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Puccinia psidii: a threat to the Australian environment and economy – a review

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Abstract. *Puccinia psidii* causes a rust disease on a broad range of hosts in the Myrtaceae and Heteropyxidaceae. 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. This paper reviews the taxonomy, biology, impact and options for control of *P. psidii*. It also discusses the probable impact if an incursion were to occur in Australia and the preparations that must be made to mitigate adverse consequences.

Introduction

Several papers calling attention to *Puccinia psidii* as a biosecurity threat to Australia and New Zealand have been published in recent years (Navaratnam 1986; Ridley *et al*. 2000; Mireku and Simpson 2002; Tommerup *et al*. 2003). *Puccinia psidii* is a native of South and Central America where it was first described on guava (Winter 1884), hence its vernacular name of guava rust. In recent years, research has been conducted into the taxonomy, biology, host range, actual and potential distribution and options available for control of *P. psidii.* This review attempts to encapsulate the reasons why this organism is considered to be a threat to Australia and to summarise the research that has been published since the last review (Coutinho *et al*. 1998).

Taxonomy

Puccinia psidii Winter and its anamorph *Uredo psidii* J.A. Simpson, K. Thomas & C.A. Grgurinovic have many synonyms (Table 1). A recent review of species of Uredinales pathogenic on species of Myrtaceae (Simpson *et al*. 2006) described eight rust species, including *U. psidii*, *U. rangelii* and *U. seclusa*, which are all anamorphs of *P. psidii sensu lato* (J. A. Simpson, pers. comm.). The remaining four species are *Phakopsora rossmaniae* and its anamorph *Physopella jueli, Physopella xanthostemonis* and *Puccinia cygnorum*. The only two species that are known from Australia are *P. xanthostemonis* (= *Uredo xanthostemonis*), on *Xanthostemon species* in the Northern Terrritory, and *Puccinia cygnorum*, which was unknown in Australia until it was detected by New Zealand quarantine on a shipment of cut flowers and was subsequently found on *Kunzea ericifolia* near Perth in Western Australia (Shivas and Walker 1994). Shivas and Walker (1994) were of the opinion that *P. cygnorum* is quite distinct from *P. psidii* and more similar to other Australian species of *Puccinia* such as *P. boroniae*; rDNA sequences support this (M. Glen, unpubl. data). It thus seems possible that other undiscovered rusts exist on Myrtaceae hosts in Australia.

Uredo rangelii is a newly described species, recorded on *Myrtus communis* from Argentina and on *Syzygium jambos* from Jamaica (Simpson *et al*. 2006). Its status as a species distinct from *U. psidii* is based on the presence of a tonsure on the urediniospores and subtle differences in size, shape and wall thickness of the urediniospore. However, molecular phylogenetic and morphological analyses of further collections should be pursued to support the morphological distinction. *Uredo seclusa* is known only from the type collection, on an unidentified species of Myrtaceae from São Paulo, Brazil. *Phakopsora rossmaniae* and *Physopella jueli* are also known only from Brazil, on species of *Campomanesia*. A full description of spores and a key to rusts on Myrtaceae is provided in Simpson *et al*. (2006).

Symptoms on susceptible hosts

Symptoms on a range of hosts have been described and illustrated (Coutinho *et al*. 1998; Tommerup *et al*. 2003; Alfenas *et al*. 2004) and appear on various websites (Agricultural Research Service USDA 2006; PaDIL 2006; University of Hawaii 2006). Lesions are produced on young, actively growing leaves and shoots, as well as on fruits and sepals (Figs 1 and 2). Lesions are brown to grey with masses of bright yellow or orange-yellow urediniospores. Occasionally, lesions have sori containing dark brown teliospores or a mixture of the two spore types. Older lesions have 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 their 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

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Table 1. Synonyms of *Puccinia psidii* or its anamorph *Uredo psidii* (Walker 1983; Sótão *et al.* 2001; Hennen *et al.* 2005; Simpson *et al.* 2006)

Synonym	Reported host
Aecidium glaziovii P. Henn.	Myrtaceae, indeterminate
Bullaria psidii G.Winter (Arthur & Mains)	*Psidium guajava*, reported as *P. pomiferum*
Caeoma eugeniarum Link	–
Puccinia actinostemonis H. S. Jackson & Holway	Myrtaceae, indeterminate, erroneously reported as *Actinostemon* sp.
P. barbacensis Rangel	Myrtaceae, indeterminate
P. brittoi Rangel	*Campomanesia maschalantha*
P. camargoi Putt.	*Melaleuca leucodendra*
P. cambucae Putt.	*Eugenia* sp., *Marlierea edulis*
P. eugeniae Rangel	*Eugenia grandis*
P. grumixamae Rangel	*Eugenia brasiliensis*
P. jambolana Rangel	*Eugenia jambolana* (= *Syzygium jambolanum*)
P. jambosae P. Henn.	*Syzygium jambos*
P. jambulana Rangel	*Syzygium jambos*
P. neurophila Speg.	Myrtaceae, genus not identified
P. rochaei Putt.	*Marlierea edulis, Myrcia jaboticaba, Myrciaria* sp.
Uredo cambucae P. Henn.	*Eugenia edulis*
U. eugeniarum P. Henn.	*Eugenia* sp., *E. uvalha*
U. flavidula Wint.	Species of *Eugenia, Marlierea, Myrcia, Psidium, Syzygium*
U. goeldiana P. Henn.	*Eugenia* sp., *Marlierea edulis*
U. myrciae Mayor	*Myrcia cf. acuminata*
U. myrtacearum Paz.	*Eugenia grandis, E. pungens, E.* sp.
U. neurophila Speg.	Myrtaceae, indeterminate
U. pitanga Speg.	*Eugenia pitanga*
U. puttemansii P. Henn.	Myrtaceae indeterminate, originally reported as *Acacia* sp.
U. rangelii J. A. Simpson, K. Thomas & C. A. Grgurinovic	*Myrtus communis*, *Syzygium jambos*
U. rochaei Putt.	*Marlierea edulis, Myrcia jaboticaba, Myrciaria cauliflora*
U. seclusa H. S. Jackson & Holway	Myrtaceae, indeterminate

branching and galling in eucalypts is a symptom of previous rust infection. Persistent localised lesions and stem swellings on *Melaleuca quinquenervia* have also been reported and illustrated (Rayachhetry *et al*. 2001). Similar symptoms may occur in other species but have not been recorded because many host species have been tested only at the seedling stage.

Symptoms on resistant hosts

On resistant plants, the pathogen may induce a hypersensitive reaction (HR) expressed as flecks or necrotic lesions generally with no sporulation (Junghans *et al*. 2003) However, depending on the level of resistance, punctiform pustules may be formed over the brown, necrotic lesions. This type of reaction is typical of a single gene controlling resistance, as previously detected in *E. grandis* (Junghans *et al*. 2003) and in several other pure species and hybrids (A. C. Alfenas, unpubl. data).

Life cycle

Puccinia psidii is considered to be an autoecious species with an incomplete lifecycle (Fig. 3). With the exception of spermogonia, all stages are produced on the same Myrtaceous host. Aecia and aeciospores are morphologically identical to uredinia and urediniospores (Figueiredo 2001; A. C. Alfenas and E. A. V. Zauza, unpubl. data). It has recently been suggested that *P. psidii* may be heteroecious with an unknown aecial host (Simpson *et al*. 2006) but this seems doubtful given the multiple observations, in independent laboratories, of infections on uredinial hosts (*E. grandis* and *S. jambos*) inoculated with teliospores or basidiospores (Figuiredo 2001; A. C. Alfenas and E. A. V. Zauza, unpubl. data).

Spore types observed in nature and in the laboratory

Under natural conditions, *P. psidii* produces abundant urediniospores. Teliospores and basidiospores are comparatively rare, although teliospores are more frequent on *S. jambos* (Ferreira 1983) and on leaves of *Eugenia jaboticaba* (A. C. Alfenas, unpubl. data) than on other hosts. Frequency on all hosts is higher in warmer months (Ferreira 1983). Aeciospores have not been observed or recognised in nature due to their similarity to urediniospores (Figueiredo 2001).

Production of teliospores can be stimulated by incubation of infected hosts at temperatures outside the optimal range for urediniospore production. Ruiz *et al*. (1989*b*) found that the number of urediniospores and teliospores produced on inoculated *E. grandis* was significantly higher at 20°C and 25°C than at 30°C. Alfenas *et al*. (2003) noted teliospore formation on *Eucalyptus globulus* and *E. viminalis* at 28°C but not at 22°C. With a variable temperature regimen, Aparecido *et al*. (2003*b*) found that incubation of inoculated *S. jambos* between 21 and 25°C resulted in greater teliospore production than incubation between 21 and 35°C.

Basidiospores have been produced free of urediniospores from leaf discs *in vitro* and used to inoculate *S. jambos* (Figueiredo 2001). Eighteen days after inoculation, aecia and aeciospores were produced that were morphologically indistinguishable from uredinia and urediniospores. Spermogonia, however, have not been observed (Figueiredo 2001). The current understanding of the *P. psidii* life cycle is illustrated in Fig. 3.

Conditions for germination and infection

Urediniospore germination and infection are affected by temperature, leaf wetness, light intensity and photoperiod (Ruiz *et al*. 1989*b*). Several studies have agreed that high humidity or leaf wetness and low light for a minimum of 6 h following inoculation are necessary for successful germination and infection (Piza and Ribeiro 1988; Ruiz *et al*. 1989*a*, 1989*c*). Several studies have determined different optimum temperatures for urediniospore germination. Lack of consistency may have been caused by variation in methodology among the studies. Variable factors included the substrate on which germination occurred, the length of incubation before germination was assessed and even the type of water (or oil) in which the spores were resuspended. Suspension of the spores in mineral oil rather than water increased the germination rate (Furtado *et al*. 2003).

This inconsistency among studies may also be due to variation among the rust biotypes. In one study, a temperature range of

Fig. 1. *Puccinia psidii* infection on eucalypt. (*a*) Urediniospores on leaves and shoots. (*b*) Urediniospore pustules. (*c*) Apical death. (*d*) Resistance reactions: (1) susceptible, (2) resistant and (3) hypersensitive response.

18–21°C gave the highest germination rate for urediniospores from *S. jambos*, whereas those from *P. guajava* had the highest germination rate at 15°C (Aparecido *et al*. 2003*a*).

Light exposure during the initial stages of infection inhibits urediniospore germination and infection, but during the post-penetration phase, more rust sori were

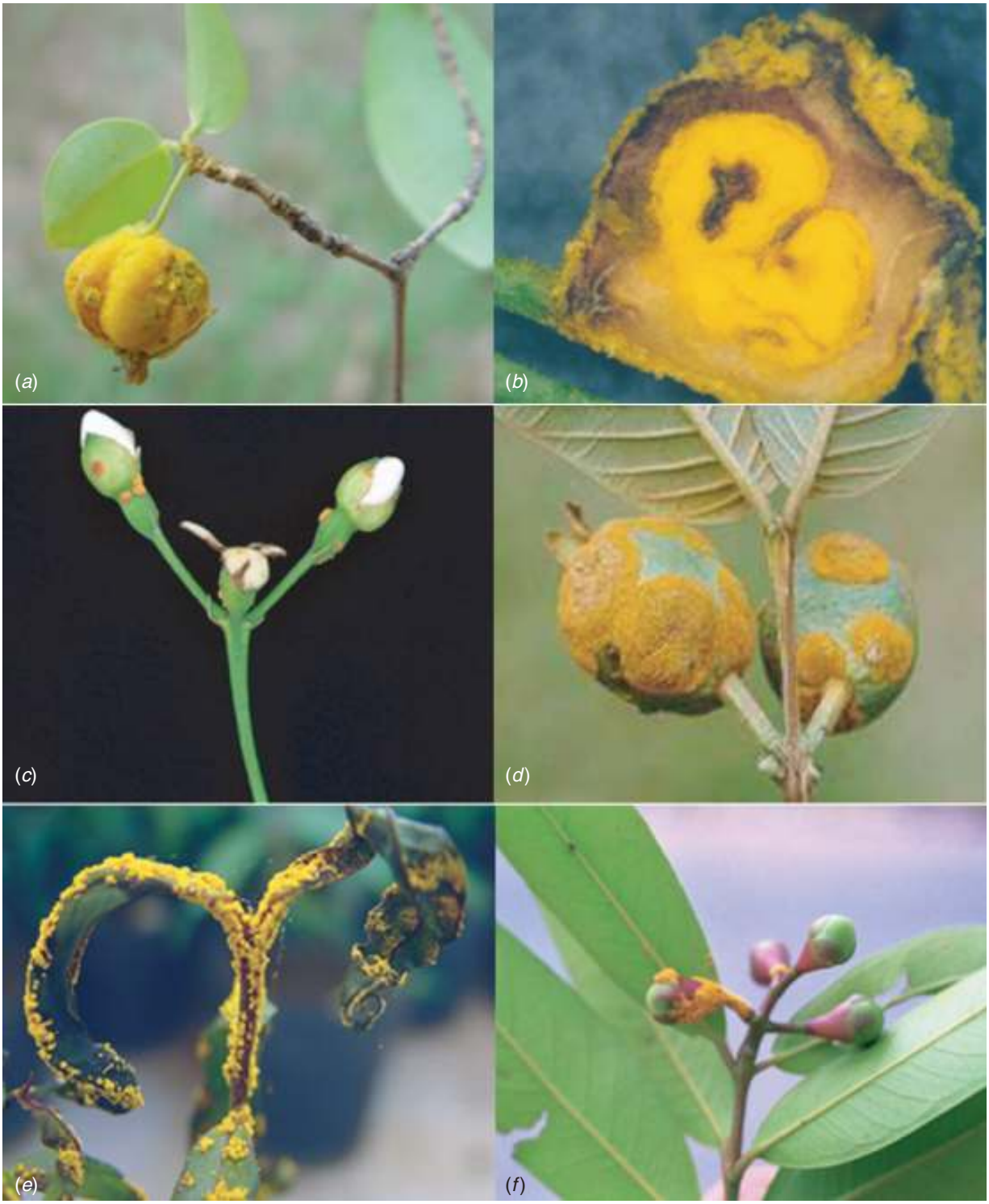

Fig. 2. Rust on other Myrtaceae species. (*a*, *b*) Infected fruit of *Eugenia uniflora*. (*c*, *d*) Flower buds and guava fruit. (*e*, *f*) Urediniosori on leaves and flower buds of *Syzygium jambos*.

produced on *E. grandis* seedlings exposed to 3640 lx than on those exposed to 1092 lx (Ruiz *et al*. 1989*a*, 1989*b*). In this study, infection and spore production were not affected by the source (host species or location) of the inoculum.

Germination on host leaves is also more prolific than on water agar. Tessmann and Dianese (2002) investigated whether plant compounds may have a stimulatory effect on urediniospore germination and found that germination was

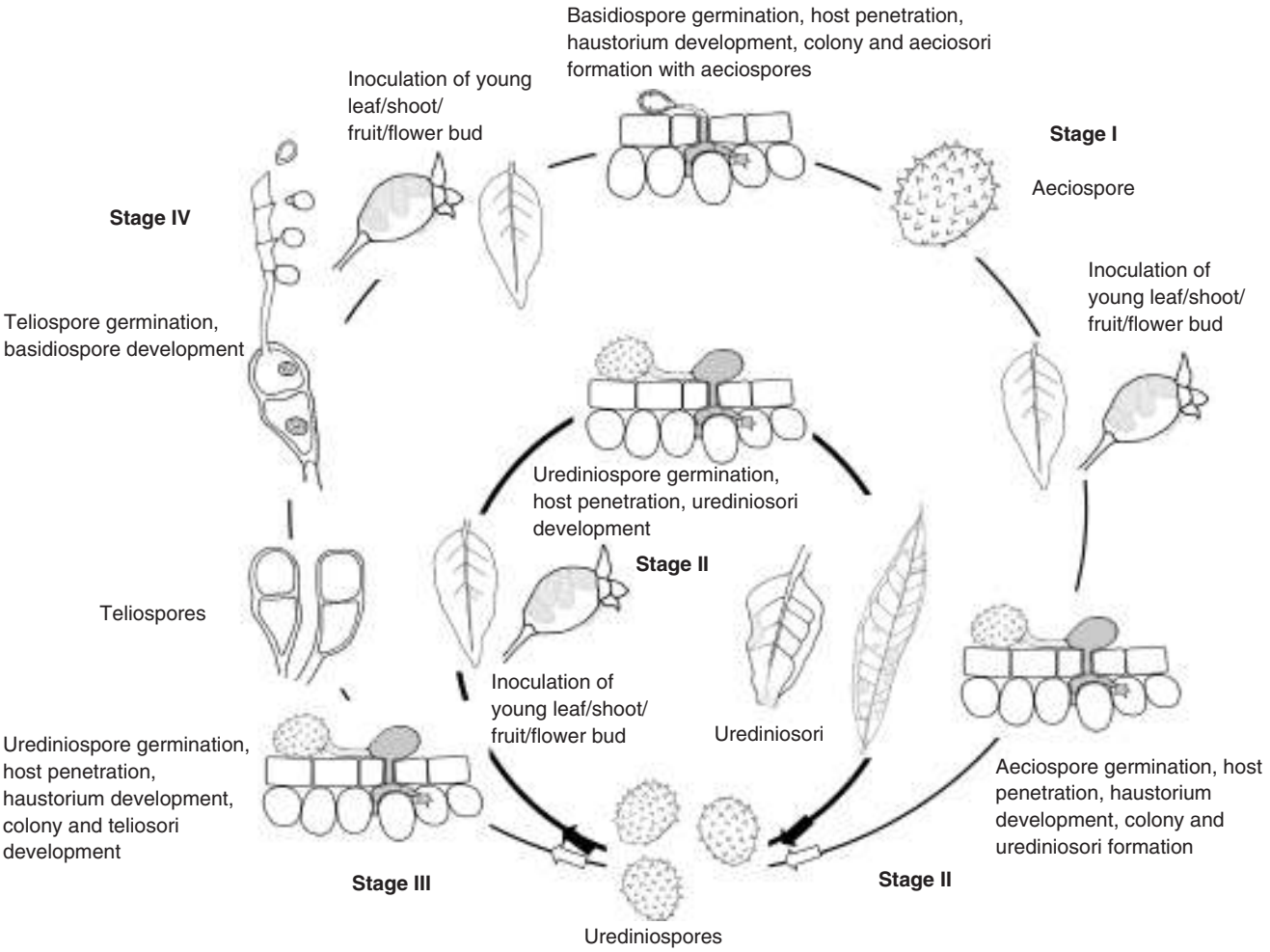

Fig. 3. Schematic life cycle of *Puccinia psidii*

enhanced by an extract from leaves of *S. jambos*. The stimulatory compound was identified as the hydrocarbon, hentriacontane. Low concentrations of hentriacontane (20–200 mg/L) almost doubled the germination rate, but higher concentrations (2000–20 000 mg/L) did not lead to a germination rate greater than could be obtained in pure mineral oil. The authors suggest that certain hydrocarbons may have a role in overcoming self-inhibition and this may account for the effect of both mineral oil and hentriacontane. In contrast, Salustiano *et al*. (2006) found that extracts of the non-host candeia (*Eremanthus erythropappus*) inhibited germination of *P. psidii* urediniospores and those of three other rusts.

Teliospores germinated *in vitro* and basidiospores were produced at temperatures ranging from 12 to 24°C (Aparecido *et al*. 2003*b*), with maximum basidiospore production at 21°C. At 12°C, basidiospore production occurred after 48 h compared with 24 h at the higher temperatures.

Infection process

A histopathological study of the infection process on detached leaves revealed no difference between susceptible and resistant *E. grandis* genotypes during the processes of urediniospore germination, appressorium formation and host penetration (Xavier *et al*. 2001). Ninety percent of the urediniospores had germinated within 6 h of inoculation and 90% of these formed appressoria within 18 h whereas a low percentage entered through stomata without appressorium formation. Infection pegs from the appressoria penetrated between the anticlinal walls of the epidermal cells and colonised the mesophyll, as previously reported for *S. jambos* (Hunt 1968). In resistant genotypes, a hypersensitive reaction was seen after 48 h, in contrast with susceptible genotypes, where macroscopic disease symptoms were observed 3–5 days after inoculation and urediniospore formation after 12 days.

Epidemiology

The environmental conditions important for the *in vitro* or *in vivo* germination of urediniospores have been shown to be strongly correlated with rust epidemics in the field (Ruiz *et al*. 1989*c*; Carvalho *et al*. 1994; Tessmann *et al*. 2001). Studies conducted on *E. cloeziana* coppice showed that rust progress and severity varied from year to year according to

the environmental conditions. Periods of high relative humidity longer than 8 h and temperatures in the range of 15–25°C were highly favourable to infection (Ruiz *et al*. 1989*c*; Carvalho *et al*. 1994). In a year-long study on *S. jambos* in central Brazil (Tessmann *et al*. 2001), disease incidence and severity were highly correlated with periods of relative humidity over 90% or leaf wetness periods greater than 6 h and nocturnal temperatures between 18 and 22°C. In another study, Blum and Dianese (2001) showed a positive correlation between the concentration of airborne urediniospores, the number of infected young *S. jambos* shoots and the number of nights with temperatures below 20°C or relative humidity above 80%. A negative correlation was observed between midday temperature and the number of airborne urediniospores.

Spore survival

Knowledge of the potential survival time of the different spore types is vital to assessing the risk associated with various pathways possible for an incursion. Aparecido *et al*. (2003*a*) examined the germination rate of urediniospores at five ages (10, 14, 21, 28 and 34 days old) from *S. jambos* and *Psidium guajava*. Germination was assessed after a 5-h incubation on water agar at temperatures ranging from 12 to 24°C. They found that urediniospores from *S. jambos* had a higher germination rate than those from *P. guajava*, and that 14-day-old urediniospores from *S. jambos*, incubated at 21°C, had the highest germination rate of 36%. The highest germination of urediniospores from *P. guajava* was 30% for 21-day-old urediniospores incubated at 15°C. No germination was recorded for 34-day-old urediniospores from either *S. jambos* or *P. guajava*. Furtado *et al*. (2003) recorded a germination rate of 3% for 31-day-old urediniospores from *S. jambos*. An even longer survival time was recorded for urediniospores from *P. guajava*, stored at 4°C and 40% relative humidity (Suzuki and Silveira 2003). Spores maintained viability, albeit low at 3%, after 100 days. In this study, germination was assessed after a 48 h incubation at 20°C and it is possible that older spores may need this longer time for germination. A factorial experiment was also conducted by Suzuki and Silveira (2003) to develop a model of survival time based on temperature and relative humidity.

In current studies, urediniospores from *Eucalyptus* spp. maintained viability after 90 days at 15°C and 35–55% relative humidity, but only for 10 days at 35 or 40°C (V. M. Lana, E. A. V. Zauza and A. C. Alfenas, unpubl. data). The survival of urediniospores during a sea voyage from South America to Australia, with temperatures around 30°C and 70% RH, is therefore likely to be low. It would also be necessary for the spores, while still viable, to reach a susceptible host in suitable environmental conditions for germination. Theoretically, teliospores survive longer than urediniospores, although less empirical information is available for teliospores of *P. psidii*.

Host range and biotypes

The host range of *P. psidii* has been studied in Florida, with a view to using *P. psidii* as a biocontrol agent for the Australian native tree *Melaleuca quinquenervia*, which has become an invasive weed in the Everglades (Rayachhetry *et al*. 2001). Of the 14 exotic and four native species of Myrtaceae tested with two collections of *P. psidii* urediniospores, 10 were asymptomatic or had symptoms without sporulation. These included *E. grandis* and *S. jambos*, which were both susceptible in other laboratory tests and in the field (Marlatt and Kimbrough 1979). The endangered native *Myrcianthes fragrans* was susceptible, but the authors suggested that the host range may have been artificially expanded by the controlled environment tests. This was because *Callistemon viminalis* and *Syzygium cumini*, which also tested positive in their experiments, were observed growing in close proximity to infected *M. quinquenervia* with no evidence of infection. However, natural infections of *C. viminalis*, *M. fragrans* and *S. cumini*, as well as *C. citrinus*, *Myrtus communis*, *S. paniculata* and *S. jambos* were subsequently reported in 2002 and 2003 (Leahy 2004). It is possible that the presence of susceptible new growth on these species had not previously coincided with the high inoculum load and environmental conditions conducive to infection.

Susceptibility of different provenances of selected Australian Myrtaceae species to *P. psidii* was tested in Brazil as part of a project funded by the Australian Centre for International Agricultural Research (ACIAR) (FST/1996/206) between CSIRO Forestry and Forest Products, Australia, Forestry and Agricultural Biotechnology Institute, South Africa, and the Federal University of Viçosa, Brazil. Of the 58 Australian species tested, 52 had some degree of susceptibility (A. C. Alfenas and E. A. V. Zauza, unpubl. data) and, for most species, the degree of susceptibility varied among provenances. Thirty-nine of the species tested were species of *Eucalyptus* or *Corymbia*, and 37 of these were susceptible.

Artificial inoculations in the ACIAR tests in Brazil extended the host range beyond the Myrtaceae when the South African species included in the study showed that *Heteropyxis natalensis* was highly susceptible (Alfenas *et al*. 2005). This tree genus belongs to the Myrtales but is classified as Heteropyxidaceae. This is, therefore, the first recorded non-Myrtaceae host of *P. psidii*.

Several races or biotypes of *P. psidii* are known to exist; although in comparison with other rusts such as those of cereal crops, very little is known of these specialised forms. For example, two strains in Jamaica infected *Pimento* spp. and *Syzygium* spp., respectively, but neither strain infected guava (MacLachlan 1938). The *Pimento* strain was able to infect *S. jambos* but did not sporulate. In Florida, the *Pimento* strain sporulated in *S. jambos* (Marlatt and Kimbrough 1979), even though it took twice as long for maturation of urediniosori in *S. jambos* than on *P. dioica*. In later tests, *S. jambos* was considered immune to rust strains from *M. quinquenervia* and *Pimenta dioica* (Rayachhetry *et al*. 2001).

In Brazil, isolates from guava did not infect eucalypts and *vice versa* (Ferreira 1983). The physiological variability of *P. psidii* was assessed by cross-inoculation on *E. grandis*, *P. guajava* and *S. jambos*, with 13 single-pustule isolates from *E. cloeziana*, *E. grandis*, *E. phaeotricha*, *P. guajava*, *S. jambos* and *Myrcia itambensis* (Coelho *et al*. 2001). Three groups of physiological specialisation (biotypes) were detected, the first compatible with *E. grandis* and *S. jambos*, the second with *E. grandis* and guava, and the third only with guava. No difference was detected in incubation period and mean latent period among the groups; however, the number of sori per unit area varied among the groups (Coelho *et al*. 2001).

In another study, Xavier (2002) evaluated the virulence of 32 isolates of *P. psidii*, obtained from different hosts and regions on five Myrtaceae species (*E. grandis*, *S. jambos*, *Eugenia jaboticaba*, *P. guajava* and *E. uniflora)*. The frequency of isolates virulent on these plants was 100, 87, 81, 31 and 4%, respectively. Virulence testing of 21 of these isolates on eight *Eucalyptus* clones showed three different interaction responses. The isolates were characterised on the basis of these responses as belonging to race 1, 2 or 3. Races 1 and 3 were from *Eucalyptus* and race 2 from guava (Xavier 2002).

Aparecido *et al*. (2003*c*) performed cross-inoculation experiments using urediniospores of *P. psidii* collected from eight different host species: jambo (*S. jambos*), *E. grandis*, three other unspecified *Eucalyptus* species, guava (*P. guajava*), jaboticaba (*Myrciaria cauliflora*) and cambuca (*Eugenia cambucae*). These were inoculated onto five different host species: *Corymbia citriodora*, guava, jambo, cereja-de-rio-grande (*Eugenia* sp.) and uvaia (*E. uvalha*); and four different groups (biotypes) were discriminated on the basis of host specificities. The relationship between the biotype from all-spice in Jamaica and Florida and those from Brazil is unknown.

Distribution and spread

Puccinia psidii had been restricted to South and Central America and the Caribbean until its appearance in Florida in 1977 (Marlatt and Kimbrough 1979). When it was first reported in Florida, the rust had already spread to several locations up to 35 km apart (Marlatt and 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). There has been a single report from Taiwan in 1992 (Wang 1992), but the pathogen was never definitively identified and it appears not to have become established (M. J. Wingfield, unpubl. data). There has also been a report from South Africa of a rust on *E. nitens* (Knipscheer and Crous 1990), although morphological characteristics were not consistent with those of *P. psidii* and it has not subsequently been detected in surveys (M. J. Wingfield, unpubl. data). Apart from these unusual reports, there is no evidence that the rust had spread between countries until April 2005, when it was detected on native Myrtacae in Hawaii (Uchida *et al*. 2006). M. J. Wingfield (unpubl. data) had undertaken surveys on the big island of Hawaii in 2000 and 2002 and there was no evidence of its presence. The introduction thus appears to have occurred very recently and it has spread throughout the Hawaiian islands rapidly, being found on all but one of the islands by December 2005 (Killgore and Heu 2005). This latest geographical expansion has increased concerns in Australia and elsewhere, where Myrtaceae are either native or used to sustain fruit or forestry industries, because it is a vivid illustration of the capacity of this organism to travel rapidly and to successfully infect new host species. The occurrence of *P. psidii* in Hawaii has brought the pathogen closer to Australia and the risk of spread through the Pacific Islands towards this continent has also been substantially increased.

Field and laboratory detection

Detection of *P. psidii* is fairly straightforward during rust epidemics as the signs and symptoms are obvious and there are few rust diseases of myrtaceous plants (Simpson *et al*. 2006). Detection and identification on less susceptible hosts, of low levels of disease when climatic conditions are unfavourable, in asymptomatic plant tissue or germplasm such as pollen, or early detection of a new incursion is likely to be complicated. More effective and sensitive techniques for detection of *P. psidii* have thus been sought.

As a sub-project in the ACIAR project FST/1996/206, a nested polymerase chain reaction (PCR) based detection assay was developed (Langrell *et al*. 2003*a*) to enable rapid detection and identification of *P. psidii* in host and non-host material (Langrell *et al*. 2003*b*). Viability of *P. psidii* propagules detected in commercial pollen by the PCR assay was verified by inoculation of *E. grandis* and *S. jambos* seedlings and subsequent disease development (Langrell *et al*. 2003*b*). In addition, *P. psidii* was detected in washings from clothing and personal effects of visitors to diseased plantations and viability was also demonstrated for these samples (Langrell *et al*. 2003*b*). Specificity of the diagnostic test has been verified against 10 other rust species, but not yet against the newly described and morphologically similar species, *Uredo rangelii*, that has been described from *S. jambos* in Jamaica and *Myrtus communis* in Argentina (Simpson *et al*. 2006). The diagnostic test has since been improved by the addition of internal amplification controls that increase confidence in negative results (Glen *et al*. 2006). The PCR diagnostic test was used to confirm the presence of *P. psidii* when unidentified spores were found on a shipment of kiln dried timber arriving in Australia from Brazil (Commonwealth of Australia 2006).

Impact

Puccinia psidii is widespread in Brazil, which has ~1000 species in 23 genera of native Myrtaceae (Landrum and Kawasaki 1997) and the pathogen has been reported on at least 10 of these genera (Marlatt and Kimbrough 1979; Walker 1983; Rayachhetry *et al*. 2001; Hennen *et al*. 2005). Despite its wide host range, there is little published information about *P. psidii* in native vegetation in Brazil. This is because the disease is not usually severe on native hosts with the exception of occasional epidemics in guava plantations (de Goes *et al*. 2004; Ribeiro and Pommer 2004). The widely planted, non-native, fruit tree *S. jambos* is highly susceptible and major epidemic outbreaks occur annually (Tessmann *et al*. 2001). The disease can also be a problem in *Eucalyptus* plantations, an important industrial crop in Brazil. In this situation, *P. psidii* occurs on seedlings in nurseries, on young trees in the field, on coppice and on shoots in clonal gardens (Alfenas *et al*. 1997; Alfenas *et al*. 2004).

Elite hybrid clones of *E. grandis* are widely used by the cellulose and paper industry in Brazil. *Eucalyptus grandis* is one of the species that is most susceptible to *P. psidii* and this disease is regarded as potentially the most damaging to *Eucalyptus* plantations in that country (Junghans *et al*. 2004). Rust has, for example, been a limiting factor in the establishment of *E. cloeziana* in the south-east of Bahia (Ruiz *et al*. 1989*c*; Carvalho *et al*. 1994). A severe incidence of *P. psidii* was

also observed in commercial plantations of *E. globulus* and *E. viminalis* in 2000 (Alfenas *et al*. 2003), a first record for these species.

Apart from Brazil, *P. psidii* is relatively common on *E. grandis* in Argentina (Acuña and Garran 2004). In the adjacent Uruguay, it has been recorded on native Myrtaceae and although it had previously been noted on *E. grandis* and *S. jambos* in that country, severe damage was recently reported on *E. globulus* in 2002 (Telechea *et al*. 2003). This report of the rust on *E. globulus* has raised considerable concern because this species is extensively planted to sustain a major pulp industry in Chile, which is geographically close to Uruguay. *Puccinia psidii* occurs on non-native *S. jambos* and *P. guajava* in Colombia (M. J. Wingfield and C. A. Rodas, unpubl. data). This is the only occurrence of the pathogen in South America west of the Andes. Despite extensive surveys (M. J. Wingfield, unpubl. data), the disease has not been found on *Eucalyptus* spp. in Colombia.

Rust is sporadic on eucalypts in South America and is dependent on annual climatic variation to provide the most suitable conditions for infection. This is in contrast to infections on *S. jambos*, which sustain annual epidemics (Tessmann *et al*. 2001). On *Eucalyptus,* the disease is only damaging on young trees, which cease to display notable damage as they extend in height. In addition, the widespread use of clonal forestry has facilitated the adoption of resistant clones in plantations.

In Florida, where it was originally found only on allspice (Marlatt and Kimbrough 1979), the range of infected species has been increasing. Previously viewed as a possible biocontrol agent for the invasive Australian species, *Melaleuca quinquenervia* (Rayachhetry *et al*. 2001), *P. psidii* now attacks native species, including a threatened species, *Myrcianthes fragrans* (Leahy 2004).

In April 2005, a rust was observed on oh'ia plants (*Metrosideros* sp.) in a Hawaiian nursery (Uchida *et al*. 2006). In May, it was found in natural populations of *M. polymorpha* on Oahu, and by July, it was found on *Eugenia koolauensis*, *E. reinwardtiana* and *P. guajava* on Oahu (Killgore and Heu 2005). It was confirmed as *P. psidii* and by December it had spread to all of the Hawaiian islands and was causing severe dieback of non-native *S. jambos* and *M. quinquenervia.* Since then, additional native hosts have included *Eugenia paniculatum* and *Rhodomyrtus tomentosa* (University of Hawaii 2006).

Potential impact in Australia

Australia has 70 genera and 1646 species of native Myrtaceae, approximately half of the world's 147 genera and 3000 species (Department of Environment and Heritage 2004*a*). 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 (de Castro *et al*. 1983; Rayachhetry *et al*. 2001; Alfenas *et al*. 2003; Tommerup *et al*. 2003; A. C. Alfenas and E. A. V. Zauza, unpubl. data). 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. No Myrtaceae species are on the critically endangered list, but there are 50 on the endangered list and 93 classed as vulnerable (Department of Environment and Heritage 2004*b*). These include species of *Syzygium*, *Kunzea*, *Melaleuca* and *Thryptomene* and 76 species of *Eucalyptus*.

Rust biotypes also show variation in host range (Coelho *et al*. 2001; Aparecido *et al*. 2003*c*), so the apparent 100% resistance found in 10 of the 83 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). Therefore, if the current ratio of susceptible to 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, although not themselves greatly threatened, may still play a significant role in maintaining the pathogen and facilitating its survival and spread.

The effects of new diseases on naïve hosts are far more unpredictable than they are on hosts that have a long association with the pathogen. The disease epidemics on the co-evolved host, *P. guajava* in Brazil, may be attributed to the establishment of large monoculture areas of a susceptible host. The same argument may also be made for the introduced eucalypts; however, another introduced species, *S. jambos*, is often grown in mixed plantings in gardens yet this species suffers frequent severe attacks (Tessmann *et al*. 2001). It is also susceptible over a larger geographic range and up to a later age than *Eucalyptus* species. Therefore, prediction of the effect on Australian ecosystems of an introduction of *P. psidii* is extremely complex.

Puccinia psidii infects young shoots, and depending on the host, floral buds and young fruits. It is unable to initiate infection on mature foliage, even though a lightly infected leaf may still have urediniosori when it is older. 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. If *P. psidii* becomes established in Australia, it is unlikely to kill mature trees, though seedling death may result in a reduced rate of regeneration of dominant species in natural forests, thus altering the biodiversity and ecology. Coppice regrowth is highly susceptible (Junghans *et al*. 2003), and this will impact on forest management practices using fire as a vegetation management tool. Secondary rotting of fruits (e.g. *Syzygium* and *Psidium*), and reduced aesthetic value in native forests and amenity plantings will most likely occur. In some areas, the physical environment could be affected as a result of canopy decline with consequential erosion and water quality loss.

The potential impact of *P. psidii* is not limited to native Australian biodiversity. The genera susceptible to the pathogen include trees grown in plantations that represent an important, expanding industry in the country. They also include native Australian species grown as ornamentals in gardens and planted commercially for wildflower production and an emerging agribusiness of essential oil production.

Although it is unlikely that entire species would be eliminated due to infection by *P. psidii*, 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 could

be adversely affected. Mature eucalypts appear resistant to the disease. On other hosts such as allspice and guava, the young tissue is susceptible even in older trees (MacLachlan 1938). Most Australian species, including most *Eucalyptus* species, have been tested only at the seedling stage, so the susceptibility of older plants is largely unknown. *Melaleuca quinquinervia* is one exception, where natural infection in Florida occurs on all ages of plants (Rayachhetry *et al*. 1997). This attribute may be important in maintaining inoculum levels.

A bioclimatic analysis of locations where the disease has occurred on *Eucalyptus* species was used as the basis for predicting the risk of *P. psidii* establishment in different climatic regions of Australia if the pathogen were introduced (Booth *et al*. 2000). A recently updated risk map by T. H. Booth and T. Jovanovic (unpubl. data) for Australia highlights the areas with climatic conditions that are considered most likely to foster epidemic disease in eucalypts if the pathogen is introduced (Fig. 4). The area with the highest risk consists of a broad strip down the east coast of Australia from north Queensland to northern New South Wales and another of lower risk across the top of the Northern Territory. These areas include a high proportion of Australia's World Heritage-listed rainforest, which has a significant component of Myrtaceae species.

The analyses by Booth *et al*. (2000) and T. H. Booth and T. Jovanovic (unpubl. data), are preliminary assessments of high risk areas, based on data for a small number of *Eucalyptus* species, and there is a need for more detailed data to develop a more accurate risk assessment for a broad range of susceptible Australian Myrtaceae. Although some further detail has been determined, such as the need for a minimum 'leaf wetness' period (Ruiz *et al*. 1989*b*), obtaining these data or accurately estimating them from currently available data for Australian sites requires further work. Thus regions outside the highlighted area may also be likely to suffer rust epidemics, although perhaps less frequently, if the pathogen becomes established in Australia.

Another caveat associated with the current risk mapping is that the presence of a highly susceptible host is important in defining the geographical range of rust. For example, in Brazil, *P. psidii* occurs on *S. jambos* wherever this tree is grown (throughout Brazil), but it only infects *Eucalyptus* spp. in the most climatically favourable regions (Tessmann *et al*. 2001). 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.

Fig. 4. Revised rust risk areas for *Puccinia psidii* in Australia (T. H. Booth and T. Jovanovic, pers. comm.). Dark blue areas represent highest risk, light blue, light green and orange areas show decreasing levels of risk. Grey areas signify lowest risk.

Susceptibility to infection by *P. psidii* 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 or 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 glasshouse tests (Tommerup *et al*. 2003; E. A. V. Zauza and A. C. Alfenas, unpubl. data).

Once established in a particular area of Australia, *P. psidii* would most likely spread very rapidly over the climatically suitable regions. This would be similar to other *Puccinia* species infecting cereal crops (Park *et al*. 2002). Such rapid spread is facilitated by the rapid urediniospore cycle, which is completed in about 10–23 days. These spores are produced in very large numbers and are repeatedly produced during the infection cycle and, like other rusts, may be wind-dispersed over large distances (Viljanen-Rollinson and Cromey 2002). There is potential for insect, bird or mammal-vectored spread.

The strain of *P. psidii* attacking allspice in Jamaica covered an area of 5000 km^2 one year after it was first reported (Smith 1935). As discussed above, a similar rapid spread of the pathogen has occurred in Hawaii. This emphasises the likelihood that the pathogen would spread very rapidly if it were introduced into Australia. Moreover, the chance of eradicating a wind-dispersed pathogen with a short reproductive cycle, once it has become established, would be very low.

The risk of an incursion of *P. psidii* into Australia is greatly increased as it becomes established in new areas. This appears to be typical of tree pathogens and pests, which, once established in new areas, begin to move to additional sites. Excellent examples of this trend are the pitch canker pathogen, *Fusarium circinatum* (Gordon *et al*. 2001), and the Eurasian wood wasp, *Sirex noctilio* (Slippers *et al*. 2002). Clearly, the closer these sites of new infestation are to Australia, the greater the risk will be. Thus if *P. psidii* becomes established in countries adjacent to Australia such as Indonesia and Papua New Guinea or southern Africa, the risk to Australia will be substantially increased. These areas are all within the windborne travelling range for rust spores and cereal rusts are known to be exchanged between South Africa and Australia as well as between Australia and New Zealand on wind currents (Viljanen-Rollinson and Cromey 2002). Incursions from these pathways are likely to establish in a remote region, delaying detection and increasing the risk of substantial spread before detection. This would also substantially decrease the probability of successful eradication.

Puccinia 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*b*). The lack of establishment after the first record in Taiwan (Wang 1992) and possible record at least of a *Eucalyptus* rust disease in South Africa (Knipscheer and Crous 1990) increases confidence in the

climatic modelling. The theoretically more durable teliospores could be an important inoculum source in regions where climatic conditions are less frequently favourable for *P. psidii*. Even in areas with a climate unsuitable for *P. psidii*, nurseries and glasshouses may provide an ideal microclimate with an on-going supply of susceptible, young foliage.

Possibilities for eradication and containment treatments in Australia

In South America, *P. psidii* does not cause major disease outbreaks in the wild, as the native hosts have co-evolved with the pathogen and do not generally occur as homogeneous stands, except in guava orchards. The situation is expected to be quite different if this pathogen becomes established in Australia. This is because pathogens are frequently far more virulent on naïve hosts, such as has been seen with many non-native tree pathogens that have been devastating after their introduction into new environments (Wingfield *et al*. 2001).

Unless an incursion of *P. psidii* is detected at an early stage in a relatively geographically isolated location, it is a disease that will be very difficult to eradicate for reasons already discussed. Eradication of rust diseases have often failed, for example, attempts to eradicate coffee rust caused by *Hemilea vastatrix* in Papua New Guinea were made three times before they ultimately failed. Likewise, eradication of coffee rust failed in Nicaragua and eradication of chrysanthemum white rust caused by *Puccinia horiana* also failed in New South Wales, Australia. Reasons cited for the failure of the attempted eradication of chrysanthemum white rust in New South Wales include inadequate spray equipment (Priest 1995).

Although eradication of chrysanthemum white rust failed in Australia, this disease has been successfully eradicated in the United States of America (United States Department of Agriculture Animal and Plant Health Inspection Service 2005). Thus the difficulty of rust eradication should not imply that eradication programs are not to be planned and preparedness to attempt this route of control should be maintained. For any rust disease, the normal process of incursion appraisal and response needs to be drastically shortened to allow effective intervention. A delay in intervention would critically reduce the probability of achieving a successful eradication. Therefore, prior agreements must be established between industry and all levels of government. The immediate response should assume that eradication is possible, until delimiting surveys have been completed.

Case studies

When considering the likelihood that an incursion of *P. psidii* might be eradicated from Australia, it is useful to draw from the experience of other rust eradication attempts in this country. Here, the example of grape vine leaf rust caused by *Phakopsora euvitis* provides an excellent case study. After this rust was detected in Darwin in 2001, a quarantine zone was established and an eradication program was initiated. This program, initially reported as successful, has been extended until mid 2007 owing to the detection of two new infections (Northern Territories Department of Business Industry & Resource Development 2006). The program's charter is first to stop this disease from spreading to central Australia's Ti-Tree table grape production area or to the major interstate wine and grape-growing areas, where it could have a devastating impact. Second, it demands that grape vine leaf rust is eradicated from Australia. For grape vine leaf rust, actions include extensive household and property surveys to locate and record every grapevine in the greater Darwin and rural areas; the removal of all diseased vines; monitoring of healthy vines in the quarantine area; ongoing identification of the location of any grapevines in the greater Darwin and rural areas; and monitoring of selected sentinel grapevines outside the quarantine area.

Sugarcane smut caused by the fungus *Ustilago scitaminea*, which is primarily spread by windborne spores and infected sugarcane cuttings, was identified by Australia as a high-risk exotic disease in a pest risk analysis conducted in 1997, and a contingency plan to deal with incursions was prepared in 1997. The disease was reported for the first time in Australia in the Ord River Irrigation Area (ORIA) of Western Australia in July 1998. Although eradication of the disease was deemed not to be feasible, quarantine regulations that were enacted reduced the risk of its spread by plant material or appliances (Croft and Braithwaite 2006). Heavily infested fields were ploughed in and all susceptible cultivars removed by 2001. Australian cultivars are now being screened offshore for resistance to sugarcane smut. The disease was contained in Western Australia for 8 years and has only recently been detected in Queensland in June 2006.

Options for eradication

Options for the eradication or containment of guava rust in Australia have been recently reviewed (Commonwealth of Australia 2006). These include consideration of (i) host removal, (ii) chemical control, (iii) biological control, (iv) physical control (heat, fire) and (v) development of host resistance. The ubiquity of the Myrtaceae, many of which are known to be susceptible, in the native Australian vegetation will also make any eradication or containment program more complex in comparison with a pathogen that has a more restricted or mainly agricultural host range such as grapevine rust or sugarcane smut.

Host removal

This treatment would only be feasible for eucalypt rust if detected early and where the disease is present only on plants within a small area. Care would need to be taken not to spread the readily wind-dispersed spores, so removal should be preceded by spore destruction using fungicides. If the plants are too large for physical removal, the application of herbicides or defoliants could effectively remove susceptible plant tissue. 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 (Ferreira 1983). Thus removal of all highly susceptible plant species (e.g. *S. jambos*, *M. quinquenervia* and *Kunzea baxteri*) in the environs of a new incursion would be essential.

Physical treatments

As for other fungi (Maloy 1993), heat treatment could provide an effective tool to kill *P. psidii* spores. However, flaming is not recommended as an eradication tool because rust spores

could be dispersed with the smoke and air currents created by fires (Maloy 1993). If the disease became established in forest areas where prescribed burning is carried out for fuel reduction or regeneration, consideration would need to be given to the strategic timing of fires. If timed correctly, fuel reduction burns could assist in local inoculum reduction, but if they are carried out at unfavourable times, they would result in a flush of susceptible new growth when climatic conditions are conducive to severe disease development. In an eradication attempt, solarisation of soil with black plastic may be useful over small areas to kill spores following fungicide or herbicide application (Stapleton 2000).

Chemical eradication treatments

Fungicides to be used in Australia for any attempts to eradicate a *P. psidii* incursion would 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 2). Chlorothalonil was reported to be the most effective, although it did not completely eliminate the rust (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 and Alfenas (1991) determined the period of protection afforded by oxycarboxin, diniconazole and triadimenol (Table 2) 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 found to be the most effective fungicide in both of these experiments, as well as in 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 with over 90% and 40%, respectively, in the controls.

Current recommendations for control of eucalypt rust in Brazil (Table 3) are based on more recent work (Alfenas *et al*. 2004) that evaluated the curative and protective effects of the systemic fungicides azoxystrobin, triadimenol, tetraconazole, tebuconazole and a mixture of epoxiconazole and pyraclostrobin. All but tetraconazole had a curative effect, as they reduced the numbers of sori per unit leaf area and the number of urediniospores per sorus when applied up to 7 days after inoculation. Azoxystrobin and triadimenol were the most effective protectants, preventing pustule formation for 21 days after spraying. Three *Eucalyptus* clones were evaluated under field conditions and fungicide sprays significantly reduced disease incidence, but no effect was detected on tree growth.

Chemical treatments are used for rust control in nurseries and high-value crops in South America. Successful control in eucalypt nurseries, in coppices of eucalypt plantations and in guava orchards has been established with a range of fungicides (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 it keeps the disease to a level that does not threaten the viability of the industry. As part of integrated management of *Eucalyptus* rust, the use of fungicides is viable in nurseries, clonal hedges and occasionally in the field for highly susceptible, elite genetic material. At the first sign of disease occurrence and during periods when environmental conditions conducive to disease (i.e. existence of juvenile tissue, temperatures of 15–25°C and 6–8 h of nocturnal leaf wetness) are present for more than five consecutive days (Ruiz *et al*. 1989*b*), one or two fortnightly sprays of azoxystrobin are recommended (A. C. Alfenas, unpubl. data). If the disease and favourable conditions persist, fortnightly sprays of triadimenol are also recommended. This regimen should be sufficient for control if the disease is detected early, but if the disease is advanced when first detected, a first spray with triadimenol should subsequently be alternated with either azoxystrobin or tebuconazol.

Experiments to chemically treat infections by *P. psidii* in Brazil have been conducted to determine a cost-effective rate of application for operational use and the aim was to achieve control rather than eradication. Eradication by chemical methods was not attempted in Hawaii due to the lack of approved pesticides (Killgore and Heu 2005) and it is vital that Australia has such pesticides available. Further experiments are needed to determine the fungicide combinations and application rates that would provide the greatest probability of a successful eradication. Trials to determine an effective spray regime for an eradication attempt are also required. 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*. 2005), myclobutanil, that is recommended by the United States Department of Agriculture Animal and Plant Health Inspection Service (APHIS) to eradicate chrysanthemum white rust, *Puccinia horiana* (United States Department of Agriculture Animal and Plant Health Inspection Service 2005), traditional curative fungicides such as copper oxychloride and the use of defoliants to assist canopy penetration. The benefit from application of 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 program.

Biological control treatments

The effect of 24 isolates of *Bacillus subtilis* on *P. psidii* urediniospore germination has been examined (dos 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 (Simpson *et al*. 2006: M. Glen, unpubl. data) and may have potential as biological control agents. Particular strains of rhizobacteria (*Pseudomonas aeruginosa*) have been demonstrated to induce systemic resistance in *Eucalyptus grandis* × *urophylla* (Teixeira *et al*. 2005). Biological control may be a tool for minimising

Table 2. Efficacy of fungicides tested for the control of *Puccinia psidii* (summarised from four separate studies) and their environmental toxicities

For Demuner and Alfenas (1991), Ferrari *et al*. (1997) and Ruiz *et al*. (1991), the quantity per ha 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. For Alfenas *et al*. (1993), three applications were sprayed at 20-day intervals and the amount given is per application

Fungicide	Chemical group	Mode of action	Commercial example	Application rate [g/L or (g/ha)] and efficacy				Environmental toxicity	Half-life in soil
				Demuner and Alfenas (1991)[A]	Alfenas *et al*. (1993)[B]	Ferrari *et al*. (1997)[C]	Ruiz *et al*. (1991)[D]		
Chlorothalonil	Benzo-nitriles	Non-systemic, protectant (group Y)	Bayer Chlorothalonil 500 SC	—	—	1.5 (300), <10%	—	Classified ecotoxin; toxic to aquatic organisms; non-toxic to bees and earthworms	~13 weeks
Copper oxychloride	Mineral	Non-systemic (group Y)	Chemspray copper oxychloride	—	—	1.0 (200), 10–20%	—	Highly toxic to zooplankton and aquatic Annelida; slightly toxic to fish; Terrestrial ecotoxicity poorly known	
Diniconazole	Azole	Systemic (group C)	None registered in Australia	0.075 (15), 14 days	(30), 65%	—	—	Very toxic to aquatic organisms	
Mancozeb	Carbamate	Non-systemic (group Y)	Barmac Mancozeb DG	—	—	1.6 (320), 10–20%	—	Highly toxic to amphibians; moderately toxic to fish	1–7 days
Oxycarboxin	Aniline/anilide	Systemic (group G)	Crompton Plantvax 750 WP	1.125 (225), 7 days	(210), 90%	—	0.75 (150), ++	Slightly toxic to amphibians and fish	20 days
Triadimenol	Triazole	Systemic (group C)[E]	Bayer Bayfidan 250 EC	0.4 (80), 28 days	(100), 40%	—	0.75 (150), +++	Toxic to aquatic organisms	43 weeks
Triforine	Amine/amide	Systemic (group C)[E]	Yates Triforine Rose fungicide	—	—	—	0.053[F] (10.6), +	Low toxicity to fish; earthworms and bees; some bio-accumulation	~3 weeks

[A]Efficacy is given as the time interval (days) required between sprayings as determined experimentally to control *P. psidii* in *Eucalyptus cloeziana* seedlings (Demuner and Alfenas 1991).

[B]Efficacy is given as the percentage of infected leaves 90 days after commencement of the treatment in *E. cloeziana* coppice (Alfenas *et al*. 1993).

[C]Efficacy is given as a rating determined by the area of affected guava fruit after spraying every 15 days (Ferrari *et al*. 1997).

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

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

[F]The concentration is 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 about one-seventh of the recommended application rate for Yates Triforine rose fungicide.

Table 3. Fungicides and spraying regimes currently recommended for control of *Puccinia psidii*

Active ingredient	Chemical group	Mode of action	Commercial example	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

disease incidence if it should become established in Australia, but this would not provide a suitable means for an eradication attempt. Much further work is also necessary before any fungus or bacterium can be recommended as a biological control agent.

Host resistance

Resistance represents the most economically viable approach to controlling *P. psidii* where it has become established. Guava cultivars have been evaluated for resistance and resistant progeny selected for breeding (Vasconcelos *et al*. 1998; Ribeiro and Pommer 2004). Selection and breeding for resistance is the only economically and environmentally feasible means of control of *P. psidii* in eucalypt plantations in Brazil (Alfenas *et al*. 2004). 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 the young tissues of guavas, jambos and allspice are susceptible on trees of all ages (MacLachlan 1938; Ferrari *et al*. 1997; Blum and Dianese 2001).

Selection of resistant species and clones has been a high priority in Brazil (Xavier *et al*. 2001). Using bulked segregant analysis and random amplification of polymorphic DNA (RAPD), Junghans *et al*. (2004) identified a marker tightly linked to a major resistance gene, designated PprI, in *E. grandis*. The availability of a molecular marker to assist selection of resistant offspring will expedite introgression of this trait into breeding programs. Resistance was confirmed against 21 single pustule isolates of *P. psidii* from seven different host species.

Resistance will be all important for industries based on Myrtaceae plants, e.g. eucalypt and melaleuca plantations, wildflower growers and guava orchards, if *P. psidii* were to become established in Australia. Resistance would also be important for natural selection in native vegetation, but this could 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.

Only a limited number of *Eucalyptus* species are grown in plantations in Brazil. Thus, most susceptibility testing performed in that country to date on Australian Myrtaceae has been carried out on seedlings, so susceptibility of older plants is unknown. This is with the exception of a few species that are grown as ornamentals or occur as weeds in rust-affected areas, such as *M. quinquenervia*, that is susceptible at all ages (Rayachhetry *et al*. 1997). Resistance testing of different seed-lots and provenances has been carried out for some *Eucalyptus* and *Corymbia* species (Tommerup *et al*. 2003; A. C. Alfenas and E. A. V. Zauza, unpubl. data) 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.

An approach that has been used to evaluate the susceptibility of planting stock to tree disease not present in a particular country is to plant this material in an area where the disease occurs naturally. This has been done with *Pinus radiata* from Australia and New Zealand to test for resistance to western gall rust caused by *Endocronartium harknessii* (Old *et al*. 1986). Some South African *Eucalyptus* clones have been tested for resistance to infection by *P. psidii* in Uruguay (M. J. Wingfield, unpubl. data). Most of the clones that represented *E. grandis* or *E. grandis* × *E. urophylla* hybrids were lost due to severe rust infection. Opportunities exist for forestry companies in areas where the disease does not occur to engage in such tests. Although this would be of little value to protect trees in natural ecosystems, plantation companies would be able to reduce the likely negative impacts of the disease if it were to appear and they would have access to breeding stock known to harbour resistance. In the event of establishment of *P. psidii* in Australia, control of disease in plantations would be vital to minimise impact in native vegetation.

Summary

The biological characteristics of *P. psidii* make it a pathogen that can easily move to new environments. Its introduction in recent years to new areas such as Florida and Hawaii suggest that it is also a pathogen on the move. All indications are that it is likely to move to new areas of the world and these could very easily include Australia.

If *P. psidii* were to become established in Australia, eradication would prove very difficult and any delay in commencement of an eradication program would significantly reduce the likelihood of success. Development of resistant lines represents a practical, long-term solution for commercially valuable plants. This is well illustrated in Brazilian forestry, where clonally propagated material facilitates the deployment of resistant planting stock. The accomplishment of this may be more difficult in Australia, where propagation is from seed. Such a solution, however, is not available for the many species of indigenous Myrtaceae that form a significant component of Australia's biodiversity, including dominant trees in many ecosystems.

Quarantine efforts such as pathway risk analysis and the surveillance of high risk sites must be rigorously applied. These must all be strongly supported with access to the knowledge, skills and resources to ensure an early detection of any incursion. A concerted strategy including countries adjacent to Australia that have native Myrtaceae or forestry industries based on eucalypts is required to raise awareness, preparedness and

establish surveillance of high risk sites offshore, and is one of the best strategies that could be implemented to protect Australia from damage due to *P. psidii*.

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