Plant Pathology (2015) Doi: 10.1111/ppa.12380

Risk assessment for *Puccinia psidii* becoming established in South Africa

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Puccinia psidii, the causal agent of myrtle rust, was first recorded from Latin America more than 100 years ago. It occurs on many native species of Myrtaceae in Latin America and also infects non-native plantation-grown *Eucalyptus* species in the region. The pathogen has gradually spread to new areas including Australia and most recently South Africa. The aim of this study was to consider the susceptibility of selected *Eucalyptus* genotypes, particularly those of interest to South African forestry, to infection by *P. psidii*. In addition, risk maps were compiled based on suitable climatic conditions and the occurrence of potential susceptible tree species. This made it possible to identify the season when *P. psidii* would be most likely to infect and to define the geographic areas where the rust disease would be most likely to establish in South Africa. As expected, variation in susceptibility was observed between eucalypt genotypes tested. Importantly, species commonly planted in South Africa show good potential for yielding disease-tolerant material for future planting. Myrtle rust is predicted to be more common in spring and summer. Coastal areas, as well as areas in South Africa with subtropical climates, are more conducive to outbreaks of the pathogen.

Keywords: *Eucalyptus*, GIS, guava rust, Myrtales, risk analysis, Uredinales

Introduction

The rust pathogen *Puccinia psidii* (Urediniales, Pucciniaceae) was first described in 1884 from *Psidium guajava* (*Psidium pomiferum*) in Brazil (Coutinho *et al.*, 1998). It gained international notoriety as a pathogen when it was described as the cause of a serious disease of plantation-grown *Eucalyptus* trees in Brazil in the 1970s (Ferreira, 1983). The wide host range of *P. psidii* is unusual for rust fungi, which tend to be highly host-specific, and this has added considerably to the quarantine importance of the pathogen. It has been reported from more than 38 genera and 165 species, spanning 11 tribes, in the Myrtaceae (Carnegie & Lidbetter, 2012; Morin *et al.*, 2012; Graça *et al.*, 2013; Pegg *et al.*, 2014b). Although the disease has commonly been referred to as guava (*Psidium* spp.) rust and eucalyptus rust, the name myrtle rust is preferred because it effectively illustrates the fact that the fungus infects many species in the Myrtaceae.

Puccinia psidii was known only from Latin America for many years, but more recently the pathogen has begun to move globally (Uchida *et al.*, 2006; Glen *et al.*, 2007; Morin *et al.*, 2014). This has raised concern not only for commercial *Eucalyptus* plantation forestry, which relies on many susceptible species, but also for the survival of native Myrtaceae in areas where *P. psidii* has appeared as an invasive pathogen. In Australia, where *P. psidii* has recently appeared (Carnegie *et al.*, 2010), it has been described as the 'biggest threat to the ecosystem' (Dayton & Higgins, 2011) and an 'ecological disaster' (Booth, 2011).

Puccinia psidii is hypothesized to be native to South and Central America (de Castro *et al.*, 1983; Tommerup *et al.*, 2003; Alfenas *et al.*, 2005; Graça *et al.*, 2013), but it is now known from many other locations, including Africa, Asia (China, Japan), Australia, North America (California, Florida, Hawaii, Puerto Rico), South America (Brazil, Colombia, Ecuador, Paraguay, Uruguay, Venezuela) and the Caribbean (Cuba, Jamaica, Trinidad) (Marlatt & Kimbrough, 1979; Telechea *et al.*, 2003; Uchida *et al.*, 2006; Morin *et al.*, 2012; Roux *et al.*, 2013). In Africa the pathogen was first detected in South Africa in May 2013 (Roux *et al.*, 2013), on the KwaZulu-Natal south coast on an ornamental, non-native *Myrtus communis* plant. Although there have been no additional reports of *P. psidii* from South Africa, this situation is likely to change in future because many susceptible tree species are planted in the country and the climate in some areas is known to be conducive to infection.

Myrtle rust affects young, succulent and actively growing parts of trees including foliage, developing flowers and fruits of susceptible plants (Glen *et al.*, 2007). Economically important damage by *P. psidii* occurs in nurseries and plantations of non-native *Eucalyptus* species in countries such as Brazil where severe damage has been reported (Ferreira, 1983; Junghans *et al.*, 2003a). Occasional

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epidemic outbreaks also occur in commercial guava (*P. guajava*) orchards in Brazil (Ribeiro & Pommer, 2004). Furthermore, *P. psidii* is reported to have decimated the allspice (*Pimenta dioica*) industry in Jamaica within 2 years of its first detection in that country (MacLachlan, 1938). In Hawaii the introduction of *P. psidii* has resulted in the wide-scale mortality of non-native *Syzygium jambos* trees (Loope & La Rosa, 2008). Furthermore, the pathogen is of great concern in countries, such as Australia, that have large numbers of native Myrtaceae and thus a threatened natural ecosystem (Glen *et al.*, 2007; Carnegie *et al.*, 2010; Pegg *et al.*, 2014b).

The recent appearance of *P. psidii* in South Africa is of great concern due to the negative impact it could have on commercial forestry and agriculture, as well as on the natural environment. This country has a significant plantation industry based heavily on Australian *Eucalyptus* species, which contribute considerably to the country's GDP (Forestry South Africa, 2013). Furthermore, South Africa is home to a number of native plants in the Myrtaceae (Table 1), of which several are endemic (Palgrave & Palgrave, 2002). Studies in South America have shown that South African seed sources of *Eucalyptus grandis* were highly susceptible to *P. psidii*, with severe damage recorded to these genotypes in Brazil (Junghans *et al.*, 2003a). Similarly, the only native South African myrtaceacous plant tested to date, *Heteropyxis natalensis*, was found to be one of the most susceptible plants tested under greenhouse conditions (Alfenas *et al.*, 2005).

Climatic requirements for *P. psidii* have been described, predominantly in terms of temperature, rainfall, relative humidity (RH) and soil moisture (Ruiz *et al.*, 1989a,b; Booth *et al.*, 2000; Aparecido *et al.*, 2003a,b; Glen *et al.*, 2007; Kriticos *et al.*, 2013). In its current distribution range, *P. psidii* is favoured by moist climate and moderate temperature, with cold and drought stress limiting its distribution range in areas where susceptible plants are found (Kriticos *et al.*, 2013). Temperature and humidity are also strongly correlated with spore survival and germination (Glen *et al.*, 2007). Controlled environment inoculation studies have highlighted the importance of leaf wetness, light intensity and photoperiod for successful infection and germination (Ruiz *et al.*, 1989a,b).

In this study, the potential threat of *P. psidii* to forestry and natural ecosystems in South Africa is considered, based on inoculation studies on *Eucalyptus* species of interest to the South African forestry industry and on the distribution of native Myrtales in the country. Furthermore, environmental factors conducive to infection and the survival of the pathogen are considered.

Materials and methods

Eucalyptus species, provenances and families tested for susceptibility

Species used in these studies included those most commonly used for plantation establishment worldwide, but especially those of interest to South African forestry. This included the

Table 1 Species of Myrtaceae and Heteropyxidaceae native to South Africa

Species	Family/tribe	Geographic distribution[a]
Eugenia albanensis	Myrtaceae	Eastern Cape, KwaZulu-Natal
E. capensis subsp. *a*	Myrtaceae	Eastern Cape (Transkei), KwaZulu-Natal, Mpumalanga, Limpopo
E. capensis subsp. *capensis*	Myrtaceae	Eastern Cape, KwaZulu-Natal, Mpumalanga, Limpopo
E. capensis subsp. *gueinzii*	Myrtaceae	KwaZulu-Natal
E. erythrophylla	Myrtaceae	Eastern Cape (Transkei), KwaZulu-Natal
E. incerta	Myrtaceae	KwaZulu-Natal
E. natalitia	Myrtaceae	Eastern Cape (Transkei), KwaZulu-Natal, Mpumalanga, Limpopo
E. simii	Myrtaceae	Eastern Cape (Transkei), KwaZulu-Natal (South Coast)
E. umtamvunensis	Myrtaceae	Eastern Cape (Transkei), KwaZulu-Natal (South Coast)
E. verdoorniae	Myrtaceae	Eastern Cape, KwaZulu-Natal
E. woodii	Myrtaceae	Eastern Cape (Transkei), KwaZulu-Natal, Mpumalanga, Limpopo
E. zeyheri	Myrtaceae	Eastern Cape, KwaZulu-Natal, Limpopo
E. zuluensis	Myrtaceae	Eastern Cape, KwaZulu-Natal, Mpumalanga
Heteropyxis canescens	Heteropyxidaceae	Mpumalanga
H. dehniae	Heteropyxidaceae	Limpopo
H. natalensis	Heteropyxidaceae	Eastern Cape (Transkei), Gauteng, KwaZulu-Natal, Mpumalanga, Limpopo
Metrosideros angustifolia	Myrtaceae	Western Cape
Syzygium cordatum subsp. *cordatum*	Myrtaceae	Eastern Cape, KwaZulu-Natal, Mpumalanga, Limpopo
S. cordatum subsp. *gracile*	Myrtaceae	KwaZulu-Natal (South Coast)
S. gerardii	Myrtaceae	Eastern Cape, KwaZulu-Natal, Mpumalanga, Limpopo
S. guineense subsp. *afromontanum*	Myrtaceae	KwaZulu-Natal, Mpumalanga, Limpopo
S. guineense subsp. *guineense*	Myrtaceae	KwaZulu-Natal, Mpumalanga, North West, Limpopo
S. intermedium	Myrtaceae	KwaZulu-Natal, Mpumalanga, Limpopo
S. legatii	Myrtaceae	KwaZulu-Natal, Mpumalanga, Limpopo
S. pondoense	Myrtaceae	Eastern Cape (Transkei), KwaZulu-Natal (South Coast), Mpumalanga

[a]Occurrences and species names according to South African National Biodiversity Institute SIBIS database.

four most commonly planted *Eucalyptus* species: *E. camaldulensis* (including *E. camaldulensis* subsp. *simulata*), *E. grandis*, *E. pellita* and *E. urophylla* (Table 2) for which seed were available from the Department of Agriculture, Fisheries & Forestry, Queensland (Qld), Australia. Provenances and families representing a broad range of origins and for which sufficient numbers of seeds were available were selected for each species.

Establishment of plants for inoculations

Seeds were sown in 40-cell hyco trays (70 mL) in a glasshouse at the Sunshine Coast University in Queensland (Qld), Australia, using a potting medium consisting of 50% pine bark fines (0–10 mm), 25% pine bark peat, 25% coarse perlite, a mix of 12- to 14-month slow release Osmocote (N 17·9: P 0·8: K 7·3) fertilizer at a rate of 4 kg m^{-3}, gypsum (1 kg m^{-3}), Micromax (1 kg m^{-3}) and a granular wetting agent Hydroflo2 (1 kg m^{-3}). Plants were irrigated twice daily for 10 min using an overhead spray system. Seedlings were thinned following germination with only a single seedling remaining per cell and considered ready for inoculation when they reached *c.* 10–20 cm in height with actively growing young shoots.

Table 2 *Eucalyptus* species, provenances and seed sources used in *Puccinia psidii* screening trials

Species	Provenance[a]	Seed lot	No. of seedlings
E. camaldulensis	Grey River, WA, AUS	15441a (6 tree bulk)	9
	Katherine, NT, AUS	13922a (10 tree bulk)	4
	Laura, Qld, AUS	10531 (61 tree bulk)	96
	Petford, Qld, AUS	9666 (156 tree bulk)	101
	Quilpie, Qld, AUS	13264a (5 tree bulk)	6
	Salique, ZAF	10547 (10 + tree bulk)	67
E. camaldulensis subsp. *simulata*	Holmes, Qld, AUS	5616 (10 families)	244
	Palmer River, SA, AUS	5621 (10 families)	276
	Walsh River, Qld, AUS	5617 (6 families)	141
E. grandis	Bellthorpe, Qld, AUS	5539 (10 + tree bulk)	93
	Copperlode, Qld, AUS	5970 (8 families)	147
	EB50 (SO), ZWE	16017 (15 tree bulk)	68
	Sappi (SO), ZAF	10518 (10 + tree bulk)	74
	Yabbra, NSW, AUS	10544 (6 + tree bulk)	89
E. pellita	Helenvale, Qld, AUS	1106 (10 families)	172
	Kuranda, Qld, AUS	1113 (10 families)	184
	Serisa, PNG	18955 (24 tree bulk)	44
	South Kiriwo, PNG	18198 (21 tree bulk)	53
E. urophylla	Aek Nauli, TLS	11121 (10 families)	150
	Flores Island, IDN	12895 (23 tree bulk)	76
	Samosir, IDN	11121 (7 families)	103
	Wetar Island, IDN	17832 (25 tree bulk)	95

[a]Qld, Queensland; NT, Northern Territory; NSW, New South Wales; SA, South Australia; WA, Western Australia; SO, seed orchard. AUS, Australia; IDN, Indonesia; PNG, Papua New Guinea; TLS, Timor; ZAF, South Africa; ZWE, Zimbabwe.

Experimental design

Twenty-two sources of seed were accessed for the trial (Table 2) and were established using a randomized incomplete block (RICB) design. Some of the seed sources (provenances) were represented by multiple single-tree seed lots (e.g. *E. grandis* Copperlode, Qld, Australia with eight families = eight individual tree seed lots) and some sources consisted of multiple tree bulks (e.g. *E. camaldulensis* Laura, Qld, Australia with a 61 tree bulk seed lot) in which seeds were obtained from multiple trees and collected into the same bag. The single-tree seed lots were allocated once into the RICB design (1 allocation × 15 replicates = 15 seedlings) whereas the sources from multiple-tree bulks were allocated between 4 and 10 times into the design depending on seedling availability (e.g. 4 allocations × 15 replicates = 60 seedlings). This ensured realistic provenance means for resistance were obtained for these bulk seed lots. *Rhodamnia rubescens* seedlings were placed randomly within the trial as a positive control (Pegg *et al.*, 2014b).

Preparation of inoculum

A single-pustule isolate of *P. psidii* (BRIP 57793) was collected from *Rhodamnia sessiliflora* growing in the Chapel Hill suburb of Brisbane, Qld, Australia. Urediniospores from this pustule were collected using a fine-bristled paintbrush and washed into 5 mL sterile distilled water (SDW) to which one drop of the surfactant Tween 20 had been added. This solution was then applied onto *S. jambos* and *R. rubescens* seedlings to produce large numbers of urediniospores for inoculations.

Inoculated *S. jambos* and *R. rubescens* plants were covered with black plastic bags, which were sealed to maintain a high humidity (RH) level, and placed in an incubator at 20°C for 24 h. After 24 h plants were grown in a controlled environment room using a 12 h photoperiod and maintaining temperatures of 26°C in the light and 20°C in the dark. Urediniospores were collected using a vacuum line 10–12 days after inoculation, continuing until spores were no longer produced. These urediniospores were then inoculated back onto *S. jambos* and *R. rubescens* seedlings, a process repeated until sufficient numbers of spores had been collected for large-scale inoculation of *Eucalyptus* species. Urediniospores were then placed into a desiccator for 48 h before being placed into Nunc tubes and stored at −80°C until required for inoculation.

Inoculation procedure

Urediniospores were removed from −80°C storage and allowed to warm to room temperature prior to being added to SDW. Two drops of Tween 20 were added per 100 mL SDW and the spore suspension was stirred to ensure an even distribution of the inoculant. Spore counts were conducted using a haemocytometer and the suspension adjusted to a concentration of 10^5 spores mL^{-1} for use in subsequent inoculations.

Seedlings were inoculated as described by Pegg *et al.* (2014a). After 24 h the plastic covering was removed and plants were placed in a shade-house and hand-watered as required. Disease symptom progression was monitored daily.

Disease assessments

Seedlings were assessed 14 days after inoculation for incidence of disease (% of seedlings with symptoms) and severity of infection on new shoots and expanding leaves. A disease rating scale was used as follows: 1 = no symptoms evident or presence of chlorotic flecking; 2 = presence of a hypersensitive reaction (HR) with flecks or necrosis; 3 = small pustules, <0·8 mm diameter, with one or two uredinia; 4 = medium-sized pustules, 0·8 to <1·6 mm diameter with about 12 uredinia; 5 = large pustules, ≥1·6 mm diameter, with 20 or more uredinia on leaves, petioles and/or shoots (Junghans *et al.*, 2003b). Ratings of 1–3 were considered as indicative of resistance.

Statistical analysis

A broad examination of all species and taxa assessed was undertaken. Comparisons were made among species, populations within the species, and families within provenances of the species. Provenances with fewer than 20 individuals were included within species comparisons but not used when comparing provenance or family data. Chi-square analysis was used to compare nonparametric disease rating data and seedling resistance frequencies between species, provenances within species, and families within provenances.

Risk mapping

A bioclimatic model of the potential distribution of *P. psidii* in South Africa was developed in ArcGIS v. 10.1 (ESRI). Bioclimatic models describe the potential geographic distribution of a species based on the climatic range limits of the known geographic range. Climatic predictors selected for this analysis were limited to average monthly minimum temperature (T_{min}) and average monthly RH, available as $1' \times 1'$ national gridded data sets (Schulze, 2007). Other climatic predictors were excluded from the model due to the unavailability of defined optimal ranges.

Because *P. psidii* has been recorded at only one location in South Africa, thus providing only a single data point, model validation was achieved using records where the pathogen has been found in Australia. High and medium risk models for Australia were developed by applying high and medium risk climate thresholds to $6' \times 6'$ monthly average RH and T_{min} gridded data sets (Bureau of Meteorology, Commonwealth of Australia, 2008). Verified records of presence of *P. psidii* (G. S. Pegg, unpublished data) were spatially intersected to the risk models to verify if points of known presence of *P. psidii* were in locations modelled as climatically suitable.

The climatic model highlights areas that could support *P. psidii*; however, the establishment of a viable population depends on the presence of a susceptible host plant. The spatial distribution of key native and commercially grown non-native Myrtaceae species in South Africa was used to identify areas within the climatically suitable range where known susceptible plant species are found.

High risk was defined by average RH ≥ 80% and average T_{min} of 18–22°C (Blum & Dianese, 2001; Glen *et al.*, 2007). Average RH ≥ 70% and average T_{min} ≥ 10°C (Kriticos *et al.*, 2013) were set as thresholds for the identification of medium risk areas. Risk maps were compiled showing the seasonal potential risk of *P. psidii* in South Africa.

Risk for *Eucalyptus* growing areas

The potential impact of *P. psidii* on the industrial production of *Eucalyptus* species in South Africa was estimated by spatially intersecting the commercial forestry cadastral data set with the medium and high risk model outputs. Forestry cadastres included areas planted to non-Myrtaceae species as well as unplanted areas. For this reason the analysis more appropriately represents a quantification of the areas climatically suitable for *P. psidii* infection within the boundaries where stands of *Eucalyptus* species occur or could be established in the future. The percentage area of commercial forestry cadastres falling within high and medium *P. psidii* risk areas was calculated as an indication of the potential impact of *P. psidii* on eucalypt fibre and timber production.

Risk to native Myrtales

The evaluation of the potential threat of *P. psidii* to native Myrtaceae was limited to four species. These species were selected because they are endemic and localized (*Metrosideros angustifolia*), because they are known to be susceptible to the pathogen (*H. natalensis*, *Eugenia natalitia*), or because they are widespread and abundant in forestry areas (*Syzygium cordatum*). The evaluation of the potential impact of *P. psidii* on these four species was performed by spatially intersecting the known species distribution to the *P. psidii* high and medium risk model outputs. Species geographic distribution consisted of $1' \times 1'$ gridded data sets. These data sets were derived from the rasterization of vector data sets developed by the South African National Biodiversity Institute (BGIS, South African Biodiversity Institute, 2013). The extent of the potential impact of *P. psidii* on these species was expressed as the percentage area of each species distribution range where climatic requirements for *P. psidii* are met.

Results

Symptom development and assessment of *Eucalyptus* spp. susceptibility

Symptoms were first observed 5 days after seedlings had been inoculated and these were seen as red-brown lesions, necrotic and chlorotic spots on leaves. On susceptible plants, sori with uredinia were first observed 7 days after inoculation. All *Eucalyptus* species evaluated in the screening experiment exhibited some level of symptom development, although there were clear differences for the different test materials. Resistant and susceptible plants were identified for all species and all provenances, apart from the Wetar provenance of *E. urophylla* where all seedlings were assessed as being resistant.

Species

Eucalyptus camaldulensis was the most susceptible of the four species assessed, with more than 30% of the seedlings rated as being susceptible to *P. psidii*. However,

E. camaldulensis in general was not significantly more susceptible than *E. camaldulensis* subsp. *simulata* (Table 3). *Eucalyptus urophylla* was most resistant, with 96% of seedlings tested rating as resistant to the strain of *P. psidii* in Australia used in this study (Table 3).

Provenances

Significant differences between provenances within species were identified for *E. camaldulensis*, *E. pellita* and *E. urophylla* (Table 4). Provenances represented by fewer than 10 seedlings were not used in these comparisons. The most susceptible source, with only 50% of seedlings recorded as resistant, was the *E. camaldulensis* seed material originating from Salique in South Africa (Table 4), significantly lower than Australian *E. camaldulensis* provenances of Laura ($P = 0{\cdot}001$) and Petford ($P = 0{\cdot}0001$).

The most susceptible *E. pellita* provenance was South Kiriwo, with 86·8% of seedlings identified as resistant in comparison to the Helenvale provenance from Queensland, where more than 96% of the seedlings were rated as resistant (Table 4). All provenances tested within *E. urophylla* had resistance levels greater than 90%. However, all seedlings of the Indonesian Wetar Island provenance were resistant, in comparison to Flores Island, also in Indonesia, where a small percentage (6·6%) of seedlings was recorded as being susceptible.

Families

Within *E. camaldulensis* subsp. *simulata* provenances, resistance to *P. psidii* varied among and within families for all but one family within the Palmer River provenance, where 100% of seedlings were rated as resistant (Fig. 1a). There were also significant differences between the most resistant and most susceptible families for the three *E. camaldulensis* subsp. *simulata* provenances tested.

Table 3 Comparison of susceptibility of *Eucalyptus* species to *Puccinia psidii* infection under greenhouse conditions

Species	Disease rating[a]	Resistant seedlings (%)[b]
E. urophylla	1·4 ± 0·04	96·0 a
E. pellita	1·6 ± 0·04	93·4 a
E. grandis	1·9 ± 0·05	85·3 b
E. camaldulensis subsp. *simulata*	2·6 ± 0·05	69·6 c
E. camaldulensis	2·5 ± 0·09	68·6 c

[a]Disease rating scale: 1 = no symptoms evident or presence of yellow flecking; 2 = presence of a hypersensitive reaction (HR) with flecks or necrosis; 3 = small pustules, <0·8 mm diameter, with one or two uredinia; 4 = medium-sized pustules, 0·8–1·6 mm diameter with about 12 uredinia; 5 = large pustules, >1·6 mm diameter, with 20 or more uredinia on leaves, petioles and/or shoots (Junghans *et al.*, 2003b).

[b]Resistant seedling percentage values followed by the same letters indicates means that do not differ significantly (pairwise comparison, $P < 0{\cdot}05$).

Within the two provenances of *E. pellita* assessed, nine families showed total resistance to infection by *P. psidii* (Fig. 1b). There was no significant difference between the most resistant and most susceptible families in both Helenvale (families 1106d-k6522, 100% seedlings resistant and 1106d-k6634, 88·2% resistant) and Kuranda provenances (families 1113d-k6634, 100% seedlings resistant and 1113d-k6635, 80% resistant).

Similar to *E. pellita*, nine families within the two *E. urophylla* provenances assessed (Aek Nauli, Samosir) had 100% of seedlings rated as resistant to *P. psidii* (Fig. 1c). No significant differences were identified between families from Samosir provenance despite a range in resistance levels from 87·5 to 100% (families 11121-a1621 and 11121-a1625, respectively). However, a significant difference between families was identified for Aek Nauli where resistance levels ranged from 100% (five families) to 75% (family 11121-a1646).

Two families from the Copperlode provenance of *E. grandis* were rated as being resistant to *P. psidii* infection (families 5970-k5168 and 5970-k5181; Fig. 1d). Four of the eight families tested had resistance levels below 80% and were significantly different from the most resistant families, with the lowest being 66·7% (family 5970-k5184).

Risk mapping

Coastal areas of South Africa, stretching from the Northern Cape in the west, to the KwaZulu-Natal coast in the east, were shown to be most conducive to the occurrence

Table 4 Susceptibility of *Eucalyptus* species to *Puccinia psidii* infection

Species	Provenance/ source	Resistant seedlings (%)[a]	Resistant families (*n*/10)
E. urophylla	Aek Nauli	94·7 b	5
	Flores Island	93·4 b	N/A
	Samosir	96·0 ab	4
	Wetar Island	100·0 a	N/A
E. pellita	Helenvale	96·5 a	6
	Kuranda	92·4 ab	3
	Serisa	93·2 ab	N/A
	South Kiriwo	86·8 b	N/A
E. grandis	Bellthorpe	87·1 a	N/A
	Copperlode	83·0 a	2
	EB50	82·3 a	N/A
	Sappi	83·7 a	N/A
	Yabbra	91·0 a	N/A
E. camaldulensis subsp. *simulata*	Holmes	68·0 a	0
	Palmer River	70·5 a	1
	Walsh River	70·9 a	0
E. camaldulensis	Laura	75·5 a	N/A
	Petford	79·2 a	N/A
	Salique	50·7 b	N/A

[a]Resistant seedling percentage values followed by the same letter indicates means that do not differ significantly (pairwise comparison, $P < 0{\cdot}05$).

of *P. psidii* during most of the year (Fig. 2). The period of widest possible geographic risk was found to be during the summer months, when several inland areas, in the KwaZulu-Natal, Limpopo and Mpumalanga Provinces, were also found to be conducive to *P. psidii* survival (Fig. 2).

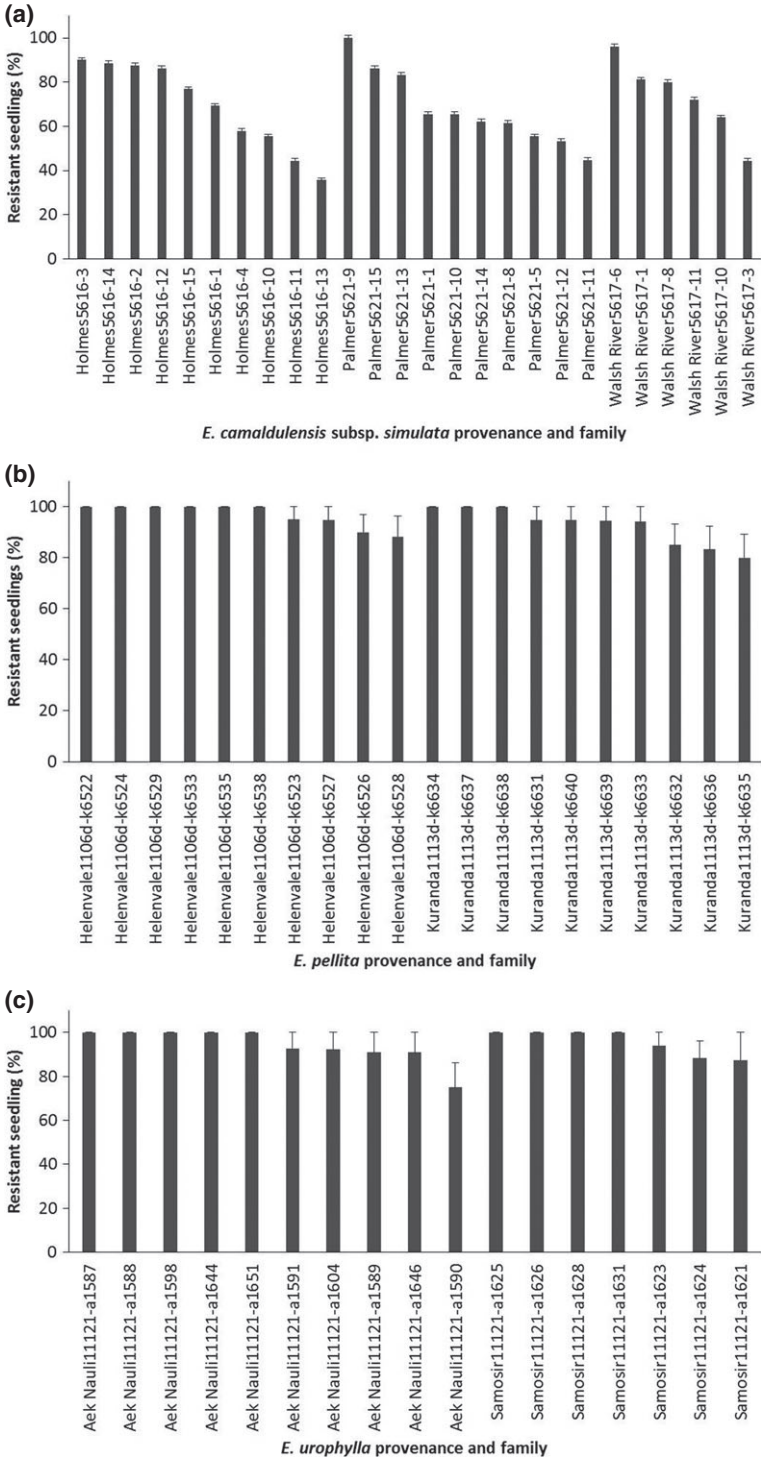

Figure 1 Comparison of tested *Eucalyptus* families of different provenances to infection by *Puccinia psidii*. (a) *E. camaldulensis* subsp. *simulata*, (b) *E. pellita*, (c) *E. urophylla*, (d) *E. grandis* families of Copperlode (Australia) provenance.

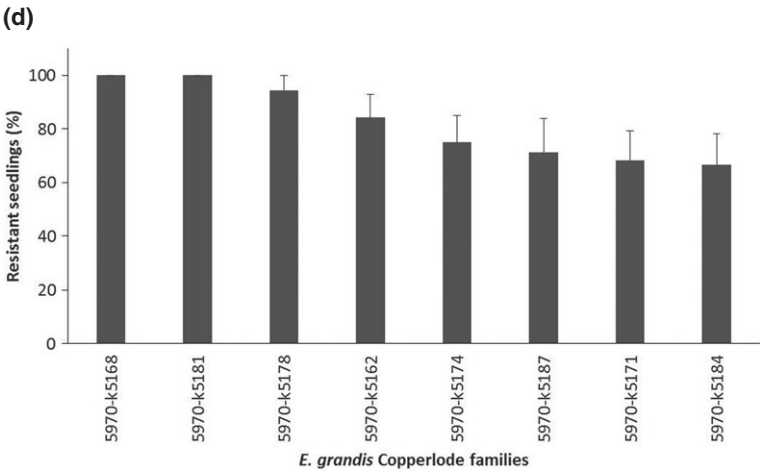

Figure 1 Continued

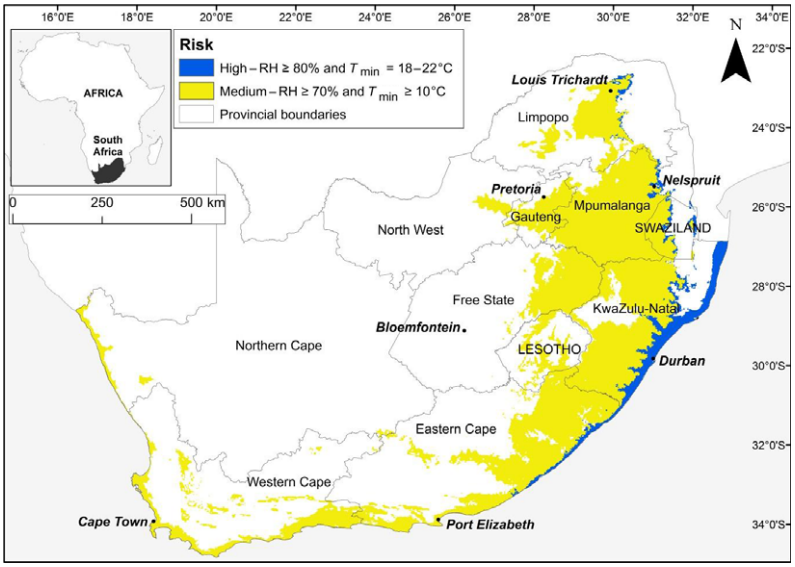

Figure 2 Risk map generated using ArcGIS, showing risk of *Puccinia psidii* occurrence in South Africa during the summer season (December to February), based on minimum temperature (T_{min}) and relative humidity (RH) requirements for survival of the pathogen. High risk areas are defined by RH ≥ 80% and T_{min} = 18–22°C; medium risk areas are defined by RH ≥ 70% and T_{min} ≥ 10°C.

Most areas where commercial *Eucalyptus* plantations occur would be at medium risk during summer, with high risk areas restricted to a narrow band along the coast (Fig. 3). For the remainder of the year, the risk areas would apparently shrink to only those close to the coast.

All four species of native Myrtaceae and Heteropyxidaceae considered occur within regions with medium to high risk of *P. psidii* successfully becoming established (Fig. 4). More than half of the area where endemic *M. angustifolia* occurs is at medium risk of *P. psidii* establishment. The other native Myrtaceae for which risk maps were produced showed limited areas of medium risk and even smaller areas with high risk of damage occurring (Fig. 4).

Discussion

Results of this study show that some species of *Eucalyptus* commonly grown in South Africa, or used as breeding stock in hybridization, are susceptible to infection by *P. psidii*. This includes some seed sources that have been used in commercial planting operations in the country in the past. Furthermore, many of the areas in which *Eucalyptus* is deployed in the country have climatic conditions that are suitable for the pathogen to infect trees. These areas also correspond with regions where native Myrtaceae occur in South Africa. The recent detection of *P. psidii* in South Africa (Roux *et al.*, 2013), and the added possibility that additional biotypes of the pathogen could be introduced into the country, highlights the fact that *P. psidii* represents an important threat to commercial forestry as well as to the natural biodiversity of South Africa.

Of the four *Eucalyptus* species tested, *E. urophylla* showed the highest overall levels of resistance to *P. psidii*. These plants included the Wetar Provenance of *E. urophylla*, for which all seedlings tested, represented by a bulk of seeds taken from 25 trees, were rated as

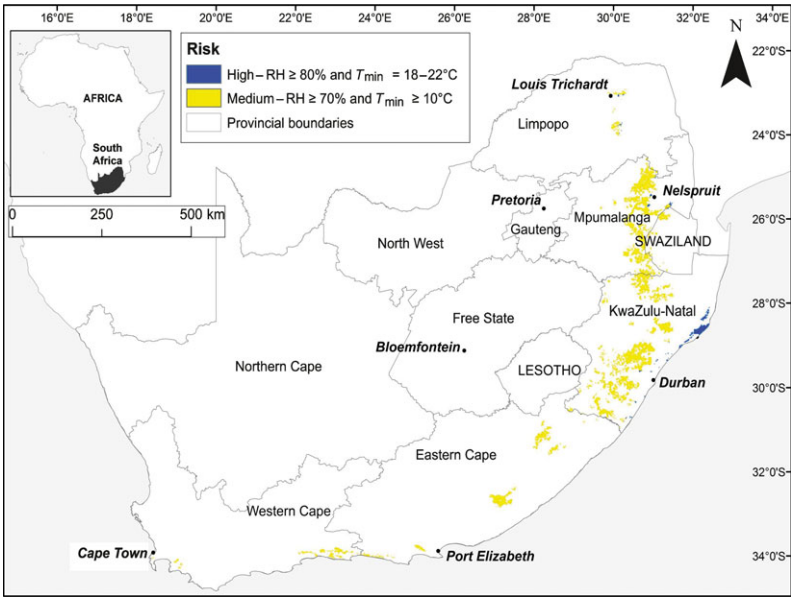

Figure 3 Risk map generated using ArcGIS, showing risk of *Puccinia psidii* occurrence in the *Eucalyptus* plantation forests in South Africa during the summer season (December to February), based on minimum temperature (T_{min}) and relative humidity (RH) requirements for survival of the pathogen. High risk areas are defined by RH ≥ 80% and T_{min} = 18–22°C; medium risk areas are defined by RH ≥ 70% and T_{min} ≥ 10°C.

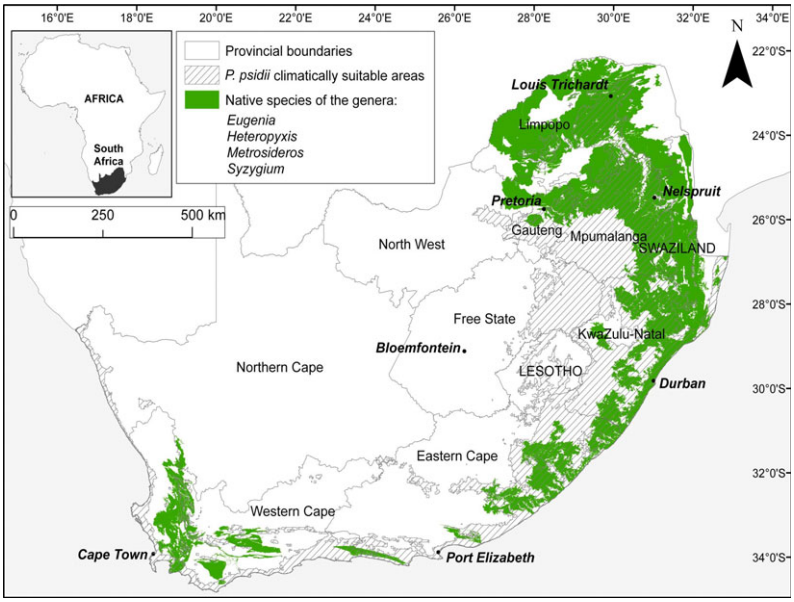

Figure 4 Risk map generated using ArcGIS, showing risk of *Puccinia psidii* occurrence in the distribution range of four species of native Myrtaceae and Heteropyxidaceae in South Africa. High risk areas are defined by relative humidity (RH) ≥ 80% and minimum temperature (T_{min}) = 18–22°C; medium risk areas are defined by RH ≥ 70% and T_{min} ≥ 10°C.

resistant. This should therefore provide a good indication of the possible impact of the disease on this provenance/species.

Eucalyptus camaldulensis was the most susceptible species tested and the most susceptible seed lot tested was one from South Africa. The susceptibility of South African *Eucalyptus* material has been reported previously, with the first epidemic outbreak of rust on *Eucalyptus* occurring on *Eucalyptus* genotypes planted in Brazil (Ferreira, 1983; Coutinho *et al.*, 1998), including material from South Africa. Several other studies have also reported *P. psidii* infection of *Eucalyptus* species and clones of interest to South Africa (Dianese *et al.*, 1986; Morin *et al.*, 2012; Guimarães *et al.*, 2013; M. J. Wingfield, unpublished data).

Variation between seed lots from different provenances (Tommerup *et al.*, 2003) is not surprising and caution should be exercised when interpreting the results obtained in this and other studies. Breeding programmes for resistance to *P. psidii* should be based on the testing of a broad range of *Eucalyptus* genotypes and the selection of resistant individual trees, to establish clonal lines for commercialization. To date, studies investigating resistance to *P. psidii* within eucalypt (*Eucalyptus* and *Corymbia*) species, including those presented here, have identified resistance in all species, with the possibility of

selection at the family level demonstrated (Dianese *et al.*, 1986; Morin *et al.*, 2012; Guimarães *et al.*, 2013; Pegg *et al.*, 2014a).

Care needs to be taken when selecting for a single trait, as resistance to *P. psidii* does not necessarily indicate resistance to other pathogens of eucalypts. For example, Pegg *et al.* (2014a), when studying *P. psidii* resistance within species of spotted gum, determined that correlations between breeding value predictions for rust resistance and *Quambalaria pitereka*, a significant foliage pathogen in Australia (Pegg *et al.*, 2009), were not significantly different from zero. This implies that there was little to no relationship between resistance mechanisms associated with the different pathogens.

At least four biotypes of *P. psidii* have been reported (Graça *et al.*, 2013). These biotypes have been shown to vary in their capacity to infect hosts and cause disease, with physiological specialization reported from cross-inoculation experiments (Ferreira, 1983; Coelho *et al.*, 2001; Aparecido *et al.*, 2003c). Graça *et al.* (2013) provided genetic evidence for host-associated multilocus genotypes in *P. psidii*. Infection experiments performed in the current study, therefore, provide only a broad indication of the possible impact of *P. psidii* to the South African *Eucalyptus* industry and the survival of native Myrtaceae in the region. Only a single genotype of *P. psidii* was used in this study as this is the only one known to occur in Australia. The Australian biotype, however, has been shown to be capable of causing disease on a wide range of hosts (Morin *et al.*, 2012), including all genera of Myrtaceae that occur in South Africa. The results should thus provide at least a strong indication of the relative susceptibility of the species tested.

It is not known yet which biotype of *P. psidii* occurs in South Africa. In Australia and Hawaii, the rust represents a single multilocus genotype and this is different to those that have been studied in Brazil (Graça *et al.*, 2013). *Puccinia psidii* studies in Brazil have shown that there is considerable genetic diversity present, but that distinct multilocus genotypes are uniquely associated with specific hosts across diverse geographic locations.

Trials should be initiated to evaluate the susceptibility of native South African Myrtales to infection by *P. psidii*. Alfenas *et al.* (2005) showed that *H. natalensis* is highly susceptible. Pegg *et al.* (2014b), when assessing susceptibility of Myrtaceae in subtropical and tropical regions of Queensland, Australia, identified seedlings of *Eugenia zeyheri* and established trees of *E. natalitia* as being moderately susceptible. No information is available regarding other native South African species or the variability in susceptibility that might exist within native populations.

Based on the modelling data obtained in this study, survey programmes to monitor for the presence of *P. psidii* in South Africa can be optimized. Surveillance will be more likely to detect rust outbreaks in spring and summer, when incidence is predicted to be highest. Furthermore, coastal areas, as well as the areas in South Africa with subtropical climates should be focused on. Not only are these areas conducive to *P. psidii* infection, but they also have high concentrations of native and non-native Myrtales (SIBIS: SABIF database, SANBI, http://sibis.sanbi.org/), including various *Syzygium* and *Eugenia* species from Asia and Australia, *P. guajava* from South America, and other Myrtaceae. Significantly, even areas in the Gauteng Province, where the country's largest international airport is located, were found to represent areas of medium risk for *P. psidii* establishment.

For the containment of introduced pathogens to be effective, it is crucial that the factors driving invasion by these pathogens are known (Gonthier & Garbelotto, 2013). Three major determinants of pathogen invasiveness are: the relative susceptibility of naïve hosts, environmental conditions, and the pathogen's ability to be transmitted between hosts. It is, therefore important for South Africa to, as a matter of priority, screen native and non-native Myrtales for resistance to various *P. psidii* genotypes for deployment. This is especially important in the case of the myrtle rust pathogen, because rust fungi are well adapted for transmission through air currents, on infected plant material and on clothing and equipment (Langrell *et al.*, 2008).

Although *P. psidii* has been found on only a single tree in South Africa, the risk of the pathogen spreading and becoming widely established is high. The results of this study provided strong evidence of this fact based on the availability of susceptible host material and climatic conditions conducive to infection. Although only limited information is available regarding the susceptibility of South African Myrtaceae to infection by *P. psidii*, numerous susceptible Australian and Asian woody plants are commonly planted, often in high density, throughout South Africa. Efforts to detect and reduce inoculum levels in the country should be intensified. At the same time both non-native commercially planted as well as native Myrtaceae should be screened for resistance and subsequent deployment in order to reduce the future impact of this pathogen.

Acknowledgements

This work is based on research partially supported by the National Research Foundation of South Africa (grant specific unique reference number UID 83924). The grant holders acknowledge that opinions, findings and conclusions or recommendations expressed in any publication generated by the NRF-supported research are that of the authors and that the NRF accepts no liability whatsoever in this regard. J.R. thanks the Harry Oppenheimer Trust of South Africa for funding her research visit to Australia. The seed used in the screening study was sourced from the Queensland Department of Agriculture, Fisheries and Forestry. This contribution is greatly appreciated.

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