inspect the entire plant if it has less than 6 leaves, or from larger plants select six leaves from all parts of the plant (upper, lower, middle) and examine them individually
If any leaves show suspect disease symptoms (refer to Section 4.2.3) take a sample (refer to Section 5.2.4) to be formally diagnosed (refer to Section 4.3)
Routinely inspect growing areas and remove alternate hosts and reservoirs of the pathogen, including weeds, crop residues and old plants that will not be marketed

Additional information is provided by the IPPC (1995) in Requirements for the Establishment of Pest Free Areas. This standard describes the requirements for the establishment and use of pest free areas as a risk management option for phytosanitary certification of plants and plant products.

Establishment and maintenance of a PFA can vary according to the biology of the pest, pest survival potential, means of dispersal, availability of host plants, restrictions on movement of produce, as well as PFA characteristics (size, degree of isolation and ecological conditions).

5.3 Availability of control methods

In South America, *P. psidii* does not cause major disease outbreaks in the wild, as the native hosts have co-evolved with the pathogen. The situation is expected to be quite different if this pathogen becomes established in Australia, as pathogens are frequently far more virulent on naïve hosts. The ubiquity of the Myrtaceae, many of which are known to be susceptible, in native Australian vegetation will also make control more difficult in comparison to a pathogen with a more restricted or mainly agricultural host range. In general, control options may include:

- Host removal (Section 5.3.2)
- Chemical control (Sections 5.3.6 and 9.6)
- Biological control (Section 5.3.3)
- Physical control (Section 5.3.4)
- Development of host resistance (Section 5.3.5)

5.3.1 General procedures for control

- Keep traffic out of affected areas and minimize movement in adjacent areas
- Stop irrigating affected (irrigated crops) areas and use bunding to divert water if necessary
- Adopt best-practice property hygiene procedures to retard the spread of the pest between fields and adjacent properties
- After surveys are completed, destruction of the infected crop is an effective control[18]
- On-going surveillance of infected areas to ensure pathogen is eradicated
- Do not use any material from infected crops for propagation of next crop

5.3.2 Host removal

Host removal may be feasible in a small area. Care must be taken not to spread the spores, which are readily wind-dispersed, so removal should be preceded by spore destruction using fungicides or disinfectants. If the plants are too large for physical removal, the application of herbicides or defoliants may just as effectively remove susceptible plant tissue. Removal of highly susceptible plant species (e.g. *S. jambos, K. baxteri*) in the vicinity of valuable crops may reduce the spore inoculum load and hence disease severity for those crops.

[18] Spore destruction using fungicides or disinfectants should be completed before the removal of host species

5.3.3 Biological control

The effect of 24 isolates of *Bacillus subtilis* on *P. psidii* urediniospore germination has been examined (Santos *et al.,* 1998). Liquid cultures, both live and autoclaved, and culture supernatant of all isolates, were all effective in reducing *in vitro* germination of *P. psidii* from an average of 34% in controls to 0-4%. There are no reports of field or pot experiments using *B. subtilis* against *P. psidii* and *in vitro* results may be difficult to reproduce *in vivo* (Van Toor *et al.,* 2005), however the initial results are promising and deserve further investigation. *Fusarium decemcellulare* has been demonstrated to be a hyper-parasite of *P. psidii* (Amorim *et al.,* 1993). Other fungi also co-occur in rust pustules (Glen, unpublished) and may have potential as biological control agents. Particular strains of rhizobacteria have been demonstrated to induce systemic resistance in *E. grandis x urophylla* (Texeira *et al.,* 2005). Biological control may be a tool for minimising disease incidence once it is established in Australia, but will not be suitable for an eradication attempt. Further work is necessary before any fungus or bacterium can be recommended as a biological control agent.

5.3.4 Physical control

Heat may be an effective tool to kill *P. psidii* spores, but fire is not recommended as a tool for eradication as rust spores may be dispersed on air currents created by fires. If the disease becomes established in forest areas where prescribed burning is carried out for fuel reduction or regeneration, consideration will need to be given to the strategic timing of fires. If timed correctly, fuel reduction burns may assist in local inoculum reduction but if they are carried out at unfavourable times, they may result in a flush of susceptible new growth when climatic conditions are conducive to severe disease. Solarisation of soil with black plastic may be useful over small areas to kill spores following fungicide and/or herbicide application.

5.3.5 Host resistance

Host resistance will be important for industries based on Myrtaceae plants (e.g. eucalypt and melaleuca plantations, wildflower growers and guava orchards) if *P. psidii* becomes established in Australia. It will also be important for natural selection in native vegetation, but this may be at the cost of species loss and environmental degradation. Importation of resistant breeding material is unlikely to be approved due to the risk of importation of (additional strains of) *P. psidii*, however there is potential to use molecular detection to demonstrate that germplasm meets the required phytosanitary standards. Resistance testing of different seed-lots and provenances has been carried out for some *Eucalyptu*s and *Corymbia* species (Tommerup *et al.,* 2003; Alfenas, Zauza, unpublished) and has revealed that seed-lots of the same species may vary greatly in disease susceptibility. This information will be vital in identifying resistant germplasm for industrial use in Australia. A genetic marker linked to a major resistance gene in *E. grandis* has also been developed for application in breeding programs (Junghans *et al.,* 2004).

Selection and breeding for resistance is the only economically and environmentally feasible control of *P. psidii* in eucalypt plantations in Brazil. Under field conditions in Brazil, *Eucalyptus* spp. appear to have an age-related resistance. Plants over 2 years old are not affected by the disease (Ferreira, 1983), unless coppiced, whereas guavas, jambos and allspice are susceptible at all ages (McLachlan, 1936; Ferrari *et al.*, 1997; Blum & Dianese, 2001). Only a limited number of *Eucalyptus* species are grown in plantations in Brazil, however, and most susceptibility testing performed to date on Australian Myrtaceae has been carried out on seedlings, so susceptibility of older plants is unknown,

with the exception of a few species that are grown as ornamentals or occur as weeds in rust-affected areas. Mature plants of *M. quinquenervia* are susceptible (Rayachhetry *et al*., 1997).

5.3.6 Chemical control

Chemical control is used in nurseries and high-value crops in South America. Successful control in eucalypt nurseries, in coppice of eucalypt plantations, and in guava orchards has been established with a range of fungicides (Demuner *et al.,* 1991; Ruiz *et al.,* 1991; Ferrari *et al.,* 1997; de Goes *et al*., 2004). The control afforded by weekly fungicide spraying in guava is not complete, but keeps the disease to a level that does not threaten the viability of the industry. Details of fungicides and their application rates from four different studies of rust control in eucalypt seedlings and coppice and in guava orchards are summarised in Table 11.

Once *P. psidii* is established in Australia, and eradication attempts have failed, fungicide control would not be economically or environmentally feasible in anything except high value crops or nurseries. All nurseries with Myrtaceae plants in the rust risk area (Figure 4), including both horticultural and forestry nurseries, would need to immediately include suitable chemical controls for *P. psidii,* as their current regime would probably not include fungicides that could control *P. psidii*. Nurseries outside the rust risk area may also need to implement controls as the environmental conditions within nurseries are similar to those preferred by the rust.

All fungicides currently recommended for use against guava rust overseas that have been listed in this document are registered for use in Australia against other fungal pathogens by the Australian Pesticides & Veterinary Medicines Authority (APVMA, PO Box E240, Kingston, ACT 2604; ph. 02 6272 5158; **www.apvma.gov.au**), unless otherwise stated. If guava rust is detected in Australia, an additional permit would need to be required to enable the use of these chemicals for its management and/or destruction. Additional permits would be required from the Civil Aviation Safety Authority (CASA, phone 131 757, **www.casa.gov.au**) if it was intended the pesticide be aerially applied.

Detailed discussion on chemical control and eradication of *P. psidii* is contained within Appendix 6 (Section 9.6).

Table 11. *Environmental toxicities of and efficacy of fungicides tested for the control of P. psidii*

Fungicide[20]	Application rate and efficacy[19]				Environmental toxicity
	Time interval[21]	Infected leaves[22]	Rating[23]	Urediniospore rating[24]	
Chlorothalonil Benzo-nitriles Non-systemic protectant (group Y) Bayer Chlorothalonil 500 SC			1.5 g/L (300 g/ha[25]) ++		Classified ecotoxin, toxic to aquatic organisms, non-toxic to bees and earthworms
Copper oxychloride Mineral Non-systemic (group Y) Chemspray copper oxychloride			1.0 g/L (200 g/ha[25]) +		Highly toxic to zoo plankton and aquatic Anelida, slightly toxic to fish. Terrestrial ecotoxicity poorly known
Diniconzole Azole Systemic (group C)[26] None registered	0.075 g/L (15 g/ha[25]) 14 days	30 g/ha[27] 65%			Very toxic to aquatic organisms
Mancozeb Carbamate Non-systemic (group Y) Barmac mancozeb DG			1.6 g/L (320 g/ha[25]) +		Highly toxic to amphibians, moderately toxic to fish
Oxycarboxin Aniline/anilide Systemic (group G) Crompton Plantvax 750 WP	1.125 g/L (225 g/ha[25]) 7 days	210 g/ha[27] 90%		0.75 g/L (150 g/ha[25]) **	Slightly toxic to amphibians and fish
Triadimenol Triazole Systemic (group C)[26] Bayer Bayfidan 250 EC	0.4 g/L (80 g/ha[25]) 28 days	100 g/ha[27] 40%		0.75 g/L (150 g/ha[25]) ***	Toxic to aquatic organisms
Triforine Amine/amide Systemic (group C)[26] Yates Triforine rose fungicide				0.053 g/L[28] (10.6 g/ha[25]) *	Low toxicity to fish, earthworms and bees, some bioaccumulation

[19] Concentrations and application rates area ll given as the amount of the active ingredient per litre or per hectare. Efficacy is presented in four ways, reflecting the different data produced in four different studies (see footnotes 21 to 24)

[20] Fungicide column lists the fungicide name, chemical group, mode of action and a commercial example

[21] The time interval required between sprayings as determined experimentally to control *P. psidii* in *Eucalyptus cloeziana* seedlings (Alfenas *et al.,* 1993)

[22] Percent of infected leaves 90 days after commencement of treatment in *E. cloeziana* coppice (Alfenas *et al.,* 1993)

[23] A rating determined by the area of affected guava fruit after spraying every 15 days (++ <10%, + 10-20%, Ferrari *et al.,* 1997)

[24] Rating based on the concentration of urediniosori on guava leaf surfaces after a single application of fungicide (* being less effective than ***; Ruiz *et al.,* 1991)

[25] The quantity per hectare is based on ground spraying and calculated assuming an application rate of 200 L/ha at the concentration that was applied to individual trees or seedlings

[26] Group C fungicides are dimethylation inhibitors. Fungicides in this group should be used in combination with others to avoid build-up of resistance in the pathogen population.

[27] Three applications were sprayed at 20 day intervals, the amount given is per application (Alfenas *et al.,* 1993)

6 Course of action

Additional information is provided by the IPPC (1998) in Guidelines for Pest Eradication Programmes. This standard describes the components of a pest eradication programme which can lead to the establishment or re-establishment of pest absence in an area. A pest eradication programme may be developed as an emergency measure to prevent establishment and/or spread of a pest following its recent entry (re-establish a pest free area) or a measure to eliminate an established pest (establish a pest free area). The eradication process involves three main activities: surveillance, containment, and treatment and/or control measures.

6.1 Destruction strategy

For attempted eradication of an initial incursion into a very restricted and isolated area, destruction of infected plants may be a feasible option. In order to prevent spore dispersal caused by the eradication process, plants must be sprayed either with a back pack or aerially with a contact fungicide and the pathogen killed before any physical manipulation and destruction of plants. Once an incursion of *P. psidii* has spread beyond one or two plants, fungicide application (refer to Section 9.6), perhaps in combination with host removal or defoliation, represents the only possibility of successful eradication. If sufficiently isolated it may be possible to remove susceptible plants within a certain radius around the infected plants and to aerially spray within this radius (an environmentally friendly fungicide should be used for this purpose). Eradication by chemical methods was not attempted in Hawaii due to the lack of approved pesticides (Killgore & Heu, 2005) and it is vital that Australia has such pesticides available (refer to Section 9.6).

However, if the size of affected plants or the affected area renders removal impractical, repeated aerial fungicide spraying may be the only possible method of containment or eradication. Killing of spores with a contact fungicide (e.g. copper, sulphur, mancozeb), possibly followed by a defoliant and a second application of fungicide to maximise canopy penetration in the diseased area and application of protectant fungicides in a large, surrounding area would be required (refer to Section 9.6). If the rust has been present for any length of time before detection, has established on a moderately or highly susceptible host with heavy infection and urediniospore production, and has spread over a distance greater than a few metres, or into urban areas where widespread spraying may not be acceptable, the chance of eradication will be very slim, as the urediniospores can rapidly travel large distances on wind currents or on animal or insect vectors. Containment is not a realistic option for this reason.

In addition to a permit from APVMA for fungicide use, a range of other permits will be required under legislation administered by agencies such as Local Government, Water Boards and Health Authorities. These approvals should be in place before an incursion is detected. Permission may be required to:

- Discharge a „contaminant" into water or into land in circumstances where it may enter water
- Discharge a „contaminant" into the air

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[28] The concentration as given at 0.28 mL/L of Saprol BR. The Agro-Fauna product inventory lists Saprol as containing 190 g/L triforine. The concentration calculated here is approximately 1/7th of the recommended application rate for Yates Triforine rose fungicide

- Overspray residential area with pesticide
- Overspray sensitive environments (such as water supplies, hospitals, schools) with pesticide
- Use pesticides in biologically sensitive areas such as areas with rare insect populations

Spores are aerially dispersed and may also „hitchhike" on any person, animal, plant or object that is transported from within the infested area. Therefore, disinfestation methods for machinery, clothing, etc., are also necessary to establish successful eradication strategies. Heat treatment (including steam), sodium hypochlorite or quaternary ammonium compounds may all be useful in this context, though no detailed studies of spore destruction have been published. Table 12 lists sources for approved, commercially available disinfectants in Australia. Many other disinfectants are available that contain glutaraldehyde as well as quaternary ammonium compounds, however the glutaraldehyde is toxic and mainly effective against bacteria and viruses rather than fungi. These disinfectants may also be needed for disinfecting glasshouses or other buildings that have housed infected plants.

Table 12. *Disinfectants*

Manufacturer/distributor	Product	Active ingredient
Ecolab Pty Ltd	Grower"s Supersan New Generation Sanitiser	100 g/L quaternary ammonium
Pascoe"s Pty Ltd	Outdoor Mould Clean Concentrate Mould and Algae Killer	142.5 g/L quaternary ammonium
Timtech Chemicals Pty Ltd	Timtechq Timber Treatment	56.4 g/L quaternary ammonium 90.24 g/L copper as ammonium carbonate 15 g/L ammonium
Mcguire Corp. & Klen Pty Ltd	Evans Swimming Pool Algaecide and WInteriser	480 g/L quaternary ammonium
Ionics Australasia Pty Ltd	Algae Free (swimming pool algaecide)	150 g/L quaternary ammonium
Dominant (Australia) Pty Ltd	Hyper-san Sanitiser	100 g/L quaternary ammonium
Ecolab Pty Ltd	Growers Virusan QT Broad-Spectrum Disinfectant	100 g/L quaternary ammonium
Seajay Industries Pty Ltd	Agriquat Disinfectant-Sanitiser-Deodorant	250 g/L benzalkonium chloride (quaternary ammonium)
NCH Australia Pty Ltd	Chemene Disinfectant	128 g/L benzalkonium chloride
Rowe Scientific Pty Ltd	Rowe Liquid Pool Chlorine	125 g/L chlorine as sodium hypochlorite
Chem Supply	Sodium Hypochlorite solution	10.5-15% available chlorine

6.1.1 Destruction protocols

- Disposable equipment, infected plant material or growing media/soil should be disposed of by autoclaving, high temperature incineration or deep burial
- Any equipment removed from the site for disposal should be double-bagged

6.1.2 Decontamination protocols

Machinery, equipment, vehicles in contact with infected plant material or soil or present within the Quarantine Area, should be washed to remove soil and plant material using high pressure water or scrubbing with products such as a degreaser or a bleach solution in a designated wash down. When using high pressure water, care should be taken not to spread plant material or spores by aerosol water droplets. Combining degreaser or detergent or using steam with high pressure water would be preferred and high pressure water should be used in wash down areas which meet the following guidelines:

- Located away from crops or sensitive vegetation
- Readily accessible with clear signage
- Access to fresh water and power
- Mud free, including entry and exit points (e.g. gravel, concrete or rubber matting)
- Gently sloped to drain effluent away
- Effluent must not enter water courses or water bodies
- Allow adequate space to move larger vehicles
- Away from hazards such as power lines
- Waste water, growing media/soil or plant residues should be contained (see Appendix 18 in PLANTPLAN)
- Disposable overalls and rubber boots should be worn when handling infected soil or plant material in the field. Boots, clothes and shoes in contact with infected soil or plant material should be disinfected at the site or double-bagged to remove for cleaning
- Skin and hair in contact with infested plant material or soil should be washed

Procedures for the sterilisation of plant containers and growing media are provided within the BioSecure *HACCP* Guidelines however, in the event of a guava rust incursion, procedures outlined in the BioSecure *HACCP* Guidelines may not be effective for the destruction of the pathogen. Any sterilisation procedure must be approved for use in the endorsed Response Plan.

6.1.3 Priorities

- Confirm the presence of the pest
- Prevent movement of vehicles and equipment through affected areas
- Stop the movement of any plant material that may be infected with the pathogen
- Determine the strategy for the eradication/decontamination of infected host material
- Determine the extent of infection through survey and plant material trace back

6.1.4 Plants, by-products and waste processing

- Any growing media/soil or infected plant material removed from the infected site should be destroyed by (enclosed) high temperature incineration, autoclaving or deep burial
- As the pathogen can be mechanically transmitted, killed plant material should not be transported unless the *P. psidii* has been destroyed
- Infested areas or nursery yards should remain free of susceptible host plants until the area has been shown to be free from the pathogen

6.1.5 Disposal issues

- Particular care must be taken to minimize the transfer of infected plant material from the area
- Burning of plant material is not recommended as a destruction strategy as the air updrafts caused by the heat of the fire could disperse the pathogen spores
- Resistance of the pathogen to a number of potential fungicides that are effective against *P. psidii* is known to increase with repeated use (refer to Section 9.6 for more details)

6.2 Containment strategies

For some exotic pest incursions where eradication is considered impractical, containment of the pest may be attempted to prevent or slow its spread and to limit its impact on other parts of the state or country. For *P. psidii*, containment is expected to be very difficult due to the ease of spore dispersal by wind currents. The practicality of local containment will depend upon the presence of natural barriers such as a large surrounding area without susceptible hosts or with an unsuitable climate. If *P. psidii* becomes established in such a location, there may be an opportunity to delay its spread to other regions by local quarantine measures such as a ban on export of plants from the area, compulsory wash-down stations for vehicles leaving the infested area and encouragement of individual measures to reduce the likelihood of inadvertent dispersal by residents and visitors (refer to Section 5.3).

In addition, reduction of local inoculum levels in and around susceptible crops may be effective in controlling disease levels. This may be achieved by the removal of highly susceptible hosts in the vicinity of vulnerable crops. In Brazil, the widespread occurrence of *S. jambos*, a popular introduced garden plant that is highly susceptible to *P. psidii*, probably contributes to disease in other hosts. *M. quinquenervia* and *K. baxteri* are two Australian natives that are known to be highly susceptible to *P. psidii*, but there may be others that will become apparent if an incursion occurs.

6.3 Quarantine and movement controls

Consult PLANTPLAN (Plant Health Australia, 2008) for administrative details and procedures

If control is to be effective, immediate action is necessary once the presence of *P. psidii* is suspected, due to its demonstrated ability to spread rapidly (Smith, 1935; Killgore & Heu, 2005). The likelihood of a false alarm is very slim as only two other rusts have been recorded on Myrtaceae in Australia and these both have narrow host ranges and geographical distributions (Walker, 1983; Shivas & Walker, 1994). Quarantine restricted and control areas should be established immediately, without waiting for

either confirmation of the diagnosis or completion of the delimiting survey. The size of these areas may be increased on completion of the delimiting survey. A delimiting survey (refer to Section 5.2.3) should be instigated immediately but eradication/containment procedures should also commence without waiting for the final results of the delimiting survey. Trace back and trace forward procedures should also be initiated immediately, without waiting for confirmation of the diagnosis.

6.3.1 Quarantine priorities

- Plant material and growing media/soil at the site of infection to be subject to movement restrictions
- Machinery, equipment, vehicles and disposable equipment in contact with infected plant material or soil to be subject to movement restrictions

6.3.2 Movement controls

A minimum area of 1 km radius should be established as the Restricted Area and 10 km radius for the Control Area, from the primary infection point. These may be increased as results from the delimiting survey and trace forward/trace back procedures become available, but should not be reduced.

Movement of people, vehicles, equipment and plant material, from and to affected properties or areas, must be controlled to ensure that the pathogen is not moved off-property. Movement controls can be achieved through the following, however specific measures must be endorsed in the Response Plan:

- Signage to indicate quarantine area and restricted movement into and within these zones
- Fenced, barricaded or locked entry to quarantine areas
- Movement of equipment, machinery, plant material or growing media/soil by permit only. Therefore, all non-essential operations in the area or on the property should cease
    - Where no dwellings are located within these areas, strong movement controls should be enforced
    - Where dwellings and places of business are included within the Restricted and Control Areas movement restrictions are more difficult to enforce, however avoidance of contact with diseased plants is necessary
        - If a production nursery is situated within the Restricted Area, all nursery operations must cease and no material may be removed without permission, due to the high likelihood of pathogen spread. Movement restrictions would be imposed on both host and non-host material.
        - Residents should be advised on measures to minimise the inadvertent transport of spores from the infested area to disease-free zones. For example, following the treatment of infected plants with fungicide to kill rust spores (Section 9.6), additional decontamination procedures should be carried out, such as fungicide treatments for plants showing no symptoms, and treatment of buildings, vehicles, clothing and other effects (Section 6.1.2). Decontamination procedures should be repeated daily for 3-5 days as viable spores may still be present in the air
    - Roads that cross the Restricted Area should be closed if alternative routes can be identified. If not, quarantine information stations should be set up at each point where

roads enter the control area, with instructions for motorists. These should include „No stopping within the control area. Keep windows closed. No getting out of vehicles." Information about the pathogen may also be included on signs

- Clothing and footwear worn at the infected site should either be double-bagged prior to removal for decontamination or should not leave the farm until thoroughly disinfected, washed and cleaned
- Plant material or plant products must not be removed from the site
- All machinery and equipment should be thoroughly cleaned down with a high pressure cleaner (see Section 6.1.2) or scrubbing with products such as a farm degreaser or a 1% bleach solution, prior to leaving the affected area. The clean down procedure should be carried out on a hard surface, preferably a designated wash-down area, to avoid mud being re-collected from the affected site onto the machine. Care should be taken when using high pressure water to contain all plant material, mud and pathogen spores dislodged during the cleaning process
- Any beehives that have been in or near the infested area during the time since the incursion occurred should also be quarantined as bees have been observed to collect *P. psidii* spores (Chapman, 1964) and eucalypt pollen may also be contaminated with *P. psidii* urediniospores (Langrell *et al.,* 2003b). Decontamination of hives and broods or DNA testing to demonstrate absence of contamination will be necessary before bees can be released in disease-free areas

6.4 Zoning

The size of each quarantine area will be determined by a number of factors, including the location of the incursion, biology of the pest, climatic conditions and the proximity of the infected property to other infected properties. This will be determined by the National Management Group during the production of the Response Plan. Further information on quarantine zones in an Emergency Plant Pest incursion can be found in PLANTPLAN, Appendix 10 (Plant Health Australia, 2008). These zones are outlined below.

6.4.1 Destruction Zone

All possible host plants should be destroyed after the level of infection has been established. The delimiting survey will determine whether or not neighbouring plants are infected and need to be destroyed. Non-host plant material within this zone may be decontaminated or destroyed, based on recommendations in the Response Plan. The Destruction Zone may be defined as contiguous areas associated with the same management practices as the infected area (i.e. the entire nursery, property or forest area if spread could have occurred prior to the infection being identified).

Particular care needs to be taken to ensure that plant material (including non-hosts) are not moved into surrounding areas.

6.4.2 Quarantine Zone

The Quarantine Zone is defined as the area where voluntary or compulsory restraints are in place for the affected property(ies). These restraints may include restrictions or movement control for removal of plants, people, growing media/soil or contaminated equipment from an infected property.

6.4.3 Buffer Zone

A Buffer Zone may or may not be required depending on the incident. It is defined as the area in which the pest does not occur but where movement controls or restrictions for removal of plants, people, soil or equipment from this area are still deemed necessary. The Buffer Zone may enclose an infested area (and is therefore part of the Control Area) or may be adjacent to an infested area.

6.4.4 Restricted Area

The Restricted Area is defined as the zone immediately around the infected premises and suspected infected premises. The Restricted Area is established following initial surveys that confirm the presence of the pest. The Restricted Area will be subject to intense surveillance and movement control with movement out of the Restricted Area to be prohibited and movement into the Restricted Area to occur by permit only. Multiple Restricted Areas may be required within a Control Area.

6.4.5 Control Area

The Control Area is defined as all areas affected within the incursion. The Control Area comprises the Restricted Area, all infected premises and all suspected infected premises and will be defined as the minimum area necessary to prevent spread of the pest from the Quarantine Zone. The Control Area will also be used to regulate movement of all susceptible plant species to allow trace back, trace forward and epidemiological studies to be completed.

6.5 Decontamination and farm clean up

Decontaminant practices are aimed at eliminating the pathogen thus preventing its spread to other areas.

6.5.1 Decontamination procedures

General guidelines for decontamination and clean up

- Refer to PLANTPLAN (Plant Health Australia 2008) for further information
- Keep traffic out of affected area and minimize it in adjacent areas
- Adopt best-practice property hygiene procedures to retard the spread of the pathogen between growing areas/fields and adjacent properties
- Machinery, equipment, vehicles in contact with infected plant material or growing media/soil present within the Quarantine Area, should be washed to remove growing media/soil and

plant material using high pressure water or scrubbing with products such as a degreaser or a bleach solution in a designated wash down area as described in 6.1.2

- Only recommended materials are to be used when conducting decontamination procedures, and should be applied according to the product label

6.5.2 General safety precautions

For any chemicals used in the decontamination, follow all safety procedures listed within each MSDS.

6.6 Surveillance and tracing

6.6.1 Surveillance

Detection and delimiting surveys are required to delimit the extent of the outbreak, ensuring areas free of the pest retain market access and appropriate quarantine zones are established.

Initial surveillance priorities include the following:

- Surveying all host growing properties and businesses in the pest quarantine area
- Surveying all properties and businesses identified in trace-forward or trace-back analysis as being at risk
- Surveying all host growing properties and businesses that are reliant on trade with interstate or international markets which may be sensitive to pathogen presence
- Surveying production nurseries selling at risk host plants
- Surveying other host growing properties and backyards

6.6.2 Survey regions

Establish survey regions around the surveillance priorities identified above. These regions will be generated based on the zoning requirements (see Section 6.4), and prioritised based on their potential likelihood to currently have or receive an incursion of this pest. Surveillance activities within these regions will either allow for the area to be declared pest free and maintain market access requirements or establish the impact and spread of the incursion to allow for effective control and containment measures to be carried out. Detailed information regarding surveys for *P. psidii* have been outlined elsewhere in this plan (refer to Sections 5.2 and 9.5).

Steps outlined in Table 13 form a basis for a survey plan. Although categorised in stages, some stages may be undertaken concurrently based on available skill sets, resources and priorities.

Table 13. *Phases to be covered in a survey plan*

Phase 1	• Identify properties that fall within the buffer zone around the infested premise • Complete preliminary surveillance to determine ownership, property details, production dynamics and tracings information (this may be an ongoing action)
Phase 2	• Preliminary survey of host crops in properties in buffer zone establishing points of pest detection
Phase 3	• Surveillance of an intensive nature, to support control and containment activities around points of pest detection
Phase 4	• Surveillance of contact premises. A contact premise is a property containing susceptible host plants, which are known to have been in direct or indirect contact with an infested premises or infected plants. Contact premises may be determined through tracking movement of materials from the property that may provide a viable pathway for spread of the disease. Pathways to be considered are: o Items of equipment and machinery which have been shared between properties including bins, containers, irrigation lines, vehicles and equipment o The producer and retailer of infected material if this is suspected to be the source of the outbreak o Labour and other personnel that have moved from infected, contact and suspect premises to unaffected properties (other growers, tradesmen, visitors, salesmen, crop scouts, harvesters and possibly beekeepers) o Movement of plant material and growing media/soil from controlled and restricted areas o Storm and rain events and the direction of prevailing winds that result in air-borne dispersal of the pathogen during these weather events
Phase 5	• Surveillance of production and retail nurseries, gardens and public land where plants known to be hosts of pathogen are being grown
Phase 6	• Agreed area freedom maintenance, pest control and containment

6.6.3 Post-eradication surveillance

The period of pest freedom sufficient to indicate that eradication of the pest has been achieved will be determined by a number of factors, including cropping conditions, the previous level of infection and the control measures applied.

Specific methods to confirm eradication of *P. psidii* may include:

- Monitoring of sentinel plants
    - Sentinel plants are to be grown in pots or small plots at the affected site. Plants are to be grown *in situ* under quarantine conditions and monitored for symptoms of infection
    - If symptoms are detected, samples are to be collected and stored and plants destroyed
- Surveys comprising host plant sampling for *P. psidii* should be undertaken for a minimum of three years after eradication has been achieved
- Alternate non-host crops should be grown on the site and any self-sown plants sprayed out with a selective herbicide

7 Technical debrief and analysis for stand down

Refer to PLANTPLAN (Plant Health Australia, 2008) for further details

The emergency response is considered to be ended when either:

a) Eradication has been deemed successful by the lead agency, with agreement by the Consultative Committee on Emergency Plant Pests and the Domestic Quarantine and Market Access Working Group

b) Eradication has been deemed impractical and procedures for long-term management of the disease risk have been implemented

A final report should be completed by the lead agency and the handling of the incident reviewed.

Eradication will be deemed successful only after two years with favourable climatic conditions and no detections of *P. psidii,* despite comprehensive surveys of previously infested areas and host plants, including sentinel plants, in their vicinities. If the source of the incursion can be determined by trace-back procedures, a review of current quarantine procedures may be required to decrease the risk of further incursions.

Eradication will be deemed impractical if, at any stage, the results of the delimiting surveys lead to a decision to move to containment/control. In this instance, it will still be desirable to prevent incursions of additional biotypes of *P. psidii*, therefore a review of current quarantine procedures may still be necessary to decrease this risk.

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9 Appendices

9.1 Appendix 1: Standard diagnostic protocols

For a range of specifically designed procedures for the emergency response to a pest incursion refer to Plant Health Australia"s PLANTPLAN (**www.planthealthaustralia.com.au/plantplan**).

9.2 Appendix 2: Host range discussion

Information on susceptibility to *P. psidii* is available for 83 native Australian Myrtaceae species, only 5% of the total, with an emphasis on economically valuable plantation tree species. In the largest study (Alfenas, Zauza, Tommerup, Craven & Burdon unpublished), 47 species had some degree of susceptibility, ranging from 5% to 100% susceptible plants. Susceptibility varied within host species depending on the provenance. For example, seedlots of *E. grandis* from Qld produced 40-94% susceptible plants while seedlots from NSW produced 12-27% susceptible plants (Tommerup *et al*., 2003). Rust biotypes also show variation in host range (Aparecido *et al.,* 2003a), so the apparent 100% resistance found in 11 of the 58 species so far tested against a single rust biotype may not be robust if challenged with a different biotype of the pathogen. There are 1646 native Australian Myrtaceae species (with 143 of them on the endangered or threatened species list), so if the current ratio of susceptible:resistant species persists with further testing of species susceptibility, it could be projected that 1447 species of native Australian plants are potential hosts. Species with low levels of susceptibility, while not greatly threatened per se, may still play a significant role in maintaining the pathogen and assisting its spread.

The effect of new diseases on naive hosts may also be far more unpredictable than on hosts that have a long association with the pathogen. There is little published information about the severity of the disease in native vegetation in Brazil, but the disease is not usually severe on native hosts with the exception of occasional epidemics in guava plantations (de Goes *et al.,* 2004; Ribeiro & Pommer, 2004). This may be attributed to the establishment of large monoculture areas of a susceptible host. The same argument could also be made for the introduced eucalypts, however another introduced species, *Syzygium jambos*, is often grown in mixed plantings in gardens yet this species suffers frequent severe attacks (Tessman *et al*., 2001). It is also susceptible over a larger geographic range and up to a later age than *Eucalyptus* species.

P. psidii appears to have switched hosts on more than one occasion, with devastating impact on the new host. In spring of 1934, *P. psidii* was first observed on allspice (*Pimenta dioica*) in Jamaica and within two weeks blighted a crop that had promised to provide a record yield (Smith, 1935). In 1935, the disease re-appeared covering a larger area. Smith, the government microbiologist, hypothesized that the strain attacking the allspice trees was a mutant or „sport" of recent origin, as *P. psidii* had been present on *S. jambos* in close proximity to allspice for some years. He was optimistic, however, that conditions would improve and that the disease would quickly establish a balance with its new host, similar to that observed on the rose-apple (*S. jambos*). His optimism was not borne out and the allspice industry in Jamaica never recovered.

S. jambos, unlike *P. psidii,* is not native to South America. Therefore the rust must have switched from a native host to *S. jambos* some time previously. A more recent host switch was from allspice in Florida to *M. quinquenervia* (an Australian native that has become a noxious pest in Florida). *P. psidii* has been present in Florida since 1977, but occurred almost exclusively on allspice (Leahy, 2004). In 1997-1998, a natural epiphytotic occurred on *M. quinquenervia* (Rayachhetry *et al.,* 1997). Since

2001, *P. psidii* has infected a dramatically increasing number of host species in Florida, until it has become a serious problem for commercial nurseries, home-owners and native Myrtaceae (Leahy, 2004). Whether the host range expansion was due to subsequent incursion of another *P. psidii* biotype, or a mutation that occurred within the Florida population which enabled the pathogen to expand its host range, is uncertain. The ability of the pathogen to expand its host range therefore makes the full effect of the disease on native Australian Myrtaceae difficult to predict. Host testing has been carried out with one or a few provenances of a limited number of host species and one or a few strains of the pathogen. This has involved considerable effort but there are still large uncertainties in prediction of the likely impact on native biodiversity.

In addition to the impact on native biodiversity, the susceptible genera include valuable forestry trees as well as native species grown for use in urban gardens and being planted on a commercial scale for wildflower production and for the emerging agribusiness of essential oil production. Mature eucalypts are resistant to the disease, though it also occurs on coppice (Junghans *et al*., 2003). On other hosts such as allspice and guava the young tissue is susceptible even in older trees. Most Australian species have only been tested at seedling stage, so the susceptibility of older plants is largely unknown. The natural infection of *M. quinquinervia* in Florida occurs on all ages of plants. This attribute may be important in maintaining inoculum levels.

Different strains or biotypes of the rust are known to exist, with each biotype infecting a different, though overlapping, group of species. Two strains in Jamaica infected *Pimento* spp. and *Syzygium* spp., respectively, but neither infected guava (McLachlan, 1938). The *Pimento* strain was able to infect *S. jambos* but did not sporulate. In Florida, the *Pimento* strain sporulated in *S. jambos* (Marlatt & Kimbrough, 1979), though it took twice as long for maturation of urediniosori in *S. jambos* than in *Pimenta dioica*. In later tests, *S. jambos* was considered immune to rust strains from *M. quinquenervia* and *P. dioica* (Rayachhetry *et al.,* 2001). In Brazil, isolates from guava did not infect eucalypts, and *vice versa* (Ferreira, 1983). More recently Aparecido *et al.* (2003a) distinguished four groupings based on host susceptibility tests. More work is needed to enable a clear understanding of the *P. psidii* biotypes and their relationships.

9.2.1 Tested host range in Australia

A summary of tested Australian and introduced species and their susceptibility/resistance to *P. psidii*, and their presence in native ecosystems, amenity plantings and commercial use is shown in Table 14. Australian species names are the names currently (March 2006) accepted by the Australian National Botanic Gardens as provided in the What‟s Its Name database (**www.cpbr.gov.au/win/index.html**).

Table 14. *Susceptibility/resistance of Australian and introduced species to* P. psidii

Species tested	Susceptibility[29]	Native vegetation	Amenity/nursery[30]	Commercial use
Angophora costata	S	+	+	
Astartea fascicularis	S	+	+	
Astartea heteranthera	S	+	+	
Asteromyrtus magnifica	R	+		

[29] S indicates that at least some of the tested plants were susceptible

[30] Use in amenity plantings was determined by the availability of species at plant nurseries within the rust risk zone, as indicated by the results of internet searches, the list may therefore be incomplete

Species tested	Susceptibility[29]	Native vegetation	Amenity/nursery[30]	Commercial use
Beaufortia schaueri	R[31]	+	+	
Beaufortia sparsa	R[31]	+	+	
Callistemon citrinus	S	+	+	+
Callistemon glaucus	S	+	+	
Callistemon pachyphyllus	S	+	+	
Callistemon speciosus	S	+	+	
Callistemon viminalis	S	+	+	
Corymbia calophylla (rosea)	R	+	+	
Corymbia maculata[32]	S	+	+	
Corymbia tessellaris	R	+		+
Eremea asterocarpa	S	+		
Eremea pauciflora	S	+		
Eucalyptus acmenoides	S	+		
Eucalyptus agglomerata	S	+		+
Eucalyptus alba	S	+		+
Eucalyptus amplifolia subsp. *amplifolia*	S	+		+
Eucalyptus andrewsii[32]	S	+		
Eucalyptus brassiana	S	+		
Eucalyptus camaldulensis subsp. *simulata*	S	+	+	+
Eucalyptus camaldulensis var. *obtusa*	S	+	+	+
Eucalyptus citriodora	S	+	+	
Eucalyptus cloeziana	S	+		+
Eucalyptus diversicolor	S	+		+
Eucalyptus dunnii	S	+	+	+
Eucalyptus elata	S	+		
Eucalyptus globulus subsp. *globulus*	S	+	+	+
Eucalyptus grandis[31]	S	+	+	+
Eucalyptus guilfoylei	S			

[31] These species were classified as resistant on the basis of >70% resistant plants, with no further information available. It is therefore possible that they have a low level of susceptibility.

[32] The natural range of these species falls completely or mainly within the high rust risk zone as identified by climate modeling

Species tested	Susceptibility[29]	Native vegetation	Amenity/nursery[30]	Commercial use
Eucalyptus gummifera[32]	S	+		
Eucalyptus intermedia[32]	S	+		
Eucalyptus marginata ssp. *marginata*	R[31]	+		+
Eucalyptus melanophloia	S	+		+
Eucalyptus microcorys	S	+	+	
Eucalyptus moluccana[32]	S	+		
Eucalyptus montivaga	S	+		
Eucalyptus nigra	S	+		
Eucalyptus nitens	S	+		+
Eucalyptus obliqua	S	+		
Eucalyptus paniculata	S	+		+
Eucalyptus pellita	S	+		+
Eucalyptus phaeotricha	S	+		
Eucalyptus pilularis[32]	S	+		
Eucalyptus punctata	S	+	+	
Eucalyptus pyrocarpa	S	+		
Eucalyptus regnans	S	+		
Eucalyptus resinifera	S	+		+
Eucalyptus robusta	S	+		+
Eucalyptus rubiginosa	S	+		
Eucalyptus saligna[32]	S	+		+
Eucalyptus scias subsp. *scias*[31]	S	+		
Eucalyptus sieberi	S	+		
Eucalyptus tereticornis[32]	S	+		+
Eucalyptus tetrodonta	S	+		
Eucalyptus tindaliae[32]	S	+		
Eucalyptus viminalis ssp. *viminalis*	S	+	+	
Eugenia brasiliensis	S		+	
Eugenia reinwardtiana	S	+		
Eugenia uniflora	S		+	
Feijoa sellowiana	R[31]		+	

Species tested	Susceptibility[29]	Native vegetation	Amenity/nursery[30]	Commercial use
Hypocalymma robustum	S	+		
Kunzea baxteri	S[33]	+	+	+
Kunzea ericoides	S	+		
Kunzea recurva	S	+		
Leptospermum laevigatum	R	+	+	
Lophostemon confertus	R	+	+	
Lophostemon suaveolens	R	+		
Melaleuca alternifolia	S	+		+
Melaleuca cajuputi	S	+		+
Melaleuca decora	S	+		
Melaleuca ericifolia	R[31]	+	+	
Melaleuca hypericifolia	S	+	+	
Melaleuca leucadendra	S	+		+
Melaleuca nesophila	S	+	+	
Melaleuca quinquenervia	S	+		+
Melaleuca squarrosa	S	+		
Myrcia jaboticaba	S		+	
Pericalymma ellipticum	S	+		
Pimenta dioica	S		+	
Psidium guajava	S		+	
Regelia ciliata	S	+	+	
Syncarpia glomulifera	S	+		
Syncarpia hillii	S	+		
Syzygium cumini	S		+	
Syzygium jambos	S		+	
Syzygium malaccensis	S	+	+	
Syzygium paniculata	S	+	+	
Thryptomene australis	S	+		

[33] The *Kunzea baxteri* seedlot tested had no resistant plants

9.3 Appendix 3: Additional host symptom images

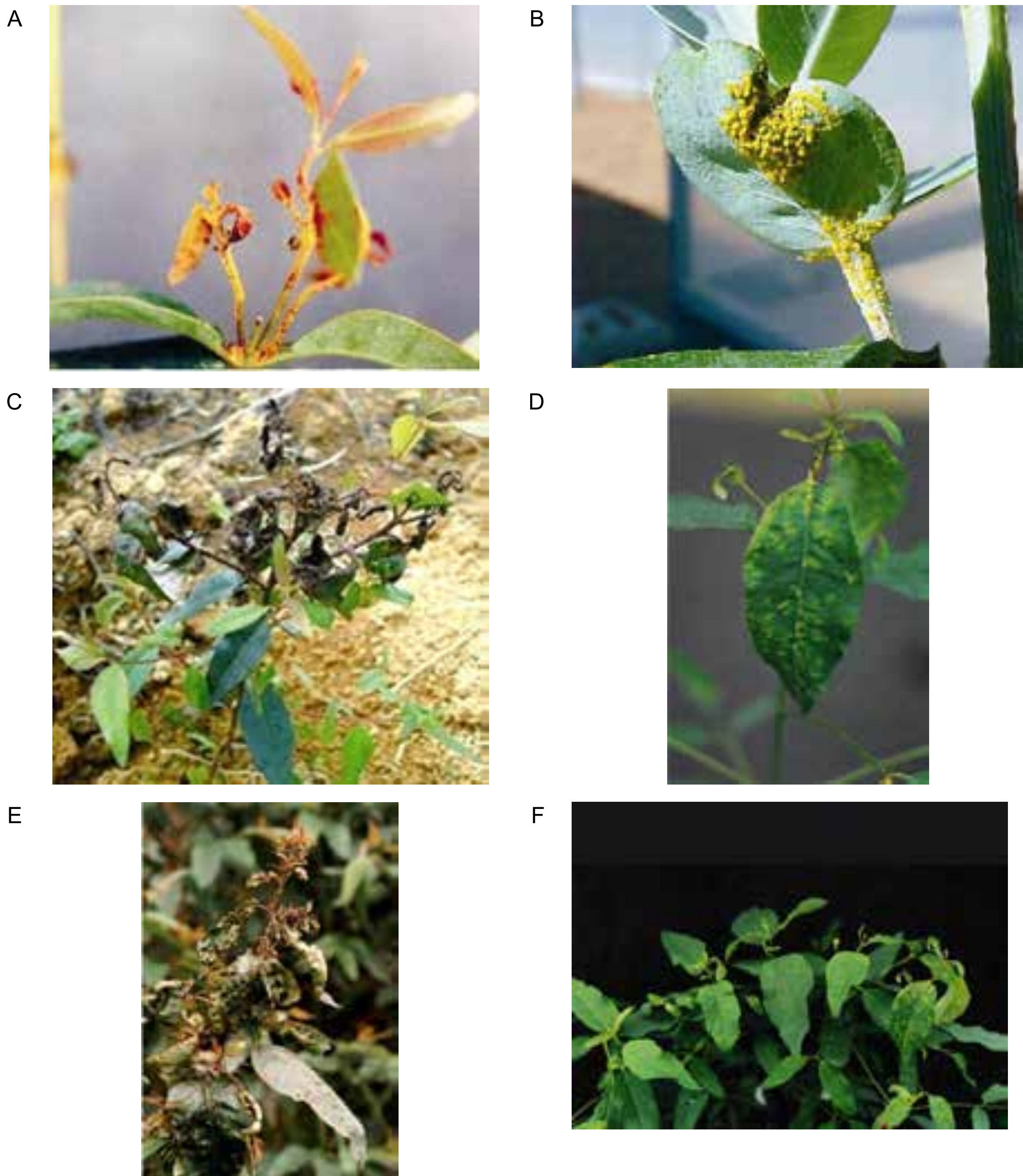

Figure 8. *Symptoms of guava rust on eucalyptus leaves. Images © Forest Pathology and Genetics of Plant Pathogen Interactions Laboratory, Federal University of Viçosa (A-C), or taken from PaDIL (D-F;* ***www.padil.gov.au****).*

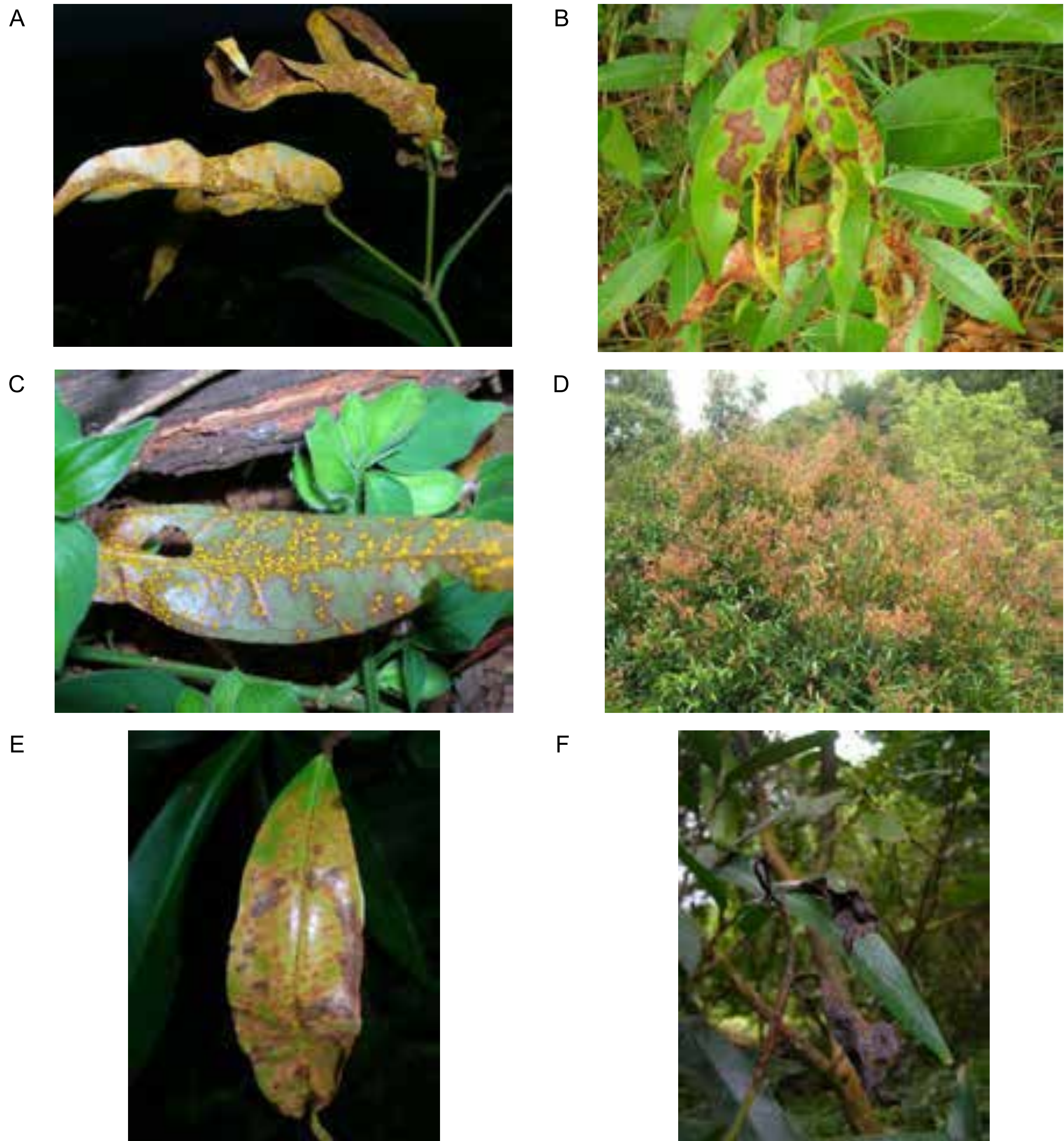

Figure 9. *Symptoms of guava rust on* S. jambos*. Images courtesy of Forest & Kim Starr, United States Geological Survey, Biological Resources Division.*

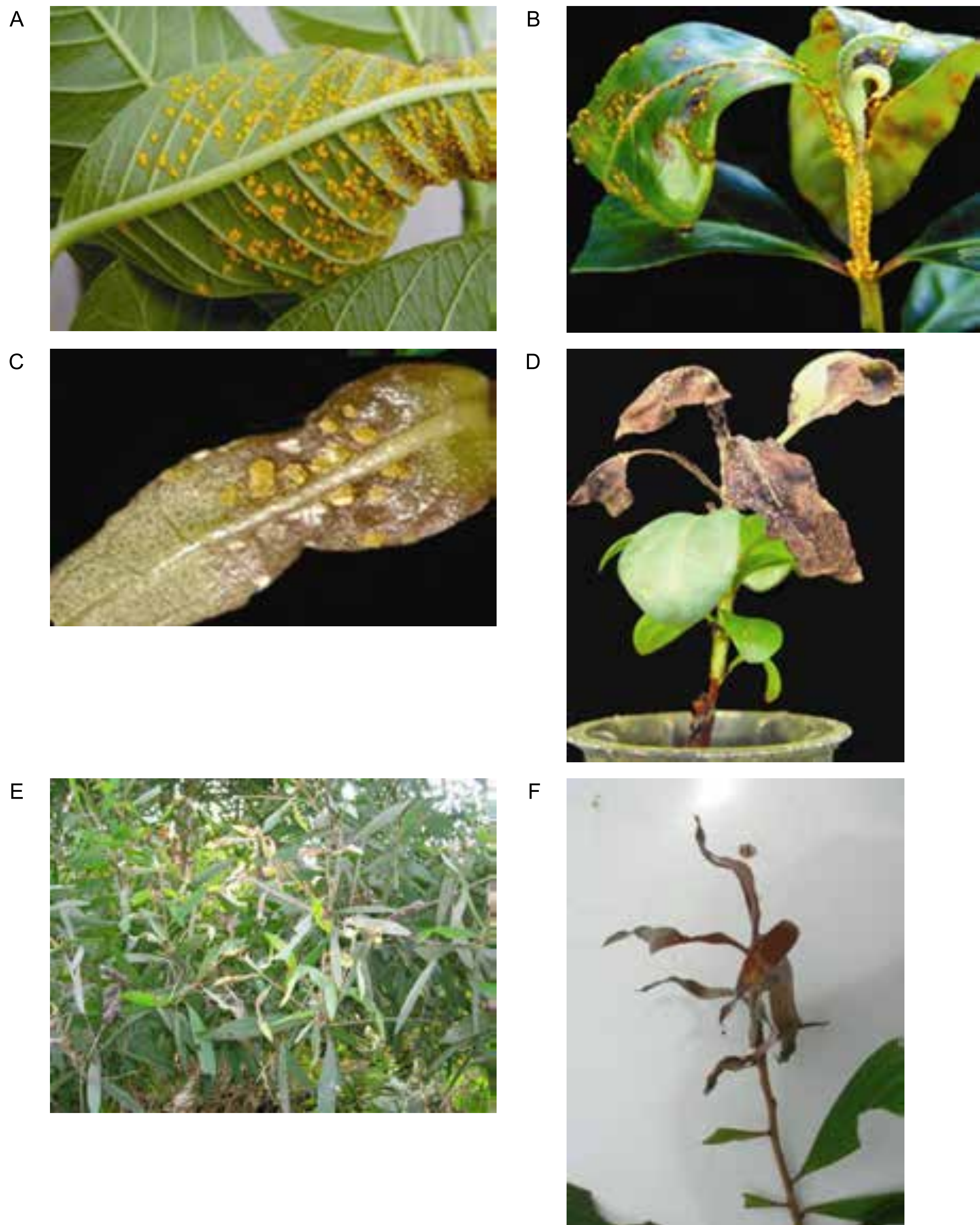

Figure 10. *Symptoms of guava rust on P. guajava (A),* Heteropyxis natalensis *(B-D) and M. quinquenervia (E-F). Images © Forest Pathology and Genetics of Plant Pathogen Interactions Laboratory, Federal University of Viçosa (A-D) or courtesy of Forest & Kim Starr, United States Geological Survey, Biological Resources Division.*

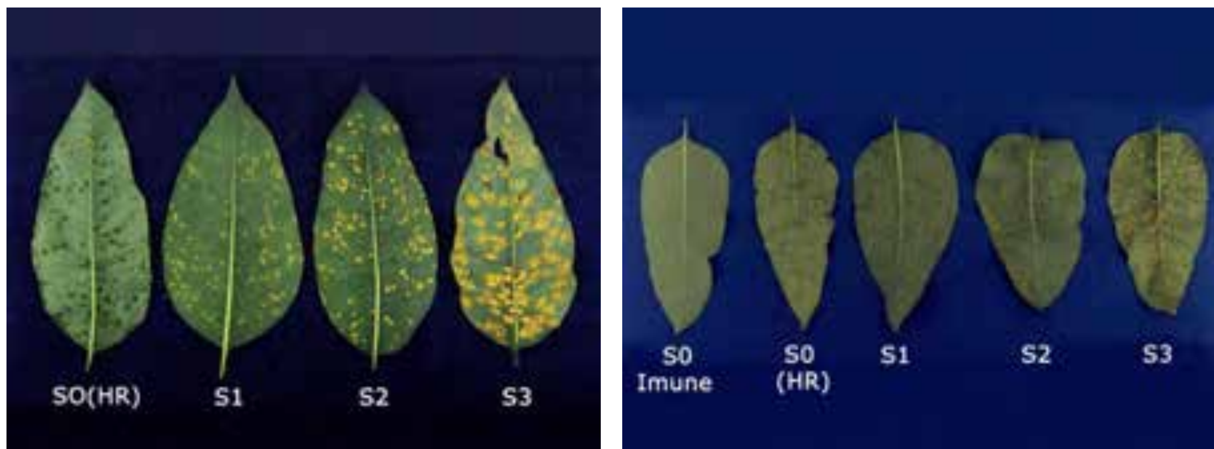

Figure 11. *Rating scale for evaluation of rust severity (Junghans* et al.*, 2003). Images taken from PaDIL (**www.padil.gov.au**).*

Figure 12. *Hypersensitive response to* P. psidii *infection in a eucalyptus leaf. Images taken from PaDIL (**www.padil.gov.au**).*

9.4 Appendix 4: Detailed diagnostic information

9.4.1 Microscopic examination of spores

9.4.1.1 EQUIPMENT

- Dissecting microscope
- Light microscope fitted with high quality 100X oil immersion objective and preferably a 1.5-2X optivar

9.4.1.2 PROCEDURES

To avoid dispersal of spores wear disposable gloves and handle specimens with care. Place a large sheet of paper or laboratory bench coating on the work area. Autoclave or incinerate packaging material, gloves, bench coating and utensils afterwards. Under a dissecting microscope, gently scrape lesions with a disposable scalpel or needle to remove spores and place onto a drop of water on a glass slide. Cover with a coverslip. Use water to view spore characters initially and stains (e.g. cotton blue; Waller *et al.,* 1998) only if characters are not distinct. If only urediniospores are present, it will be necessary to check for marginal paraphyses in the urediniosorus to rule out *Uredo* spp. (see Figure 11 in Walker 1983). Cut thin cross-sections through the urediniosori, and examine under a compound light microscope (Ruzin, 1999; Xavier *et al.,* 2001; Lux *et al.,* 2005).

9.4.1.3 DIAGNOSTIC CHARACTERS

Positive diagnosis depends upon visualisation of teliospores or urediniospores. Diagnosis is positive when spores matching the description of *P. psidii* teliospores are seen. Reproduction is mainly by repeated generations of urediniospores, though teliospores are occasionally produced. In the absence of teliospores, examination of cross-sections of urediniosori to confirm the absence of paraphyses is necessary, as well as confirmation of urediniospore characters. Discrimination of *Uredo* spp. may require determination of germpore positions on urediniospores and these can be difficult to see. Descriptions of *P. psidii* urediniospores and teliospores follow (Section 9.4.1.4), and a full description of the organism is included in Section 9.4.1.5 (page 63). Note that three uredinial species are anamorphs of *P. psidii*. These are *Uredo psidii*, *U. rangelii* and *U. seclusa*.

9.4.1.4 DESCRIPTION OF PUCCINIA PSIDII *SPORES*

Description of *Puccinia psidii* spores is derived from Laundon & Waterston (1965) and Walker (1983) with additional information from Tommerup, Zauza & Alfenas derived from fresh material, numerous cross inoculations of hosts and natural infections of a wide range of hosts, unpublished data (2002-2004). A full description of *P. psidii* and life cycle is included in Sections 4.1.2 and 9.4.1.5. A taxonomic key for rusts on Myrtaceae plants is shown in Figure 13.

Urediniospores (Figure 14 and Figure 15) are ellipsoidal to broadly obovate, 18-28 x 15-24 µm, wall uniformly 1-1.5 µm thick, finely and mostly uniformly echinulate, with short sharp spines up to 1 µm long and 0.5-1.5 µm apart and sometimes a lateral-apical plage. Teliospores (Figure 16) are medium to dark brown in mass, roughly ellipsoidal to cylindrical to broadly clavate, slightly but definitely constricted at the central septum, 26-44 µm x 15-23 µm with the upper cell often slightly broader and shorter than the lower, wall pale golden yellow, sometimes darker in the upper cell, uniformly 1.5 µm thick or very slightly thickened at the apex, pore apical in the upper cell, just below the septum in the lower cell, pedicel either deciduous or short to 15 µm long and 6-8 µm wide.

In addition to the figures provided here, characteristics of spores of several collections are illustrated as taxonomic drawings by Walker (1983) and also in Laundon & Waterson (1965). Many photographs of urediniospores are also available on the Pest and Disease Image Library (**www.padil.gov.au**) and the United States Department of Agriculture Research Services websites (**www.ars.usda.gov**). If the host is among indigenous ones known to have an indigenous rust they are well described by Walker (1983) and Shivas & Walker (1994). These include *P. cygnorum* on *Kunzea ericifolia* and *Uredo xanthostemonis* on *Xanthostemon* species.

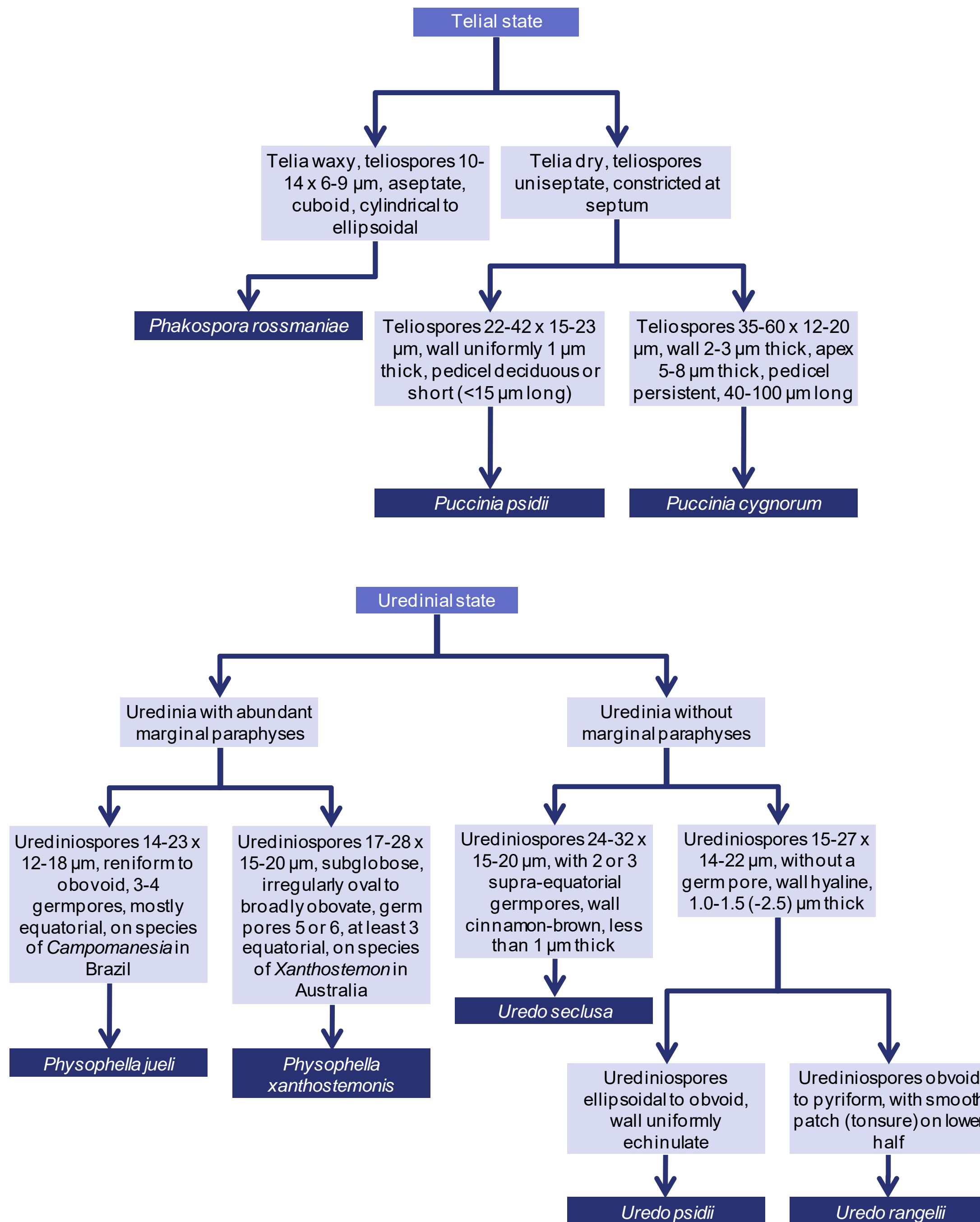

Figure 13. *Taxonomic key to rusts on Myrtaceae (from Simpson* et al.*, 2006). Note that Ureda psidii, U. rangelii and U. seclusa are all anomorphs of P. psidii.*

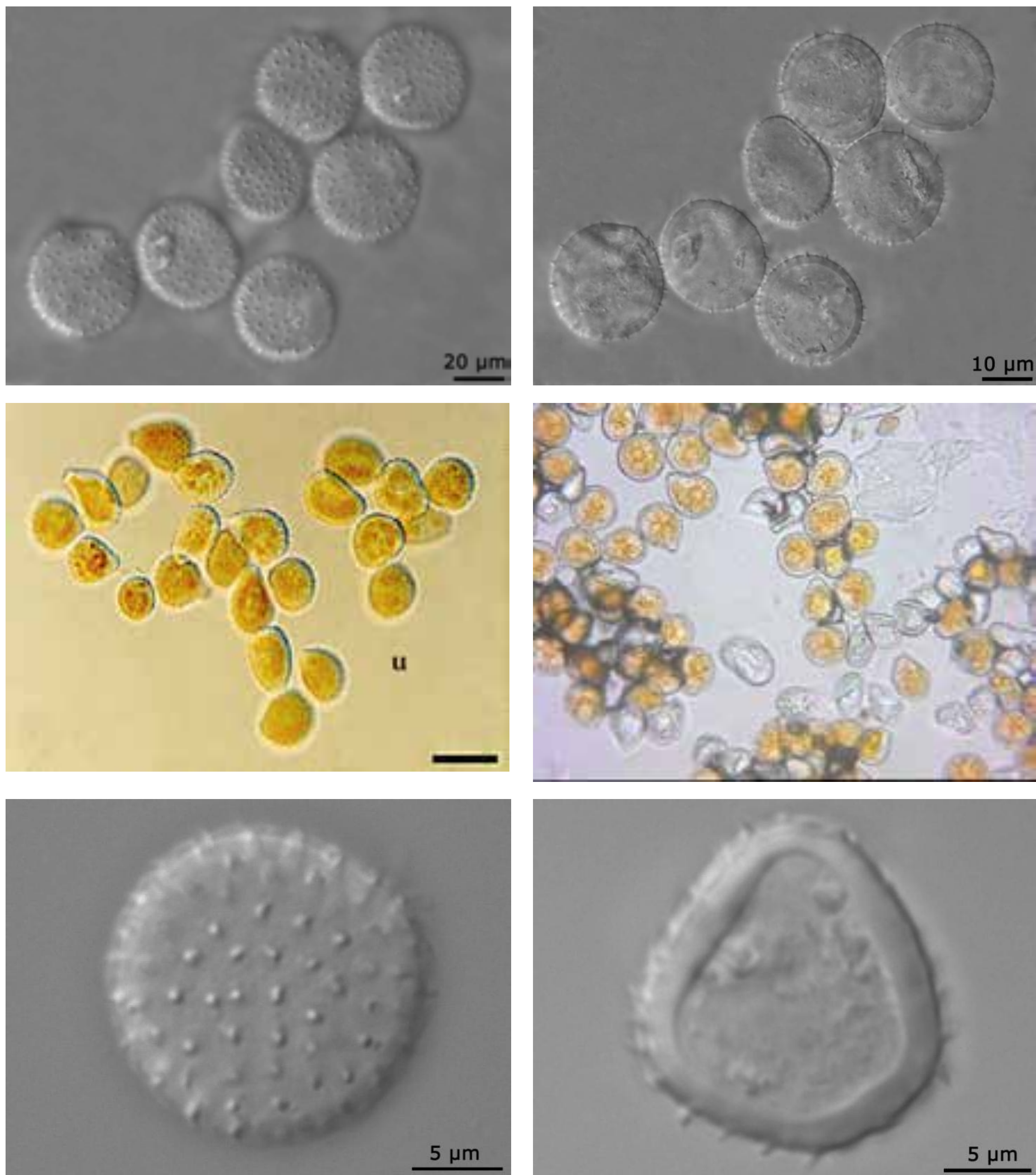

Figure 14. *P. psidii urediniospores. Images taken from PaDIL (top and bottom;* ***www.padil.gov.au****), © Forest Pathology and Genetics of Plant Pathogen Interactions Laboratory, Federal University of Viçosa (middle, left) or © Hank Dankers, Plant Diagnostic Clinic, University of Florida (middle, right).*

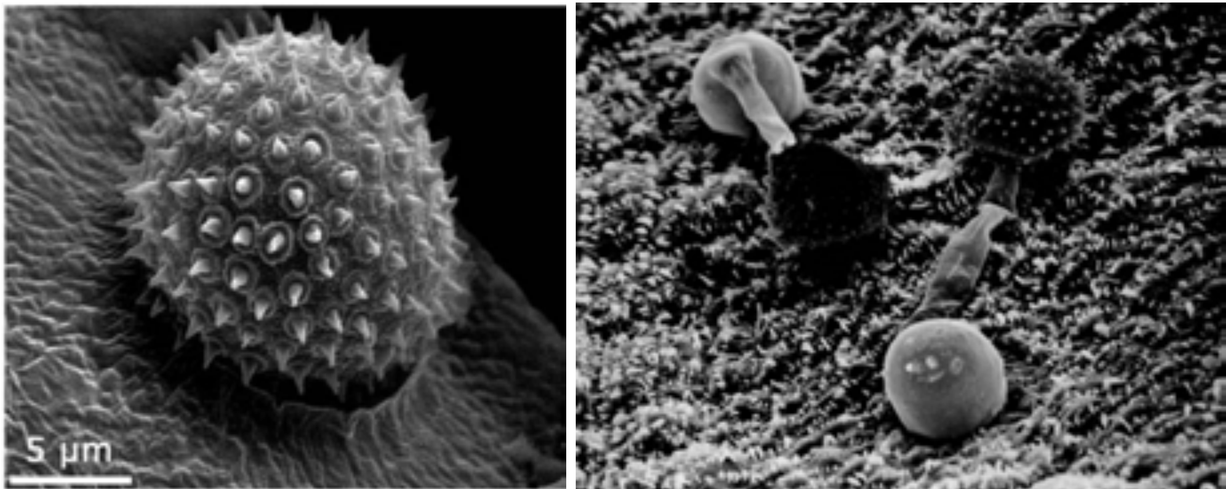

Figure 15. *Scanning electron micrograph images of P. psidii urediniospores. Germination shown in the image on the right. Images taken from PaDIL (**www.padil.gov.au**).*

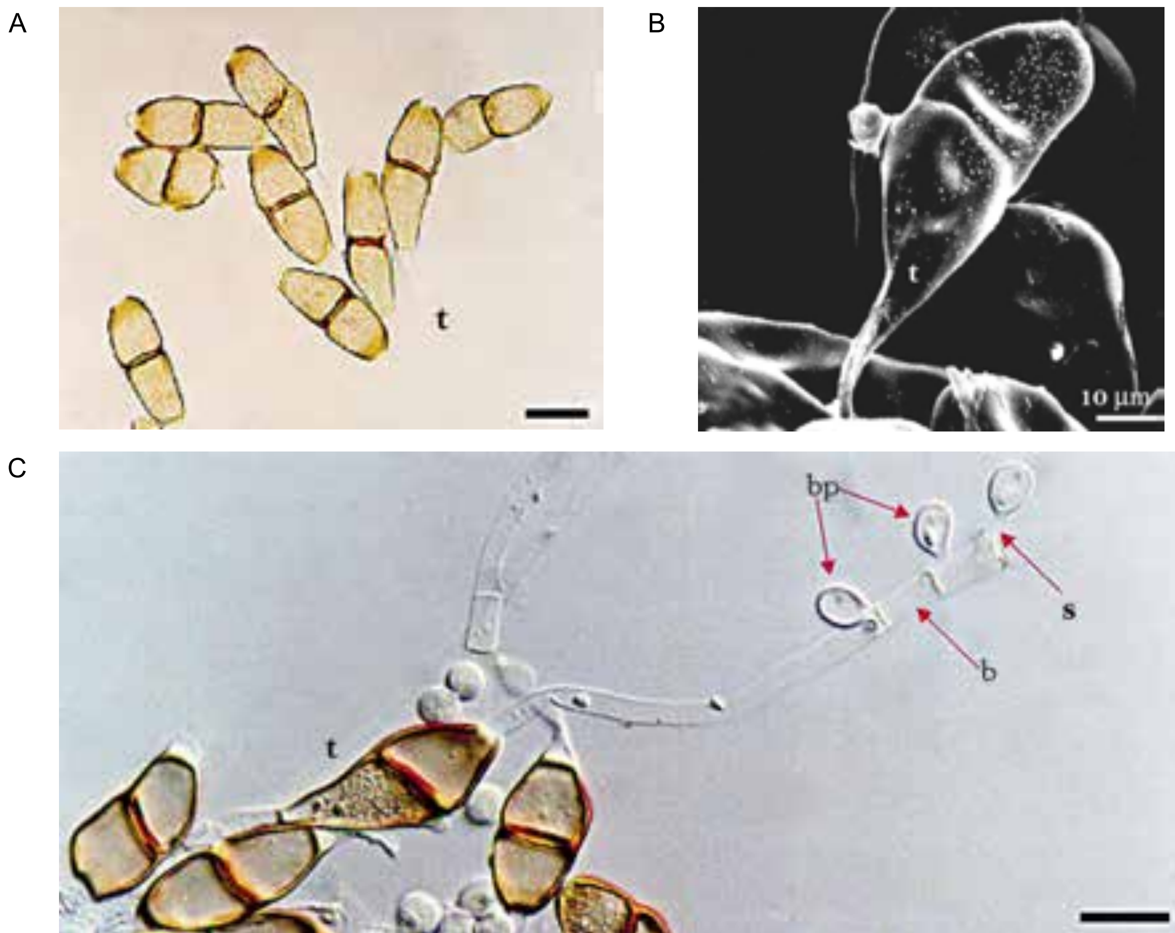

Figure 16. *Teliospores of P. psidii. Germination is shown in (C), t=teliospores, b=basidium, S=sterigma, bp=basidiospores. Images © Forest Pathology and Genetics of Plant Pathogen Interactions Laboratory, Federal University of Viçosa.*

9.4.1.5 DESCRIPTION OF PUCCINIA PSIDII *WINT. HEDWIGIA 23:171,1884*

Description of *P. psidii* Wint. is derived from Walker (1983) with additional information from Tommerup, Zauza and Alfenas derived from fresh material, numerous cross inoculations of hosts and natural infections of a wide range of hosts, unpublished data (2002-2004).

Leaf and shoot spots

Young sori are produced on green tissue and erumpent pustules may coalesce in very susceptible tissue or when natural inoculation loads are heavy (Figure 5). Urediniosori are more common than teliosori. Lesions caused by this rust are brown to grey and older lesions have purpling of lesion margins including on shoots of many eucalypt, *Melaleuca* and *Callistemom* hosts (Ferreira 1989, Walker 1983). Lesions on fleshy fruits of *Psidium*, *Syzygium* and *Eugenia* may not have obvious margins and are often distinguished by heavy spore masses when young and rot due to secondary pathogens when ripening (Figure 6).

Sori

Young sori are scattered on green tissue, leaf spots, stems, sepals and fruits and mainly hypophyllous on leaves. Urediniosori which are bright yellow or orange yellow are usually more common than teliosori which are dark brown and both are aparaphysate (Figure 7). Both teliosori and urediniosori may occur together in the same lesion.

Urediniospores

Urediniospores are bright yellow to orange in mass and singly, immature spores are colourless (Figure 7). Urediniospores are ellipsoidal to broadly obovate, 18-28 x 15-24 µm, wall uniformly 1-1.5 µm thick, finely and mostly uniformly echinulate, sometimes a lateral-apical plage, with short sharp spines up to 1 µm long and 0.5-1.5 µm apart (Figure 14 and Figure 15).

Teliospores

Teliospores are medium to dark brown in mass, roughly ellipsoidal to cylindrical to broadly clavate, slightly but definitely constricted at the central septum, 26-44 µm x 15-23 µm with the upper cell often slightly broader and shorter than the lower, wall pale golden yellow, sometimes darker in the upper cell, uniformly 1.5 µm thick or very slightly thickened at the apex, pore apical in the upper cell, just below the septum in the lower cell, pedicel either deciduous or short to 15 µm long and 6-8 µm wide (Figure 16).

Mesospores

Mesospores are cylindrical to obovate, 24-39 x 11-20 µm, sometimes with the germpore not apical but depressed to one fifth the way down the side.

Basidiospores and Aeciospores

Basidiospores are hyaline, obovate and thin walled (Figure 16C). Aeciospores are indistinguishable from urediniospores.

9.4.2 Molecular diagnostics

Two methods are available for molecular confirmation of the identity of the pathogen:

- A species-specific nested PCR
- Direct sequencing of the ribosomal DNA internal transcribed spacers (rDNA ITS) and comparison with authentic *P. psidii* sequences

The nested PCR is faster than sequencing and more sensitive but requires the availability of four specific primers. The test has been validated against Brazilian and Hawaiian sources of the rust from a wide range of hosts (Langrell *et al.,* 2008).

Sequencing requires commonly used fungal-specific primers, but it takes longer and problems in obtaining a sequence from the rust may be caused by the presence of contaminating fungi. Authentic *P. psidii* rDNA ITS sequences are available at Ensis Forest Biosecurity and Protection and on Genbank (**www.ncbi.nlm.nih.gov**) as listed in Langrell *et al.* (2008). ITS sequences from a broad geographical and host range have very limited ITS (<1%) sequence variation (Langrell *et al.,* 2008) and the *P. psidii* sequence varies by at least 5% from all other known ITS sequences of rust fungi (Glen, unpublished data).

9.4.2.1 NESTED PCR

Reference laboratory protocol for a laboratory with experience running nested PCRs

Equipment

- Three isolated work areas
    - DNA extraction
    - Laminar flow cabinet fitted with UV light for setting up PCRs. Irradiate cabinet for 1 h between each procedure
    - Electrophoresis tanks and powerpacks for running gels
- Centrifuge and water bath for DNA extraction
- Laminar flow not to be used for fungal cultures
- Electrophoresis tanks for running agarose gels
- Transilluminator and photographic equipment for visualising and recording gel results

Procedures

DNA extraction

Standard procedures such as Glen *et al.* (2002) (described below), Langrell *et al.* (2008) or a commercial DNA purification kit, such as the Mo Bio Ultraclean Microbial DNA kit may be used. Include a reagent blank in each set of extractions.

Suitability of the DNA for PCR must be demonstrated, either by inclusion of an IAC (internal amplification control) in each PCR reaction, or by prior amplification with primers that amplify DNA from a broad range of fungi (details listed in this Appendix).

The following DNA extraction procedure has been taken from Glen *et al.* (2002).

Scrape several spores into a 1.5 mL microcentrifuge tube, or cut a small section of a sorus (~5mm x 5mm) and place into the tube. Use a new blade and new paper towel for each sample. Grind with a micropestle under liquid nitrogen into a fine powder. Add 250 µL extraction buffer and incubate at 65˚C for 1 hour. Centrifuge at ~14,000 *g* for 15 min. To a 1.5 mL tube, add 7 µL silica suspension, 800 µL NaI and 200 µL supernatant – vortex. Incubate on ice for 15 min, shaking occasionally. Centrifuge 10 sec, discard supernatant and resuspend pellet in 800 µL wash buffer. Centrifuge 10 sec, discard supernatant and resuspend in 800 µL 100% ethanol. Centrifuge 10 sec, discard supernatant and invert tubes to dry. Resuspend pellet in 25 µL TE buffer and incubate at 45˚C for 10 min. Centrifuge at ~14,000 *g* for 1-2 min, remove supernatant to a fresh tube. Dilute 1/20 and use 5 µL of diluted DNA in a 25 µL PCR reaction.

Use bottled water for irrigation (Baxter Healthcare Pty Ltd) for all buffers and solutions. Ethanol is absolute ethanol (Merck), all other chemicals are analytical grade (BDH Chemicals).

Extraction buffer

(Raeder & Broda, 1985)

200 mM Tris-HCl, pH 8.5

250 mM NaCl

25 mM EDTA

0.5% SDS

NaI solution

Dissolve 100 g NaI in 100 ml water, add 1.5 g sodium sulphite. Filter through Whatman No. 1 filter paper and store at 4ºC in an opaque bottle. If the solution starts to turn yellow, add a few grains of sodium sulphite.

Silica suspension

(Modified from Boyle and Lew, 1995)

Mix 10 g silica (Sigma cat # S-5631) in 100ml of phosphate-buffered saline and allow to settle for 2 h. Remove the supernatant and repeat. Centrifuge (2,000 *g* for 2 min) and resuspend in 10 ml TE buffer. Store 1 ml aliquots at -20 or -80°C long term or 4°C short term. Vortex thoroughly after thawing and immediately before each use.

Phosphate buffered saline

NaCl 8 g/L

KCl 0.2 g/L

Na_2HPO_4 1.44 g/L

KH_2PO_4 0.24 g/L

Adjust pH to 7.4 with HCl.

TE buffer

10 mM Tris-HCl, pH 8

1 mM EDTA

Wash solution

10 mM Tris-HCl, pH 7.5

1 mM EDTA

100 mM NaCl

in 50% absolute ethanol (add last).

Store at 4°C.

Verification of amplifiability

The primer combinations ITS1-F/ITS4, ITS5/ITS4 and ITS1/ITS4 are all suitable to demonstrate DNA template quality. Each reaction should contain 2 mM $MgCl_2$, 0.25 µM each primer, 0.2 mM dNTPs, 0.02 U/µL *Taq* or *Tth* polymerase enzyme, with the appropriate buffer as supplied by the manufacturer of the enzyme in a total volume of 25 µL. Add template DNA in a 10-fold serial dilution from approximately 10 ng to 100 fg. Primer sequences (from Gardes & Bruns, 1993 and White *et al*., 1990); ITS1-F (CTT GGT CAT TTA GAG GAA GTA A); ITS4 (TCC TCC GCT TAT TGA TAT GC); ITS5 (GGA AGT AAA AGT CGT AAC AAG G); ITS1 (TCC GTA GGT GAA CCT GCG G). Thermocycler program: 94° for 3 min, 35 cycles of 94° for 30s, 55° for 30s, 72° for 30s, followed by 72°C for 10 min.

Specific PCRs

Use aerosol-resistant pipette tips for all procedures. Each reaction should contain 1.5 mM $MgCl_2$, 0.25 µM each primer, 0.2 mM dNTPs, 0.02 U/µL *Taq* or *Tth* polymerase enzyme, with the appropriate buffer as supplied by the manufacturer of the enzyme. Add template DNA (100 fg to 10 ng), at a concentration that has been determined to be amplifiable.

Positive and negative controls must be included in each set of PCR reactions (see Langrell *et al*. 2008). An IAC has been developed for each of the *P. psidii*-specific PCR reactions that produces a 1200 bp fragment which can easily be distinguished from the diagnostic fragment when electrophoresed on a 1.5% agarose gel (Glen, unpublished). These IACs can be obtained from Ensis FBP and added to each PCR reaction. In the absence of IAC, a separate PCR containing DNA from an authentic *P. psidii* sample should be included in each set of reactions. Negative controls in each set of reactions should include at least one DNA extraction blank and at least one water blank.

Template for the second round PCR consists of 1 µL of first-round PCR product per 25 µL reaction. Best results are obtained by making a 1/5 dilution of an aliquot from the first round PCR and adding 5 µL of this to the second-round PCR.

Primers

First-round PCR - Ppsi1 (TTC TAC CTT ATT ACA TGT) and Ppsi6 (GTC ATA TTG ACA GGT TAG) produce a 508 bp product only for *P. psidii* (Langrell *et al*., 2008).

Second-round PCR - Ppsi2 (ATA GTA ATT TGG TAT ACG) and Ppsi4 (GTC AAT CCA AAT CAA AGT) generate a diagnostic fragment of 379 bp only for *P. psidii* (Langrell *et al.*, 2008).

PCR Amplification conditions

Thermocycler program: 94° for 3 min, 20 cycles of 94° for 30s, 62° for 30s, 72° for 30s, followed by 72°C for 10 min (Langrell *et al.*, 2008).

Visualisation of PCR products

In 1.5% agarose, gels stained with ethidium bromide (see Figure 17 and Figure 18) (Langrell *et al.*, 2008).

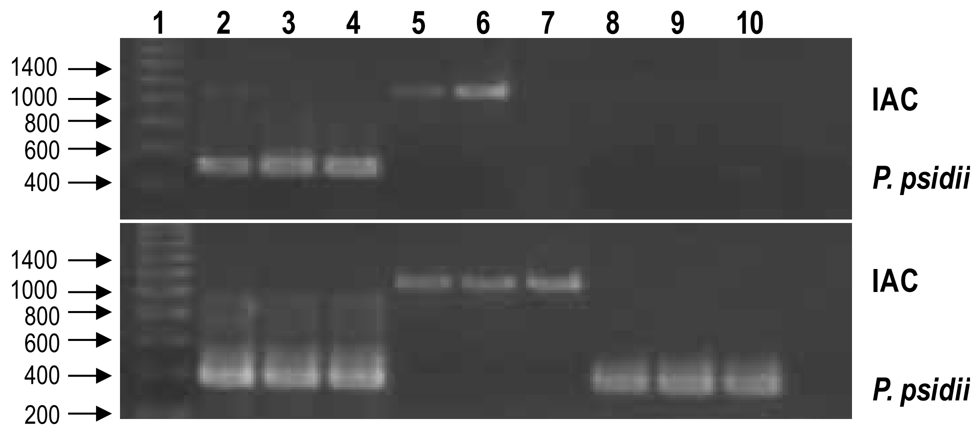

Figure 17. *Agarose gel (1.5%) of diagnostic PCR products incorporating internal amplification controls (IAC) showing first-round (upper panel) and second-round (lower panel) products. Lane 1 is a 200bp ladder (Geneworks), lanes 2-4 are positive in both rounds, lanes 5 and 6 are negative, lane 7 is a doubtful negative (no amplification of IAC in round 1) and lanes 8 to 10 are positive, despite the lack of IAC amplification in both rounds 1 and 2.*

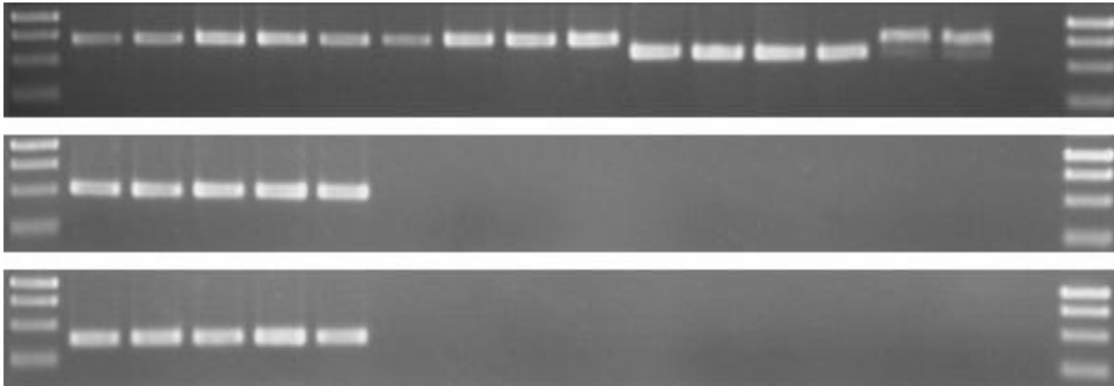

Figure 18. *Agarose gel (1.5%) of diagnostic PCR products without internal amplification controls (IAC). Upper panel shows amplification with non-specific primers ITS1-F and ITS4 to verify the quality and amplifiability of the DNA extract. The centre panel shows first-round products and the lower panel shows the second-round products. Lanes 1 and 18 contain a 1kb ladder (showing fragments of 250, 500, 750 and 1,000 bp), lanes 2-6 are positive in both rounds, lanes 7 to 16 are negative, lane 17 is a negative control (no DNA template added).*

Quality control of samples

Positive: Product of 379 bp (Langrell *et al*., 2008)

Negative: A sample is assessed as negative only if there is no product from the nested PCR and the DNA has been verified to be amplifiable, either by inclusion of an IAC (available from Ensis FBP) or by amplification in a separate PCR of a clear product from primers ITS5 and ITS4.

9.4.2.2 DNA SEQUENCING

Reference laboratory protocol for a laboratory with experience in DNA sequencing

Equipment

- Centrifuge and water bath for DNA extraction
- Laminar flow cabinet
- Thermocycler
- Electrophoresis tanks for running agarose gels
- Transilluminator and photographic equipment for visualising and recording gel results
- Access to DNA sequencing facility

Procedures

DNA extraction and sequencing of rDNA ITS

Standard DNA extraction procedures such as Glen *et al*. (2002) (refer to page 64), Langrell *et al*. (2008) or a commercial DNA purification kit, such as the Mo Bio Ultraclean microbial DNA kit may be used. Amplify the rDNA ITS using primers that will amplify fungal and not host DNA (e.g. ITS1-F

[Gardes & Bruns, 1993] and ITS4 [White *et al.,* 1990]). If co-amplification of contaminating fungi is a problem, the use of more selective rust-specific primers such as Rust1 (Liu *et al*., 1993, Kropp *et al*., 1997) or URITS4 (Glen, unpublished), in place of ITS4, sequencing of PCR product excised from an agarose gel or cloning of PCR products before sequencing, may assist in obtaining a clean sequence.

Primer sequences: ITS1-F (CTT GGT CAT TTA GAG GAA GTA A); ITS4 (TCC TCC GCT TAT TGA TAT GC); Rust1 (GCT TAC TGC CTT CCT CAA TC); URITS4 (CAA CAG ACT TAT ACA TGG TCC AGC).

Thermocycler program: 94° for 3 min, 35 cycles of 94° for 30s, 55° for 30s, 72° for 30s, followed by 72°C for 10 min.

Each PCR reaction should contain 2 mM $MgCl_2$, 0.25 µM each primer, 0.2 mM dNTPs, 0.02 U/µL *Taq* or *Tth* polymerase enzyme, with the appropriate buffer as supplied by the manufacturer of the enzyme, and 0.1 to 10 ng template DNA. Follow standard procedures for sequencing as outlined in Sambrook & Russell (2001) or use a commercial kit. Sequence with the same primers that were used for PCR.

Diagnosis

Positive: Greater than 99% similarity with authentic *P. psidii* ITS sequence (available from Ensis FBP and public databases, GenBank accession numbers AJ421800 – AJ421808, AJ535657-AJ535661, AJ536601-AJ536603)

Negative: Less than 95% sequence similarity with authentic *P. psidii* ITS sequence. However it is necessary to ascertain that a fragment from the rust fungus has been amplified, and not a fragment from a hyperparasite, e.g. *Cladosporium* or *Fusarium* spp. (Langrell *et al*., 2008).

9.4.2.3 SOURCES OF CONSUMABLES

- DNA extraction kits: Geneworks, PO Box 299, Hindmarsh, SA, 5000. Ph. 08 8234 2644 (1800 882 555 from outside SA).
- Micropestles: Fronine Laboratory Supplies, PO Box 27, Riverstone, NSW, 2765. Ph. 02 9627 3600.
- Primers: Geneworks, PO Box 299, Hindmarsh, SA, 5000. Ph. 08 8234 2644 (1800 882 555 from outside SA).
- Sequencing kits: Applied Biosystems, 52 Rocco Drive, Scoresby, Vic, 3179. Ph. 03 9730 8600, 1800 033 747. Beckman Coulter Australia, P. O. Box 218, Gladesville, NSW 1675. Ph. 02 9844 6000, 1800 060 878.
- *Taq* polymerase, *TTH* polymerase: Fisher Biotec, PO Box 169, Subiaco, WA, 6904. Ph. 08 9322 6866.
- Water: Baxter Health Care Pty Ltd, 1 Baxter Drive, Old Toongabbie, NSW, 2146. Ph 1300 789 646.
- Silica: Sigma-Aldrich Co., PO Box 970, Castle Hill, NSW, 1765. Ph. 1800 800 097.
- Ethanol: Merck Pty Limited 207 Colchester Rd Kilsyth Victoria Australia 3137. Ph 1800 335 571.
- BDH Analytical Grade chemicals: Biolab Australia, 2 Clayton Rd, Clayton, Vic, 3167. Ph 1300 735 292.

9.4.3 Key personnel for diagnosis of *Puccinia psidii*

The State Chief Plant Health Manager will select the preferred laboratory.

Table 15. *Key personnel for the diagnosis of* Puccinia psidii

Name	Contact details
Morphological diagnosis	
Professor Robert Park Plant Breeding Institute University of Sydney	107 Cobbitty Rd Cobbitty NSW 2570 Phone: (02) 9351 8806 Email: **robertp@camden.usyd.edu.au**
Dr Roger Shivas Deptartment of Primary Industries and Fisheries	80 Meiers Rd Indooroopilly Qld 4068 Phone (07) 3896 9340 Email: **shivasr@dpi.qld.gov.au**
Dr Louise Morin CSIRO Entomology	Clunies Ross St Acton GPO Box 1700 Canberra ACT 2601 Phone: (02) 6246 4355 Email: **Louise.Morin@csiro.au**
Molecular diagnosis	
Dr Morag Glen CSIRO Ensis Forest Biosecurity and Protection	Private Bag 12 Hobart Tas 7001 Phone: (03) 6226 7989 Email: **Morag.Glen@csiro.au**
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9.5 Appendix 5: Survey development background information

9.5.1 Technical information for planning surveys

The following characteristics of the pathogen provide the basic biological knowledge that informs the survey strategy.

9.5.1.1 BIOTYPES

Several races or biotypes of *P. psidii* are known to exist, though knowledge of them is limited in comparison to rusts of cereal crops. Three to four strains are known to exist in Brazil (Xavier, 2002; Aparecido *et al.,* 2003a), two in Jamaica (McLachlan, 1938) and one each in Florida and Hawaii, the one from Florida being the same as one of the Jamaican strains and the one in Hawaii probably the same as one from Brazil. There are likely to be additional biotypes that have not yet been recognised, as work in this area is limited. Fungicides are not extensively used against *P. psidii*, except in nurseries. There are no reports of fungicide-resistant strains.

9.5.1.2 CONDITIONS OF INFECTION

The urediniospores need high humidity (leaf wetness for more than 6 hours) and moderate temperatures (8-27°C, ideally 13-22°C) for germination (MacLachlan, 1938; Piza & Ribeiro, 1989). Low light conditions are also preferred, with at least 8 h of darkness required for a reasonable germination rate (Piza & Ribeiro, 1988). The fungus is not known to initiate infection in mature tissue, but a leaf that has been lightly infected when young may still bear urediniosori when mature (MacLachlan, 1936).

9.5.1.3 NATURE OF VECTOR

No specific vectors have been reported for *P. psidii*, though bees have been noted to collect urediniospores (Chapman, 1964). Birds and mammals may also disperse spores on infected fruit.

9.5.1.4 RATE OF COLONISATION

P. psidii is only known to infect young host tissue, under 30-40 days of age (Marlatt & Kimbrough, 1979). A heavily infected leaf will be killed, but a leaf with a lighter infection may survive to maturity and still show symptoms (MacLachlan, 1936). It is unknown whether the pathogen can survive asymptomatically in older tissue.

9.5.1.5 GENERATION TIME

The fungus can go through a generation (from one urediniospore to thousands) in as little as 10 days in favourable climatic conditions and a highly susceptible host. The degree of susceptibility of Australian host species in the wild is largely unknown, though some are known to be highly susceptible in glasshouse tests and in the wild (Rayachhetry *et al*., 2001).

9.5.1.6 PERSISTENCE

Urediniospores have been reported to survive for at least 22 days at 15°C (MacLachlan, 1938) and for over 100 days at lower temperature and humidity (Suzuki & de Silveira, 2003). Survival at higher temperatures (>32°C) was minimal after 11 days. Teliospores are theoretically more persistent, though no figures are available for survival times. This is the most likely spore form to survive in drier, hotter conditions. It is unknown whether, or for how long, the mycelia can survive asymptomatically in the host plant until climatic conditions are again favourable, but it can survive for several months in a lightly infected leaf (MacLachlan, 1936).

9.5.1.7 SOIL SURVIVAL

Spore survival is likely to be reduced in soil, though no hard data is available. Predation and decomposition are expected to be greater in soil than on plant surfaces.

9.5.1.8 ENDEMIC HOSTS

There are many known and many more potential hosts in most areas of Australia, including the areas identified as climatically suitable (Figure 3 and Figure 4; Table 14). Most species of Myrtaceae so far tested or unwittingly exposed to *P. psidii* have some degree of susceptibility and species in this family have high economic and environmental value in Australia.

9.5.1.9 NATURAL DISPERSAL

The spores are produced in vast numbers and readily dispersed by wind. Insect and other vectors may also transport spores, but are probably less effective than wind dispersal. Rapid spread of new incursions or new biotypes has been documented for Jamaica and Hawaii. In Hawaii, *P. psidii* had spread across all but one of the islands within a few months of its initial detection.

9.5.1.10 MECHANICAL TRANSMISSION

The risk of mechanical transmission is high. Viable spores have been detected on clothing and personal effects such as cameras and spectacles after visits to severely affected plantations (Langrell *et al.,* 2003b). The spores are sufficiently small to be well-concealed in crevices.

9.5.1.11 RISK MAPPING

The areas of Australia that have climatic conditions favourable for the rust have been mapped (Figure 3 and Figure 4). These are the areas that should be targeted with the greatest vigilance. In addition, local variation in altitude and aspect may be significant. For example, pimento trees in Jamaica were more severely affected in valleys where mists were more frequent and on northern and western slopes that received less morning sun, thereby prolonging the period of leaf wetness (MacLachlan, 1938). Fine-scale risk mapping may therefore help in targeting surveillance activities.

9.5.2 Surveys for early detection of an incursion

If an incursion of *P. psidii* is to be eradicated, it must be detected very early, before the spores have had the opportunity to disperse very far. It is therefore necessary to consider pathways and plan

surveys and/or sentinel plantings accordingly. A pathway risk analysis is currently being undertaken by Biosecurity Australia and will provide valuable information in this respect. At present, the greatest risk is probably from travellers and importations of host plants or other goods, so vigilance at ports and in populated areas is probably more essential than surveys of large areas of inaccessible rainforest. It is possible that spores have been unwittingly imported many times on tourists‟ personal effects, but have perished before finding a susceptible host in suitable climatic conditions. PCR tests on swabs taken from the clothing of incoming visitors from South America, Florida, Jamaica and Hawaii or residents returning from these countries would give an indication of the frequency of such events.

Rainforests are a popular destination for tourists, who may inadvertently bring viable spores, so park rangers and tour guides in the rust risk area should be trained on what to look for. Information should also be distributed to plant nurseries and to any other businesses (e.g. *Melaleuca* plantations, wildflower growers) that use Myrtaceae plants. Awareness and vigilance of people who are in regular close contact with potential hosts in the risk areas are likely to provide a greater degree of „ground cover‟ than it would be economically possible to attain by other means.

If *P. psidii* becomes established in Indonesia or Papua New Guinea, the threat of an incursion being initiated in a remote region by way of wind dispersal from close neighbouring islands would become greater, so more active surveillance of northern areas may then be necessary. The climate in Indonesia and Papua New Guinea is expected to be very hospitable for *P. psidii* (Figure 4). Raising awareness of this threat in neighbouring countries should also be a high priority as it will be extremely difficult to prevent establishment of the rust in Australia if it becomes established in any of our near neighbours. In that instance, the feasibility of aerial surveillance of remote regions should be examined. An ACIAR funded project is underway to raise biosecurity awareness and establish sentinel plantings in Fiji and Vanuatu (Ross Wiley & Tim Wardlaw, pers. comm.), however similar work is needed in Indonesia, Papua New Guinea, and other Pacific Islands such as New Caledonia, the Solomon Islands and the Marshall islands.

In Australia, sentinel plantings at air and sea ports and other locations such as local council offices or visitor centres in parks throughout the rust risk area, are recommended. No plant species is highly susceptible to all known biotypes of *P. psidii*, so at least two plant species should be used at each sentinel location. *S. jambos* is highly susceptible to most biotypes of the rust, therefore it would make the most suitable sentinel plant. The strain of *P. psidii* that infects allspice in Jamaica and Florida may not infect *S. jambos* (Rayachhetry *et al.,* 2001), may take longer to sporulate (Marlatt & Kimbrough, 1979) or may produce lesions without urediniospores (MacLachlan, 1938), therefore all leaf lesions should be considered suspect even without urediniospore production. DNA testing (Section 9.4.2) will confirm the presence or absence of *P. psidii* in non-sporulating lesions.

As little is known about the different races or biotypes of the pathogen, several plants of different provenances would be preferable to a single plant, or to several plants from a single seedlot. *S. jambos* is also susceptible at all ages (though only the young tissue is susceptible, so regular pruning to promote new growth is recommended), whereas eucalypts develop resistance by about 2 years of age, unless coppiced. Guava (*Psidium guajava*) is also susceptible at all ages but is less suitable for sentinel plantings as at least two races that do not infect guava are known to exist (Marlatt & Kimbrough, 1979). In addition, the strain that is commonly found on *Eucalyptus* spp. and is most virulent to *Eucalyptus* spp. rarely infects guava (Aparecido *et al.,* 2003a). *Pimenta dioica* and *M. quinquenervia* are both highly susceptible to the biotype that does not readily infect *S. jambos* (Rayachhetry *et al.,* 2001), and both are susceptible at all ages (MacLachlan, 1938; Rayachhetry *et al*., 1997), so either of these would be a suitable choice for a second sentinel species.

Sentinel plants should be inspected weekly and any suspicious or unusual symptoms should be sampled carefully to avoid dispersal of any spores (refer to Section 5.2.4) and sent immediately to a

diagnostic facility. Weekly inspection is essential as without this, the sentinel plants may end up being a convenient incubator for spores that might otherwise have perished before finding a suitable host. Fruits of *S. jambos* should be removed before maturity to prevent dispersal of seed, as this is a potentially invasive weed (Department of Environment & Heritage, 2004b). The cost of the sentinel plant monitoring may be reduced by integration with the Asian Gypsy moth trap monitoring which is done on a fortnightly basis, but frequency would need to be increased to accommodate the potentially shorter lifecycle of *P. psidii*.

9.5.3 Delimiting surveys in the event of an incursion

9.5.3.1 GENERAL POINTS

In the event of an incursion, delimiting surveys will be required to inform the decision-making process. The size of the survey area will depend on the size of the infected area and the severity of the infection as well as prevailing winds during the period prior to detection. All potential host species (i.e. all Myrtaceae; refer to Section 4.2 on page 11) should be surveyed, with particular attention paid to the species in which the pathogen was initially detected. In addition to inspection of possible host plants, sentinel plants and spore traps should be established and the trapped material tested using the PCR diagnostic protocol. Cyclone-style traps (**www.burkard.co.uk/instmts.htm**) that deposit spores into a centrifuge tube rather than onto an adhesive surface are more suitable for PCR detection. If the incursion is in a populated area, publication and distribution of information sheets and appeals for public assistance may assist.

9.5.3.2 ESTABLISHED PLANTS

If the incursion is in or near natural vegetation with a high proportion of Myrtaceae species, it will be very difficult to completely survey a large area. A sampling strategy will have to be devised. Attention should be concentrated on:

- Species with the greatest disease severity
- Any young plants of Myrtaceae species
- Any Myrtaceae species with young growth

Selection of locations for more intense surveillance should be based on local geographic and climatic variables. For example, hillsides with a southerly or western aspect are not likely to receive early morning sun and so should have longer periods of leaf wetness (MacLachlan, 1938). If current temperatures at sea-level are higher than optimum for *P. psidii*, cooler regions at higher elevations may have more favourable conditions. Wetlands and valleys that are prone to mists will also be more conducive to rust infection.

9.5.3.3 SENTINEL PLANTS

Syzygium jambos would be first choice for sentinel plantings as it is susceptible to most known biotypes of the rust and is susceptible at all ages. It is a potential invasive weed (Department of Environment & Heritage, 2004b) so care will be needed to maintain a register of all plants deployed and ensure their subsequent removal. Dispersal of seeds must also be prevented. This should not present any difficulties, as weekly inspection of the sentinel plantings is necessary. Sentinel plants must be sourced from well outside the quarantine and buffer areas to avoid inadvertently distributing inoculum factories throughout the quarantine area.

If insufficient *S. jambos* plants are available, a combination of *Kunzea baxteri* and *Callistemon citrinus* would be the second choice, as these are both highly or moderately susceptible (Tommerup *et al.,* 2003) and readily available in large quantities from many nurseries. *K. baxteri* plants tested (Alfenas *et al.,* unpublished) were 100% susceptible, though laboratory and field susceptibility do not always concur (e.g. Daly *et al.,* 2005). *Callistemon viminalis* is another highly susceptible species (Rayachhetry *et al.,* 2001, Alfenas & Zauza, unpublished) that is also readily available. *M. quinquenervia* and *Pimenta dioica* are recommended as sentinels for the biotype that infects allspice in Jamaica and Florida. *M. quinquenervia* is probably more readily available than *Pimenta dioica* in Australia and is susceptible to the biotype that infects eucalypts in Brazil (Alfenas & Zauza, unpublished), as well as the allspice biotype (Rayachhetry *et al.,* 2001).

Selection of locations for sentinel plants should be based on local geographic and climatic variables. For example, hillsides with a southerly or western aspect are not likely to receive early morning sun and so should have longer periods of leaf wetness (MacLachlan, 1938). If current temperatures at sea-level are higher than optimum for *P. psidii*, cooler regions at higher elevations may have more favourable conditions.

9.5.3.4 SPORE TRAPS

The cyclone style spore traps will be useful in detecting wind-borne spores when weather conditions are not ideal for spore germination and will be particularly useful in establishing the distance that spores may have travelled. Detection in spore traps is not dependent on suitable spore germination conditions, as is the case in plants, whether they are natural vegetation or sentinel plantings. They also may be more suitable for remote areas that cannot be visited as frequently and for deployment at the outer edges of the buffer zone where spores are not expected to be found. The material from spore traps can be tested using the DNA diagnostic, and can also be used for viability tests under controlled conditions.

9.6 Appendix 6: Chemical eradication

Fungicides to be used in Australia for any eradication attempts will need to have a curative effect rather than (or as well as) a protective effect. Non systemic fungicides tested on guava in Brazil were chlorothalonil, mancozeb and copper oxychloride (Table 11). Chlorothalonil was reported to be the most effective, even though it did not completely eliminate the disease (Ferrari *et al.,* 1997). In other experiments by Ruiz *et al.* (1991), triadimenol (0.75 g/L), triforine (0.28 mL/L) and oxicarboxin (0.75 g/L) were reported to also give protective and curative results. Demuner *et al.* (1991) determined the period of protection afforded by oxycarboxin, diniconazole and triadimenol (Table 11) in *Eucalyptus cloeziana* seedlings. Subsequent field-based experiments determined the efficacy and cost per ha of various application rates and intervals of these three chemicals on *E. cloeziana* coppice (Alfenas *et al.,* 1993). Triadimenol was determined to be the most effective fungicide in both of these experiments, as well as those of Ruiz *et al.* (1991). In the field trials, applications of 200 L/ha (0.5 g/L) at intervals of 20 days kept the infection rate to under 40% for leaves and under 20% for shoots, compared to over 90% and 40%, respectively, in the controls. Current recommendations for control of eucalypt rust in Brazil are given in Table 16.

The experiments described above were conducted to determine a cost-effective rate of application for operational use and the aim was to achieve control rather than eradication. Further experiments are needed to determine the fungicide combinations and application rates that would provide the greatest probability of successful eradication. Trials to determine an effective spray regime for an eradication attempt are needed. Such trials should include combinations of the more recently developed systemic

fungicides that are effective against wheat or soybean rusts (e.g. flutriafol or fluquinconazole; Loughman *et al.,* 1995), myclobutanil, that is specified by APHIS (APHIS, 2005) to eradicate chrysanthemum white rust, *Puccinia horiana* (NAPPO, 2004), traditional curative fungicides such as copper oxychloride and the use of defoliants to assist canopy penetration.

Table 16. *Fungicides and spraying regimes currently recommended for control of P. psidii in South America (Alfenas* et al., *2004)*

Active ingredient	Chemical group	Mode of action	Commercial example	Concentration of active ingredient (g/L)	Spray interval
Triadimenol	Triazole	Systemic	Bayfidan CE	0.125	2-3 weeks
Azoxystrobin	Strobilurin	Systemic	Amistar 500 WG	0.1	2-3 weeks
Mancozeb	Carbamate	Non-systemic	Mancozeb DG	1.6-2.0	Weekly
Copper oxychloride	Mineral	Non-systemic	Copper oxychloride	1.6-2.0	Weekly

All of the chemicals in Table 11 and Table 16 are approved active constituents for fungicide use in Australia and most are available under several trade names and formulations (Australian Pesticides and Veterinary Medicines Authority, 2006). Continued use of the DMI (demethylation inhibitor) class of fungicides may result in increased resistance in the pathogen population, so these must be used in conjunction with other chemicals. To avoid the development of resistant populations, the group C (DMI inhibitor) class of fungicide should not be used for more than three consecutive sprays or more than five sprays per season. Copper and carbamate fungicides have been used for decades with no evidence of resistance developing. Copper acts as a contact fungicide and experiments have demonstrated that cuprous oxide, copper hydroxide and copper oxychloride were effective against coffee rust (*Hemileia vastatrix*) and were not readily removed by rain (Oliveira *et al*., 2002). Their effectiveness was reduced (cuprous oxide), maintained (copper oxychloride) or enhanced (copper hydroxide) by the addition of vegetable oil to prevent evaporation (Oliveira *et al*., 2002).

The benefit from application of these fungicides must be weighed against their economic and environmental costs. In Australia it is possible that an incursion could occur in or near an area of significant environmental heritage value and this should be a consideration in the selection of fungicides on standby for an eradication event. Both chlorothalonil and triadimenol are highly toxic to aquatic organisms (Bayer 2005a, Orica 2005), though toxicity to birds, bees and earthworms is low for chlorothalonil (Bayer, 2005a). Diniconazole is not available in Australia, but another azole fungicide, tebuconazole is available (Australian Pesticides and Veterinary Medicines Authority, 2006) and is recommended for other rust diseases in wheat, sugarcane and beans (Bayer, 2005b). Tebuconazole poses a low hazard to birds, earthworms and bees, but is moderately toxic to fish and aquatic organisms (Bayer, 2005c).

If aerial spraying is deemed to be appropriate, optimisation of application rates and droplet size to ensure adequate canopy coverage is necessary. An initial estimate, based on New Zealand work aimed at control of Dothistroma in pines, would be to use 5-10 L/ha and a volume median diameter (VMD) of 100 microns (Richardson, pers. comm.), slightly larger than the 65 microns recommended for Dothistroma (Ray & Vanner, 1988) to accommodate the larger leaf size of *Eucalyptus* and other broad-leaved Myrtaceae. Also based on this work, the amount of fungicide per ha may be less in aerial application than in ground-based application. For ground-based control of Dothistroma in nurseries, the recommended application rate of copper oxychloride is 4 kg/ha in 100-500 L total spray

volume, but only 1.66 kg/ha in 5 L total spray volume for aerial application in forests. Rotary atomisers, such as the Micronair, produce droplets with a narrower range of droplet sizes than do conventional nozzles (Ray & Vanner, 1988). Determining the specific flow rate and blade angle will depend on the aircraft type, the exact formulation and application rate. At a flow rate of 5 L per minute, wind speed 180 km/h and a blade angle of 50° on a Micronair AU5000, VMD was 100 microns for fenitrothion and 90 microns for water (Hooper & Spurgin, 1995). VMD was reduced to 45 and 35 microns, respectively, when the blade angle was 35°. Smaller droplet sizes can lead to an unacceptable degree of spray-drift (Hooper & Spurgin, 1995).

Given the small droplet size, the addition of emulsifiable spray oil to the formulation is recommended to reduce evaporation. This also increases the effectiveness of copper oxychloride in controlling Dothistroma (Ray & Vanner, 1988). The addition of vegetable oil increased the effectiveness of copper oxychloride, but not copper oxide or copper hydroxide, in reducing the germination of *Hemileia vastatrix* (coffee rust) urediniospores *in vitro*, but did not lead to improved control of the disease in plants (Oliveira *et al*., 2002).

Even assuming optimal droplet size, it may be difficult to achieve complete canopy penetration, particularly in forested areas where multiple canopy layers may exist. The use of a defoliant following the initial application of contact fungicide is suggested as a follow-up to further reduce the probability of spore dispersal. In addition to reducing the likelihood of wind dispersal, spore survival in decaying leaf litter in contact with the ground is likely to be much shorter than on attached leaves, due to ingestion by invertebrates and degradation by saprotrophic fungi and bacteria. Paraquat and diquat are two fast-acting, non-residual herbicides that cause rapid defoliation (Stephan Gous, pers.comm.) These chemicals are toxic to mammals, causing central nervous system and organ damage, but are „not acutely toxic" to „slightly toxic" for most of the insects, molluscs and fish tested. The exceptions are one snail, one crayfish, one toad and two water-flea species, to which paraquat was moderately toxic, and one fairy shrimp and one leopard frog, to which it was highly toxic (Pesticide Action Network, 2006). Diquat was „not acutely toxic" or „slightly toxic" to all aquatic organisms tested (Pesticide Action Network, 2006). The defoliants dimethipin, diuron, endothal, ethephon, paraquat, quizalofop, sodium chlorate and thidiazuron are also registered for use in Australia, and used in cotton to promote leaf drop before harvesting. The results in Australian natives may differ, however. *Casuarina cristata, C. cunninghamiana* and *E. camaldulensis* were all defoliated by thidiazuron, thidiazuron + diuron, ethephon and sodium chlorate, but *Acacia pendula* was not significantly affected by any of the treatments (Downey *et al.,* 2001). Thidiazuron, quizalofop and dimethipin may be more environmentally friendly than the others, as they have only moderate toxicity to mammals, are less likely to cause blindness and are less toxic to aquatic animals (Pesticide Action Network, 2006), but all of these chemicals act much more slowly than paraquat or diquat.

Application of systemic fungicides is recommended in a buffer zone surrounding the affected area. Systemic fungicides in group C (demethylation inhibitors) have demonstrated effectiveness against *P. psidii* and other rusts, but these chemicals must be used in rotation with others to avoid the build-up of resistance. Weekly spraying is recommended for maximum protection.

9.7 Appendix 7: Resources and facilities

Table 17 provide a list of diagnostic facilities for use in professional diagnosis and advisory services in the case of an incursion.

Table 17. *Diagnostic service facilities in Australia*

Facility	State	Details
DPI Victoria Knoxfield Centre	Vic	621 Burwood Highway Knoxfield VIC 3684 Ph: (03) 9210 9222; Fax: (03) 9800 3521
DPI Victoria Horsham Centre	Vic	Natimuk Rd Horsham VIC 3400 Ph: (03) 5362 2111; Fax: (03) 5362 2187
DPI New South Wales Elizabeth Macarthur Agricultural Institute	NSW	Woodbridge Road Menangle NSW 2568 PMB 8 Camden NSW 2570 Ph: (02) 4640 6327; Fax: (02) 4640 6428
DPI New South Wales Tamworth Agricultural Institute	NSW	4 Marsden Park Road Calala NSW 2340 Ph: (02) 6763 1100; Fax: (02) 6763 1222
DPI New South Wales Wagga Wagga Agricultural Institute	NSW	PMB Wagga Wagga NSW 2650 Ph: (02) 6938 1999; Fax: (02) 6938 1809
SARDI Plant Research Centre - Waite Main Building, Waite Research Precinct	SA	Hartley Grove Urrbrae SA 5064 Ph: (08) 8303 9400; Fax: (08) 8303 9403
Grow Help Australia	QLD	Entomology Building 80 Meiers Road Indooroopilly QLD 4068 Ph: (07) 3896 9668; Fax: (07) 3896 9446
Department of Agriculture and Food, Western Australia (AGWEST) Plant Laboratories	WA	3 Baron-Hay Court South Perth WA 6151 Ph: (08) 9368 3721; Fax: (08) 9474 2658

9.8 Appendix 8: Communications strategy

A general Communications Strategy is provided in Appendix 6 of PLANTPLAN (Plant Health Australia, 2008).

9.9 Appendix 9: Market access impacts

Within the AQIS PHYTO database (**www.aqis.gov.au/phyto**), export of some plant material to New Zealand, Papua New Guinea and South Africa requires an additional declaration regarding freedom from *P. psidii* (as at February 2009; Table 18). Should *P. psidii* be detected or become established in Australia, additional countries may require a specific declaration or supplementary measures upon export. Latest information can be found within PHYTO, using an Advanced search “Search all text” for *Puccinia psidii.*

Table 18. *Export declarations required in relation to guava rust (as listed on the PHYTO database)*

Country	Commodity	Declaration
New Zealand	Feijoa (*Acca sellowiana*)	Guava rust (*Puccinia psidii*) is not known to occur in Australia
Papua New Guinea	*Eugenia* spp.	Guava rust (*Puccinia psidii*) and Pierce's disease (*Xylella fastidiosa*) are not known to occur in Australia
South Africa	*Eucalyptus* spp.	Guava rust (*Puccinia psidii*) and Chestnut blight (*Cryphonectria parasitica*) are not known to occur in Australia
South Africa	Spotted gum (*Corymbia henryi*)	Guava rust (*Puccinia psidii*) and Chestnut blight (*Cryphonectria parasitica*) are not known to occur in Australia