GUAVA RUST IN BRAZIL — A THREAT TO *EUCALYPTUS* AND OTHER MYRTACEAE*

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ABSTRACT

Puccinia psidii Wint. is a very unusual rust with an extremely wide host range within the Myrtaceae. The fungus currently occurs only in South and Central America, the Caribbean, and Florida, where it causes disease of both indigenous and introduced species. The hosts of most economic importance are *Eucalyptus* species, grown as plantations on a very large scale, especially in Brazil. The rust is a serious threat to tropical, sub-tropical, and possibly temperate plantations in Australia, and in other regions world-wide. In Australia, there is an additional threat to native vegetation as many communities are dominated by Myrtaceae. A collaborative project between Australia, Brazil, and South Africa aims to assist in the management of the disease by screening a wide range of myrtaceous genera for susceptibility to the disease, mapping areas in South America, Australia, and other areas globally in eucalypt-growing regions for potential disease hazard, and developing molecular tools for detecting the presence of *P. psidii* in seed, pollen, and other germplasm. A total of 26 *Eucalyptus* species of many provenances have so far been tested for rust resistance, along with four *Melaleuca* species and one species of each of *Angophora*, *Callistemon, Kunzea*, *Lophostemon*, and *Syncarpia*. Additional genera across the main groups of the family are currently being screened for susceptibility. Hazard maps, which can be used as a measure for evaluating risks associated with germplasm movements have been generated for South and Central America and Australasia. In addition, a highly sensitive detection system has been developed by which rust contamination has been detected in samples of asymptomatic vegetative eucalypt material, seed, and pollen, and on the surfaces of clothing and equipment.

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INTRODUCTION

Puccinia psidii, guava rust, is a serious pathogen of industrial eucalypt plantations in Brazil. The rust damages guava (*Psidium guajava* L.) fruit in commercial plantations in disease-prone regions of Brazil and is a minor pathogen of a range of Myrtaceae, including several *Psidium* species, in natural vegetation. Rust in eucalypt nurseries and guava plantations is controlled by means of fungicide applications, by planting selected resistant clones, and through nursery management. Fungicides are rarely applied in eucalypt plantations and control is by use of rust-resistant clones or seedling families in disease-prone regions. Rust disease is prevalent at generally low levels in eucalypt plantations throughout the disease-prone regions. However, *P. psidii* is widespread among guava and other fruit trees, whether grown in home gardens or as commercial plantations.

Puccinia psidii is currently known only from South America, mainly east of the Andes, including northern-most Argentina, Uruguay, Paraguay, Brazil, Venezuela, Ecuador, and Colombia; Central America and the Caribbean, including Cuba, Dominican Republic, Jamaica, Puerto Rico, Trinidad and Tobago; and Florida in the United States (Commonwealth Mycological Institute 1987; Coutinho *et al.* 1998; Booth *et al.* 2000; Alfenas *et al.* 2003). It is able to infect many Myrtaceae species across more than 15 genera and poses a potential threat to Myrtaceae-based industries worldwide in *P. psidii* disease-prone regions. The rust is not a pathogen which co-evolved with many of the genera and most of the species that have been identified as hosts so far. The new host associations and proximity of diverse hosts may be facilitating rapid evolution of the rust.

Eucalypts are the most widely grown and important international industrial hardwood forest trees. An FAO International Plant Genetic Resources Institute (IPGRI) workshop on safe movement of eucalypt germplasm highlighted *P. psidii* as a major threat (Ciesla *et al.* 1996). With a few notable exceptions (e.g., *E. urophylla* S.T.Blake), most plantation-grown eucalypts are indigenous Australian species. Australia is also a major genetic resource centre for oil-yielding melaleucas and has a huge diversity of other Myrtaceae species. Eucalypt forests once covered most of the current agricultural and valuable grazing areas of the tropical, temperate, mediterranean, and sub-alpine regions. Eucalypts are significant components of the biodiversity of such landscapes and are vital to natural resource management and maintenance of the sustainability of large tracts of Australia. Other Myrtaceae are important as forest understorey species or components of woodland and heath communities.

The threat of *P. psidii* to Australian biodiversity, eucalypt forests and plantations, and the international hardwood industry is being managed by determining the potential host range of the rust and by predicting those geographic regions favourable to rust epidemics, should the pathogen be introduced. Molecular tools have been developed to rapidly identify *P. psidii* should it reach Australia, or other regions outside its present distribution. Such tools also provide detection systems for discovering contaminated material and preventing its movement between international or within national boundaries.

DISTRIBUTION

Puccinia psidii is indigenous to Brazil, and probably also to parts of Paraguay, Uruguay and northern Venezuela, Ecuador, Colombia, and parts of Central America. The rust has spread to Florida and the Caribbean islands — whether this was by natural means such as weather events or due to human activities is unclear. By 1934 *P. psidii* had spread to and devastated the allspice industry in Jamaica and it is now found in Florida on several hosts (MacLachlan 1938; Florida Department of Agriculture 1977; Marlatt & Kimbrough 1979; Burnett & Schubert 1985; Rayachhetry *et al.* 2001). *Puccinia psidii* has been reported once in Taiwan but has not been found since. In view of the remoteness of Taiwan, and from the known distribution of the rust, its occurrence there was most probably a result of human-vectored spread (Wang 1992). Differentiating races in *P. psidii* is a challenge because of its wide host range and variation in susceptibility in co-evolved and non co-evolved hosts. For example, based on host species testing, two possible strains of *P. psidii* occur in southern Florida, and two in Jamaica, with one of the strains being similar in both regions. When tested against one strain of the rust in Jamaica, allspice (*Pimenta dioica* (L. ex Merrill)) was very susceptible, *Syzygium jambos* (L.) Alston was resistant, and *Psidium guajava* was immune. Against the second strain, *S. jambos* was very susceptible and allspice and guava immune. The rust was originally described from a variety of guava, and guavas are major hosts in Brazil (Ferreira 1989). One southern Florida strain infects both *S. jambos* and allspice (Marlatt & Kimbrough 1979). In contrast, more recently in Florida, host range tests were performed on 18 species of 11 genera of Myrtaceae by inoculating expanding leaves with urediniospores of two *P. psidii* isolates, one from *Melaleuca quinquenervia* (Cav.) S.T.Blake and another from *P. dioica*; *S. jambos* was immune to both isolates, and the isolates had similarities to one from Jamaica (Rayachhetry *et al.* 2001; MacLachlan 1938). In Brazil, *S. jambos* is considered to be very susceptible to *P. psidii* (Tessmann *et al.* 2001). Isolates from a range of hosts and locations in Brazil caused "resistant" to "susceptible" responses in a set of 10 different clones of *Eucalyptus grandis* Maiden (Xavier 2002).

THE DISEASE

Puccinia psidii causes disease of young shoots, floral buds, and young fruit, depending on the host (Fig. 1). On guava it is most severe on fruits and floral buds, rather than leaves. The disease is particularly severe on susceptible eucalypt seedlings, cuttings, juvenile trees, or coppice from stumps or damaged mature trees. Heavy infection of juvenile eucalypt shoots causes plants to be stunted and multi-branched, reducing their potential to develop into marketable trees. Very large differences in susceptibility among young eucalypt trees established from seed of landrace species and some pure species have been observed in Brazil. Highly susceptible individuals (typically 10–20%) of a stand are grossly malformed and may be killed. Infection of 20–30% of the canopies of young trees or second-rotation coppice regeneration has been reported in Brazilian plantations, enough to significantly affect growth rates and plantation profitability. A single dominant allele conferring rust resistance has been identified and mapped in a Brazilian landrace *E. grandis* using RAPD markers (Junghans 2000; Junghans, Alfenas, Brommonschenkel, Oda, Melo & Grattapaglia 2003).

FIG. 1–*Puccinia psidii* urediniosori and symptoms of rust disease on (L to R top row) *Psidium guajava* fruit, *Myrica jaboticaba* fruit, *Syzygium jambos* shoots, (L to R bottom row) *Eucalyptus grandis* leaves showing distortion, *E. grandis* shoot showing pustule development on a main stem and leaf death, and *E. grandis* showing woody warts developing on older shoots where pustules of *P. psidii* had formed when the shoots were younger.

HOSTS

Puccinia psidii has apparently formed many new associations with hosts introduced into rust-prone regions of South and Central America. The disease has been known on eucalypts in South America since the early twentieth century (Gonçalves 1929; Joffily 1944) but the first significant epidemic occurred in 1973 on seedlings of *E. grandis*, sourced from South Africa (Ferreira 1983). *Puccinia psidii* has since been found on a phylogenetically wide array of Myrtaceae tree and shrub species from Australian and Asian sources. *Puccinia psidii* has a remarkably wide host range for a rust pathogen, particularly as it is not known to have alternate hosts for the aecial phase, and spermogonia have not been reported (Ferreira 1989). Prior to this research more than 15 genera and 30 species of Myrtaceae, including 14 species of eucalypts, were recorded as hosts of *P. psidii* (Burnett & Schubert 1985; Laundon & Waterston 1965; Ferreira 1989). Fourteen species of eucalypts have been tested for susceptibility to rust and from the most to least susceptible they include *Eucalyptus cloeziana* Maiden, *E. phaeotricha* Blakely & McKie, *E. grandis*, *E. nitens* (Deane & Maiden) Maiden, *E. globulus* Labill., *E. viminalis* Labill., *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson, *E. camaldulensis* Dehnh., *E. tereticornis* Smith, *E. urophylla*, *C. maculata* (Hook.) K.D.Hill & L.A.S.Johnson, *E. paniculata* Smith, *E. punctata* DC, *E. pyrocarpa* LA.S.Johnson & D.Blaxell, *E. microcorys* F.Muell., *E. pellita* F.Muell., and *E. saligna* Smith (Coutinho *et al.* 1998; A.C.Alfenas pers. comm.; Alfenas *et al.* 2003; Telechea *et al.* 2003).

Melaleuca spp. are important sources of natural products such as tea tree (*M. alternifolia* Cheel) and cajputi (*M. cajuputi* Powell) oils. These are the basis for medicinal, cosmetic, and similar ointments and balms, and are widely used throughout South-east Asia. Other species, such as *M. quinquenervia*, have become established as noxious weeds in Florida where it now also harbours *P. psidii* (Rayachhetry *et al.* 2001).

IMPACTS ON NATIVE VEGETATION

The potential of this pathogen to cause damage in natural ecosystems as well as eucalypt plantations may be gauged by considering that the family Myrtaceae consists of approximately 155 genera distributed in Australia, South-east Asia, New Caledonia and other Pacific islands, South and Central America, and southern Africa. Should an incursion of *P. psidii* occur in any of these regions, outside its natural distribution, biodiversity and ecosystem function could be put at risk. For example, several *Syzygium* spp. native to the tropical rainforests of northern Queensland are important food sources for birds, fruit bats, and other native mammals.

RESEARCH TO MANAGE THE THREAT

Threat management research has involved:

(1) Screening a wide range of Australian Myrtaceae for susceptibility to rust by inoculation in Brazil, with particular emphasis on the economically important genera *Eucalyptus*, *Corymbia*, *Melaleuca*, *Eugenia*, *Syzygium*, and *Callistemon*;

(2) Identifying regions of high risk by climate matching to known disease-prone locations in Brazil and predicting eucalypt-growing areas of high, medium, and low risk to *P. psidii,* using climate interpolation and disease-hazard mapping methods;

(3) Developing molecular-based diagnostic assays for disease-development pathway analysis; and

(4) Developing molecular diagnostic techniques for detection of *P. psidii* in seed, tissue-cultured plants, or pollen.

Screening Eucalypts and Other Myrtaceae for Susceptibility to Rust

Myrtaceae were screened by inoculating seedlings with *P. psidii* spores, incubating under controlled conditions (Alfenas *et al.* 2003), and assessing disease development. Reaction types, based on pustule size, were scored according to the rating scale of Junghans, Alfenas & Maffia (2003). On this scale “immune, hypersensitive, and slow rusting with few pustules” are rated as resistant, and “medium to extensive pustule development” is rated as susceptible.

Screening results indicate that large differences in susceptibility to *P. psidii* occur among *Eucalyptus* spp. and among genera of Myrtaceae. Even the more susceptible eucalypts, e.g., *E. grandis*, showed variation among seedlots (Table 1). Within susceptible seedlots of this and other species some individuals had highly resistant or immune reaction types. These observations are in accord with field observations in Brazil where plantations established from *E. grandis* seed typically have a proportion of highly resistant individuals. *Melaleuca* spp. have a wide range of susceptibilities, with *M. cajuputi* being the most susceptible industrial species of this genus tested, and *M. squarrosa* Smith being virtually immune. In view of the importance of *M. cajuputi*, which is grown widely across Indonesia, Malaysia, and the South-east Asian mainland, the data indicating its relative susceptibility to rust are a cause for concern.

TABLE 1–Variation in susceptibility to *Puccinia psidii* among some eucalypt species and provenances with location given as Australian State, or other country.

Species	Susceptible seedlings (%)	Seedlots tested (No.)
E. grandis (Queensland)	40–94	3
E. grandis (New South Wales)	12–27	2
E. saligna (New South Wales)	27–50	2
E. globulus (Victoria)	25–38	2
E. globulus (Tasmania)	25–35	2
E. urophylla (Indonesia)	10–29	3
E. camaldulensis var. *obtusa* (Queensland)	4–27	3
E. camaldulensis var. *obtusa* (Western Australia)	11–15	2
E. camaldulensis var. *obtusa* (Northern Territory)	55	1
E. camaldulensis ssp. *simulata* (Queensland)	13–20	3
E. brassiana (Queensland)	9–33	4
E. brassiana (Papua New Guinea)	0	2

Although data are limited to single seedlots, some Myrtaceae, e.g., *Syncarpia glomulifera* (Smith) Niedenzu, a tall tree found at the interface of sclerophyll forest and rainforest, appear to be highly susceptible whereas other tree species (*Corymbia maculata* (Hook.) K.D.Hill & L.A.S.Johnson and *Angophora costata* Britten) showed moderate resistance (Table 2). *Melaleuca quinquenervia* susceptibility causes concern as it is a dominant species and widespread in coastal regions of sub-tropical eastern Australia. Significantly, it is susceptible to strains of the rust in both Brazil and Florida, which have different host-species reaction types. *Kunzea baxteri* (Klotzsch) Schauer, a very susceptible species, and *Callistemon citrinus* Skeels, a moderately susceptible species, are important industrial floriculture species which are widely grown in private and public gardens in disease-prone regions of Australia and parts of Africa. These and other widely dispersed susceptible hosts would facilitate rust invasion should an incursion occur.

Prediction of High-risk Regions Based on Climate

Puccinia psidii disease-prone regions were modelled using climate interpolation and disease-hazard mapping methods (Booth *et al.* 2000). They showed that areas highly prone

TABLE 2–Variation in susceptibility to *Puccinia psidii* among some species of Myrtaceae (single seedlots)

Species	Susceptible seedlings (%)
Syncarpia glomulifera	100
Kunzea baxteri	100
Melaleuca quinquenervia	80
Melaleuca cajuputi	44
Callistemon citrinus	60
Melaleuca alternifolia	30
Corymbia maculata	27
Angophora costata	15
Lophostemon confertus	0
Melaleuca squarrosa	0

to rust occur across the moist tropical and subtropical regions globally. These areas coincide with major eucalypt- and melaleuca-oil production areas. Importantly, they are also the centres of genetic diversity of many eucalypts and other Myrtaceae biodiversity, including some which are important tropical food crop and spice species.

Molecular-based Diagnostics for *Puccinia psidii*

Conventional diagnosis relies on identification of sporulating rust pustules with no capacity to detect *P. psidii* in symptomless tissue and germplasm. Therefore we have developed a fast, accurate, nested PCR system which is highly specific and sensitive and is designed to detect *P. psidii* directly in vegetative plant material (Langrell, Tommerup, Alfenas & O'Brien 2003) from forests, nurseries, plantations, orchards and wild vegetation. It detects the known strains of *P. psidii* from Brazil and elsewhere. The procedure has detected and diagnosed *P. psidii* associated with commercial germplasm, including seed and pollen, from diverse sources throughout Brazil and on personal effects of personnel leaving diseased eucalypt and guava plantations and nurseries (Langrell, Tommerup, Alfenas & O'Brien 2003; Langrell, Tommerup, Zauza & Alfenas 2003). PCR diagnosis will facilitate appropriate control measures and/or eradication procedures more quickly and effectively than traditional methods of pathogen detection should an incursion occur into Australia, South Africa, Asia, New Zealand or other regions. *Puccinia psidii* threatens Australia and other regions globally and has the capacity for international dissemination by people and as a contaminant of their apparel to plantations, nurseries, natural ecosystems, parks or gardens.

KEEPING THE RUST OUT OF AUSTRALIA AND INDUSTRIAL PLANTATIONS GLOBALLY

Puccinia psidii is a pathogen exotic to Australia and many other regions of the world where Myrtaceae are grown. Apart from impacts on natural ecosystems, which are very difficult to predict, the potential impact of an incursion on the Australian hardwood plantation industry could be impairment of early growth and stem form of plantations.

Selection of resistant seedlots and/or clonal lines would be needed in disease-prone areas and use of some highly desirable but rust-susceptible species, e.g., *E. cloeziana, E. phaeotricha* , and some provenances of *E. grandis*, would be prevented. Effective co-operation of industry globally, and adherence to rigorous quarantine policies and procedures, will be needed to prevent the entry of *P. psidii* into Australia, New Zealand, Africa, Papua New Guinea, Pacific islands, southern India, and Asia. Should *P. psidii* establish in regions to the north of Australia there is a possibility of movement into Australia by monsoonal weather systems, and if it were undetected for a period of time in native vegetation the rust would be impossible to eradicate.

The research described here, and in publications currently under preparation, has provided the most comprehensive assessment to date of the relative susceptibility of eucalypts and other Myrtaceae to this damaging disease. Climatic matching and interpolation methods indicate that a broad global swathe of the humid tropics and sub-tropics is likely to provide an environment suitable for epidemic disease. Molecular techniques have been developed for the rapid identification of *P. psidii* in the event of an incursion into new regions and the capacity has been provided for detection of the rust in germplasm. In view of the increasing movement of improved eucalypt germplasm between countries, especially into and within South-east Asia, there is a need to apply this technology to ensure that eucalypt rust remains in the Western Hemisphere.

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