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Screening *Corymbia* populations for resistance to *Puccinia psidii*

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The exotic rust pathogen *Puccinia psidii* is now widespread along the east coast of Australia from temperate Victoria to tropical far north Queensland, with a current host range exceeding 200 species from 37 myrtaceous genera. To determine the threat *P. psidii* poses to plantation and native eucalypts, artificial inoculation was used to screen germplasm of spotted gum (*Corymbia* spp.) for resistance to the biotype of *P. psidii* that has become established in Australia. The objective was to characterize resistance to *P. psidii* within the *Corymbia* species complex so that management strategies for the deployment of germplasm from existing breeding programmes of these spotted gum species could be developed. Symptom development initiated 7 days after inoculation, with resistant and susceptible seedlings identified within all species, provenances and families. Inter- and intraspecific variability in rust resistance was observed among spotted gum species. There was no apparent relationship between climatic conditions at the provenance origin and disease resistance. The heritability estimates for all assessments are moderate to high and indicate a significant level of additive genetic variance for rust resistance within the populations. The results of this study clearly identify potential to select for resistance at the family level within the tested populations. While the potential for *P. psidii* to detrimentally impact upon *Corymbia* in the nursery and in young plantations was demonstrated, estimations of the heritability of resistance suggest that efforts to enhance this trait through breeding have reasonable prospects for success.

Keywords: *Corymbia*, *Puccinia psidii*, spotted gum

Introduction

Puccinia psidii was first described from *Psidium pomiferum* in Brazil by Winter in 1884 (Coutinho *et al.*, 1998). It has since been reported from a range of hosts in South and Central America and in Florida and California in the United States (Coutinho *et al.*, 1998; Zambino & Nolan, 2011). More recently, *P. psidii* has been reported for the first time outside of the Americas, with detections in Hawaii in 2005 (Uchida *et al.*, 2006) and Japan in 2009 (Kawanishi *et al.*, 2009). Zhuang & Wei (2011) published a new report of *P. psidii* infecting *Syzygium jambos* on Hainan Island in southern China.

Outside of Australia, *P. psidii* has been reported on more than 100 species from 29 genera in the Myrtaceae (Coutinho *et al.*, 1998; Simpson *et al.*, 2006; Carnegie & Lidbetter, 2011). *Puccinia psidii* has had a significant impact on industries relying on Myrtaceae, including the all spice (*Pimenta dioica*) industry in Jamaica (MacLachlan, 1938) and the eucalypt plantation industry in Brazil (Ferreira, 1983; Furtado & Marino, 2003; Glen *et al.*, 2007).

Eucalyptus rust, originally named guava rust, has long been recognized as a significant threat to Australia's Myrtaceae-rich natural forests and industries that rely on species in this family (Glen *et al.*, 2007). In Brazil, the impact of *P. psidii* on introduced eucalypts was so significant, including reduced growth, destruction of growing shoots leading to stem malformation and tree mortality, that the term 'eucalyptus rust' was used to describe the disease (Ferreira, 1983). Infection can affect growth rates and subsequent profitability (Booth *et al.*, 2000).

Puccinia psidii was first identified in Australia in April 2010 when it was detected in a cut flower nursery in New South Wales (NSW; Carnegie *et al.*, 2010). The fungus was given the common name 'myrtle rust', while taxonomic efforts attempted to distinguish it from the well-known guava and eucalyptus rusts endemic to South America but hitherto absent from Australia (Department of Agriculture, Fisheries & Forestry, 2010). By December 2010, *P. psidii* had spread throughout NSW and was detected in Queensland for the first time (Carnegie *et al.*, 2010). By September 2012, *P. psidii* had spread along the east coast of Australia from temperate areas in Victoria to tropical regions of far north Queensland. Based on observations of the natural infection within the host range, the rust has been found on more than 200 species from 35 genera (Carnegie & Lidbetter, 2011).

Several *Eucalyptus* and *Corymbia* (spotted gum) spp. have been naturally infected by *P. psidii*, with the potential impacts on native forests of great concern. In addition, commercially important species such as *E. agglomerata*, *E. pilularis*, *E. cloeziana*, *E. grandis*, *C.*

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citriodora subsp. *citriodora*, *C. citriodora* subsp. *variegata*, *C. henryi* and *C. torelliana* have been infected and indicate the pathogen could impact upon the Australian plantation hardwood industry as well as ecologically focused reforestation efforts (Carnegie & Lidbetter, 2011).

Elite *Eucalyptus* clones are extensively used to achieve improvements in pulpwood quality, rapid growth and high yield (Junghans *et al.*, 2003a). However, one of the most commonly used *Eucalyptus* species, *E. grandis*, is highly susceptible to *P. psidii* (Junghans *et al.*, 2003a), a disease still considered one of the most damaging to eucalypt plantations in Brazil. While management of rust in nurseries may be achieved with chemical treatment, management of rust in plantations across South America relies on developing resistant cultivars (Furtado & Marino, 2003). Junghans *et al.* (2003a) identified segregation ratios in families suggesting major gene control of rust resistance. This work identified the Ppr-1 genetic marker as being useful for marker-assisted introgression of resistance and has provided an initial lead for positional cloning efforts to identify the causal resistance allele. However, Graça *et al.* (2011) recently found clones carrying the resistance gene *Ppr-1* to be susceptible to a newly discovered rust biotype and suggested multiple genes may govern resistance.

Wide variation in resistance to eucalyptus rust has been observed between and within *Eucalyptus* and *Corymbia* spp. tested with a single rust biotype in Brazil as part of a study to understand the risk the disease posed to Australia (Zauza *et al.*, 2010). Inter- and intraspecific variability was also observed among and within a range of Myrtaceae species tested. Of the species tested, accessions of *E. grandis* and *E. cloeziana* were ranked as most susceptible, while spotted gum species *C. citriodora* subsp. *citriodora* and *C. maculata* had >72% of seedlings rated as resistant (Zauza *et al.*, 2010). This confirmed earlier glasshouse and field-based assessments (Dianese *et al.*, 1984) that identified *E. grandis* and *E. cloeziana* as being more susceptible to rust infection.

Preliminary host testing of the rust in Australia has revealed that a range of species, some of which are relied upon by the plantation forest industry (*E. agglomerata*, *E. cloeziana*, *E. dunnii*, *E. globulus*, *E. grandis*, *E. pellita*, *E. pilularis*, *E. saligna* and *C. citriodora*), are susceptible to *P. psidii* (Carnegie & Lidbetter, 2011; Morin *et al.*, 2012). So far the impact of *P. psidii* on *Eucalyptus* and *Corymbia* spp. in Australia, based on field observations alone, is restricted to seedlings (e.g. *C. citriodora* subsp. *variegata*, *C. torelliana*, *E. cloeziana*, *E. tereticornis*). However, on *E. carnea* and *E. curtisii* the disease has been observed, causing dieback of mature trees and young coppice regrowth (authors' unpublished data). To date, *P. psidii* has not been detected within forest tree plantations in Queensland. This may be because the disease has not yet established in areas where the majority of Queensland plantations exist.

This study aimed to understand the threat *P. psidii* poses to plantation and native forests, through screening a diverse range of *Eucalyptus* and *Corymbia* germplasm for resistance to the biotype of *P. psidii* (Carnegie *et al.*, 2010) now widespread in Australia. A second aim was to characterize resistance to *P. psidii* within the *Corymbia* species complex, providing guidance for developing management strategies for the deployment of germplasm from existing industrial breeding programmes. Three different disease scoring methods were also compared. Spotted gum taxa tested in this study included three closely related, valuable native forest and plantation species, *C. citriodora* subsp. *variegata* (CCV), *C. citriodora* subsp. *citriodora* (CCC) and *C. henryi* (CH) (Ochieng *et al.*, 2010). In addition, resistance within seed lots of *C. torelliana* (CT) and associated *Corymbia* hybrids (Lee *et al.*, 2009) were studied. In total, nine spotted gum provenances were evaluated, with pedigreed families representing six of these provenances. This allowed the investigation of patterns of resistance within and among species of the widely distributed and environmentally significant *Corymbia* genus.

Material and methods

Species, provenance and family resistance to *Puccinia psidii*

To examine the influence of origin on rust resistance patterns, 15 seedlings from between nine and 11 open-pollinated families from a range of provenances were examined for disease levels following inoculation with *P. psidii* (Table 1). Two provenances from CCC were compared to five CCV and two CH provenances. Of the CCC provenances, one was from an inland location in far north Queensland (Mt Garnet) and a second was from a coastal location at the southern end of the range of CCC (Yeppoon) in Queensland. Presho, a CCV provenance frequently severely damaged by a native fungal foliage pathogen (*Quambalaria pitereka*), originates in the westernmost range of the species. The resistance of Presho to *P. psidii* was compared to the more coastal provenances of Woondum, Brooyar, Mt McEuan and another inland provenance, Ballon.

For CH, two provenances were selected from different rainfall zones: Lockyer and Nerang. Resistance levels were also examined at a family level for seven provenances (CCC – Mt Garnet, Yeppoon; CCV – Brooyar, Mt McEuan, Woondum; CH – Lockyer, Nerang) with a family structure. Ballon and Presho provenances were excluded from family level comparison as the seedlings originated from a bulked seed lot. Also included were families from CCV, CH and CT seed orchards. To examine repeatability of results at a family level, a second inoculation was done on seedlings from three CCV provenances, Brooyar, Mt McEuan and Woondum and results compared to the first inoculation.

In addition, seedlings from seven full-sib controlled cross *Corymbia* hybrids (details provided in Table 1) and eight commercial *Corymbia* clones selected from within control pollinated hybrid families of CT mother trees pollinated with CCV pollen, were included in the study. The clones had had a restricted genetic base, originating from three full-sib families with two to three clones being from each sib family. These clones had been commercialized based on their superior early growth, form and amenability to vegetative propagation as rooted cuttings relative to CCV. The clones were similar in size to the seedlings but

Table 1 *Corymbia* species, provenances, seed lots, hybrids and clones used in studies to characterize resistance levels to myrtle rust, *Puccinia psidii*

Taxon	Provenance or source	Seed lots or clones	Latitude (S)	Longitude (E)	Altitude (m a.s.l.)	Mean annual rainfall (mm)
Corymbia citriodora subsp. *citriodora* (CCC)	Mt Garnet	10101 (10 families)[a]	18°00′	145°11′	700	832
	Yeppoon	11246 (9 families)	23°06′	150°44′	30	1325
Corymbia citriodora subsp. *variegata* (CCV)	Woondum	11185 (11 families)	26°25′	152°81′	400	1536
	Brooyar	10248 (10 families)	26°10′	152°30′	90	1143
	Mt McEuan	12805 (10 families)	26°14′	151°39′	300	726
	Ballon	5584 (bulk of 11 trees)	26°30′	150°55′	350	656
	Presho	4928 (bulk of 50 trees)	25°11′	149°10′	470	675
	Seed orchard seed	X7370–X7379 (10 families)	–	–	–	–
Corymbia henryi (CH)	Lockyer	10250 (10 families)	27°28′	152°17′	150	840
	Nerang	10257 (10 families)	27°59′	153°19′	100	1439
	Seed orchard seed	X6088–X6097 (10 families)	–	–	–	–
Corymbia torelliana (CT)	Landrace	X15, X34, X164, X168, X169 (5 families)	–	–	–	–
Corymbia hybrids: (CT×CCC, CT×CCV, CT×CH)	Control crosses	X135, X137, X159, X161, X194, X196, X136 (7 families)	–	–	–	–
Corymbia hybrid clones: (CT×CCV)	–	1, 6, 8, 11, 13, 18, 22, 39 (8 clones)	–	–	–	–

[a]Details of the provenances with family structure are provided in Table 4.

their age was unknown, as they were supplied by a commercial nursery and grown with the seedlings for a 4 week period prior to screening the trial.

Experimental design

The trial with 112 treatments (seed lots) was established as a randomized incomplete block design with 15 replicates, of a single tree from each family treatment in each replicate. Bulk seed lots were represented by 10 allocations in the design (e.g. 10 × 15 replicates = 150 seedlings) to ensure that realistic provenance means for the assessments were obtained. In this sort of design, the number of treatments per block (a subset of the replicate, used to account for variation within the replicate) is less than the number of treatments being evaluated. This type of trial design increases precision of the analysis and reduces experimental error. Incomplete block design trials can be thought of as two-factor experiments (block and treatment) with unequal numbers and no interaction between the block and treatment factors.

Seedlings

Seeds were sown in 40-celled hyco trays (70 mL) in the glasshouse using a potting medium consisting of 50% pine bark fines (0–10 mm), 25% pine bark peat, 25% coarse perlite, a mix of 12–14 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 a day for 10 min at each irrigation time using an overhead spray. Cuttings of the *Corymbia* hybrid clones tested were obtained from Clonal Solutions Australia Pty Ltd. These clones were acclimated with seedlings of the other *Corymbia* spp. for 4 weeks prior to inoculation.

Inoculum

A single pustule isolate of *P. psidii* (BRIP# 57793) was collected from *Rhodamnia sessiliflora* growing in the Chapel Hill suburb of Brisbane, Queensland, Australia. Urediniospores from a single 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 was added. This suspension was then applied onto *Syzygium jambos* and *Rhodamnia rubescens* seedlings to further propagate spores for screening.

These plants were then covered with black plastic bags, which were sealed to maintain a high humidity level, and placed into an incubator at 20°C for 24 h. After 24 h plants were grown in a controlled environment room (CER) using a 12 h/26°C light and 12 h/20°C dark photoperiod. 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 inoculation. Urediniospores were then placed into a desiccator for 48 h before being placed into Nunc tubes and stored at −80°C until required.

Inoculation

Urediniospores were removed from −80°C storage and allowed to warm to room temperature prior to being added to SDW. The surfactant Tween 20 was added at a rate of two drops per 100 mL SDW and the spore suspension stirred to reduce clumping. Spore counts were then conducted using a haemocytometer and the suspension adjusted to a concentration of 1×10^5 spores mL^{-1} for use in subsequent inoculations.

Corymbia seedlings were inoculated using a fine mist spray (2·9 kPa pressure), generated by a compressor driven spray gun

(Iwata Studio series 1/6 hp; Gravity spray gun RG3), applied to the upper and lower leaf surfaces of the seedlings, ensuring all leaves were coated with a fine mist but run-off of the spore suspension was avoided. Ten *R. rubescens* seedlings were used as susceptible controls and placed haphazardly within replicates of the seedlings tested.

Seedling trays were placed into solid plastic tubs and then onto a metal bench layered with a plastic sheet. Immediately after inoculation, seedlings were covered with a plastic sheet for 24 h to maintain high humidity levels and leaf wetness in a controlled environment room set between 18 and 20°C in the dark. Hot tap water (60°C) was applied to the lower plastic sheet immediately after inoculation to ensure high humidity levels were achieved rapidly. After 24 h, plastic bags were removed and plants grown in a shade-house and hand watered as required. Disease symptom progression was monitored daily.

Disease assessments

Seedlings were assessed 12 days after inoculation for incidence of disease (% of seedlings with symptoms) and severity of infection on new shoots and expanding leaves using a disease rating scale: 1 = no symptoms evident or presence of yellow flecking; 2 = presence of a hypersensitive reaction (HR) with fleck 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; Fig. 1). Ratings 1–3 are considered as indicating resistance.

Disease incidence (I) was also assessed as a percentage of the four youngest inoculated leaves on seedlings showing evidence of pustule development and uredinia. Disease severity (S) was scored as a subjective assessment of the percentage of the total area of infected foliage on diseased leaves only. In total, four assessments were available for analysis as response variables: disease incidence (I) and disease severity (S), disease rating scale (1–5) and the percentage of resistant seedlings based on the disease rating scale.

Statistical analyses

Two sets of analyses were used to interrogate the data collected from the assessment of inoculated seedlings. Firstly, a broad examination of all species, taxa and type of plant (clone versus family) assessed was undertaken including CCC, CCV, CH, CT and *Corymbia* hybrids following the approach of Lee *et al.* (2011). Comparisons were then made among CCC, CCV and CH populations within the species and families within provenances of the species. These analyses excluded data from CT and its hybrids and were primarily undertaken to indicate the significance of difference between means so that comparisons could be made among experimental units, and relationships examined between resistance ratings and environmental variables at the provenance origin (e.g. mean annual rainfall), and population performance could be investigated. All proportion data were arcsine square root transformed prior to analysis using ANOVA and compared using Fisher's PLD post hoc test (STATVIEW). Reverse-transformed data are presented. Chi-square analysis was used to compare non-parametric disease rating data and seedling resistance frequencies between provenances within species and families within provenances.

Genetic parameters of rust resistance

Genetic analyses were undertaken to describe patterns of variation in disease resistance in the *Corymbia* populations with variance components estimated for provenances and families within provenances, to allow estimation of genetic parameters. Genetic parameters were estimated across and within species to provide an indication of the heritability of rust resistance as well as an estimate of the relative importance of within or among population variation for rust resistance (Brawner *et al.*, 2011). Comparisons between the three types of assessment data (incidence, severity, disease rating) were made for each of these analyses.

Genetic parameters were approximated using estimates of the causal components of variance from a mixed model fit with ASREML (Gilmour *et al.*, 2009), assuming the provenances sampled for this experiment were randomly selected races within each *Corymbia* taxon. Two separate analyses were undertaken; the first used a multivariate analysis including CCV, CCC and CH while the second set of univariate analyses considered each taxon separately. The multivariate analysis included all taxa, as CCV and CCC are taxonomically similar subspecies and CH could not be distinguished from CCV using molecular genetic approaches (Ochieng *et al.*, 2008). Therefore, data from these populations were analysed jointly to estimate genetic parameters for the greater *Corymbia* complex. The statistical model used

Figure 1 Rating scale for assessment of resistance to *Puccinia psidii* on the spotted gums (*Corymbia* taxa) with five classes of severity based on ratings developed by Junghans *et al.* (2003b). From left to right: 1 = hypersensitive reaction with yellow flecking; 2 = hypersensitive reaction with necrotic lesions and no evidence of pustules with uredinia; 3 = small pustules <0·8 mm in diameter with 1–2 uredinia; 4 = medium pustule size 0·8–1·6 mm diameter with up to 12 uredinia; 5 = pustule size >1·6 mm in diameter with 20 or more uredinia on leaves, petioles and/or shoots.

was equivalent to equation 3 detailed in Brawner *et al.* (2011). Narrow-sense heritability estimates were approximated as ratio of additive genetic variance to the within provenance phenotypic variance. A similar statistic, proportion of provenance variance, was estimated to evaluate the relative importance of among provenance variance. In addition, genetic correlations were estimated at the family and provenance level to understand the relationships between assessment traits.

Results

Symptom development

Symptoms were first observed 5 days after inoculation, presenting as blistering on leaves and shoots of *R. rubescens* and red-brown coloured lesions, necrotic and chlorotic spots on the *Corymbia* species (Fig. 2). Pustules with uredinia were first observed 6 and 7 days after inoculation on *R. rubescens* and *Corymbia* spp. seedlings, respectively (Fig. 2).

Disease assessment

With one exception, all *Corymbia* species evaluated in this screening experiment exhibited disease symptoms and incidence and severity scores greater than zero; the *Corymbia* hybrid clones were resistant to infection by rust with only chlorotic or necrotic spots observed on new shoots and expanding leaves. Resistant and susceptible seedlings were identified within all species, provenances and families.

Resistance of *Corymbia* species and subspecies

CCC was the most susceptible of the taxa but not significantly different from CH, CT and CCV based on disease rating scores (Table 2). *Corymbia* hybrids were more resistant to *P. psidii* infection than CCC, CH, CCV and *C. torelliana* when comparing disease incidence, severity and disease rating scores.

Resistance of provenances of CCC, CCV and CH

All provenances studied, based on means of families and bulk seed lots, showed symptoms of infection by *P. psidii* (Table 3). Of the nine provenances tested, Mt McEuan (CCV) had the highest percentage of resistant seedlings (Table 3) followed by Lockyer (CH) and Presho (CCV). Mt Garnet (CCC) and Ballon (CCV) had the lowest percentage of resistant seedlings.

Resistance of families within CCC, CCV and CH provenances

Resistance to *P. psidii* varied among and within families in all provenances (Table 4). No family showed total resistance or total susceptibility to *P. psidii*. However, in

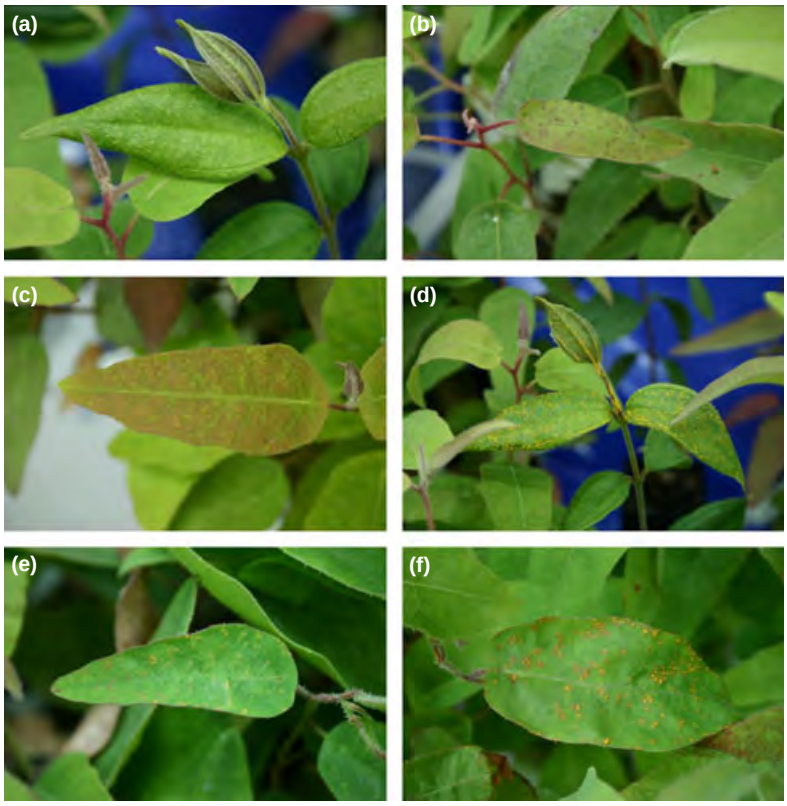

Figure 2 Symptoms first observed 5 days after inoculation appearing as blisters on *Rhodamnia rubescens* (a), and red-brown lesions (b) or yellow-coloured flecking (c) on spotted gum (*Corymbia* taxa). Pustules with yellow-coloured uredinia were first observed 6 days after inoculation on *R. rubescens* (d) and 7 days on spotted gum (e,f).

Table 2 Estimates of performance with ± standard errors by *Corymbia* taxa for the three traits assessed in the rust resistance screening trial, listed from least to most susceptible

Taxa	Incidence[a]	Severity[a]	Disease rating[b]
C. hybrid	16·9 ± 5·0 a	3·0 ± 1·4 a	2·1 ± 0·2 a
C. torelliana	34·4 ± 7·9 b	7·4 ± 2·3 ab	2·8 ± 0·3 b
C. henryi	44·4 ± 3·5 bc	10·1 ± 1·0 b	2·9 ± 0·1 b
CCV	50·2 ± 3·9 cd	9·6 ± 1·1 b	3·0 ± 0·1 b
CCC	57·1 ± 4·1 d	12·9 ± 1·2 c	3·2 ± 0·1 b

[a]Estimates for incidence and severity data followed by different letters significantly ($P < 0·05$) differ from one another according to Tukey's multiple comparison tests.
[b]Chi-square analysis was used to compare disease ratings between species (pairwise comparison $P < 0·05$).

each provenance tested there were significant differences between the most resistant family and the most susceptible family when comparing the percentage of resistant seedlings (Table 4).

Within CCC provenances, the percentage of resistant seedlings ranged from 93 to 21% for Mt Garnet and 87 to 33% for Yeppoon provenance. For each CCV provenance (Mt McEuan, Brooyar and Woondum), a single family was identified with 93% of seedlings rated as resistant to *P. psidii* with only one seedling identified as susceptible (Table 4). With Mt McEuan the most susceptible family had a higher average disease rating of 3·3 with 60% resistant seedlings in comparison to Woondum (4·3; 13%) and Brooyar (3·9; 33%) (Table 4).

A large range in resistance was also identified between Lockyer and Nerang (CH) families. Only 20% of seedlings were rated as resistant from the most susceptible Lockyer family in comparison to 93% of seedlings in the most resistant family. Similarly in Nerang provenance, the percentage of seedlings rated as resistant ranged from 87% in the most resistant family to 26% in the most susceptible family (Table 4).

Similar ranges of rust resistance were observed in families derived from seed orchards (Table 4) with CCV ranging from an average disease rating of 2·6 with 87% resistant seedlings to 3·9 and 33% resistance. Disease resistance ranged from 73% of seedlings to 0% within CH seed orchard families. All but one CT and *Corymbia* hybrid family (Table 5) had average disease ratings levels of less than 3 with the percentage of resistant seedlings ranging from 93 to 53% for CT and 100 to 33% for *Corymbia* hybrid.

When examining the repeatability of resistance screening results at the family level, variability in resistance levels was identified among families of provenances tested (Table 6). However, the only significant difference ($P = 0·03$) identified was for Woondum family 11185-115 where 80% of seedlings were rated as resistant in the first assessment and only 43% in the second assessment. Nevertheless, when comparing the most susceptible families within the three provenances across the two experiments (Tables 4 & 6), the same family was recorded for each provenance (Brooyar 10248-1; Mt McEuan 12805-20; Woondum 11185-118). For both Brooyar (10248-1) and Woondum (11185-118), the same percentage of resistant seedlings was recorded in both experiments.

To further investigate the repeatability of the screening process, data from both trials were simultaneously analysed using a reduced form of the model used to estimate the genetic parameters, where insignificant Tray effects were dropped and Screening was used to index the analysis rather than assessment traits. This was done in order to estimate a genetic correlation between the two screenings. This type-B correlation ranges from −1 to 1, with the latter estimate indicating that the relative performance of families screened in the two screenings was the same. The estimate of the type-B correlation at the family level (followed by the standard error) was 0·97 ± 0·20. The type-B correlation at the provenance level was very poorly estimated due to the limited sample size, but traditional analyses of variance confirmed the

Table 3 Spotted gum (*Corymbia* spp.) provenance susceptibility in order of least to most susceptible, based on percentage of resistant seedlings calculated from the disease severity ratings

Species	Provenance	Disease incidence (% leaves infected)[a]	Number of plants/severity class (1:2:3:4:5)	Disease rating	Resistant seedlings (%)[a]
CCV	Mt McEuan	39·2 ± 3·7 a	20:59:36:14:20	2·7 ± 0·1	77 a
CH	Lockyer	40·7 ± 3·6 a	35:40:27:21:26	2·7 ± 0·12	69 b
CCV	Presho	51·0 ± 3·6 ab	4:52:44:33:16	3·03 ± 0·1	67 b
CCV	Brooyar	53·4 ± 3·6 bc	14:37:47:27:23	3·05 ± 0·1	66 b
CCV	Woondum	52·6 ± 3·4 bc	11:46:47:36:23	3·1 ± 0·1	64 b
CCC	Yeppoon	62·4 ± 3·6 cd	7:37:46:38:20	3·2 ± 0·1	61 b
CH	Nerang	47·9 ± 3·6 ab	40:22:26:31:30	2·9 ± 0·12	59 bc
CCC	Mt Garnet	51·6 ± 3·6 b	15:40:31:35:27	3·1 ± 0·1	58 bc
CCV	Ballon	65·8 ± 3·4 d	3:32:38:39:36	3·5 ± 0·1	49 c

[a]Percentage followed by same letters shows means that do not differ significantly (disease incidence Fisher's PLSD test $P < 0·05$; resistant seedling pairwise comparison $P < 0·05$).

Table 4 Susceptibility of spotted gum (*Corymbia* spp.) families to *Puccinia psidii* ranked least to most susceptible.

Species	Provenance	Family	Number of plants/severity class (1:2:3:4:5)	Disease rating	Resistant seedlings (%)[a]
CCC	Mt Garnet	10101-11	5:6:3:0:1	2·1 ± 0·3	93 a
		10101-1	2:7:2:3:1	2·6 ± 0·3	73 ab
		10101-2	5:2:3:2:3	2·7 ± 0·4	67 abc
		10101-10	1:3:5:5:1	3·1 ± 0·3	60 bc
		10101-3	0:5:4:6:0	3·1 ± 0·2	60 bc
		10101-6	1:4:4:3:3	3·2 ± 0·3	60 bc
		10101-8	0:7:1:2:5	3·3 ± 0·4	53 bcd
		10101-4	0:3:5:4:3	3·5 ± 0·3	53 bcd
		10101-5	1:1:3:5:4	3·7 ± 0·3	36 cd
		10101-9	0:2:1:5:6	4·1 ± 0·3	21 d
CCC	Yeppoon	11246-8	1:7:5:2:0	2·5 ± 0·2	87 a
		11246-3	0:5:7:3:0	2·9 ± 0·2	80 a
		11246-6	1:5:4:5:0	2·9 ± 0·3	67 ab
		11246-2	0:5:3:5:1	3·1 ± 0·3	57 ab
		11246-5	1:1:6:5:1	3·3 ± 0·3	57 ab
		11246-7	0:3:5:4:3	3·5 ± 0·3	53 ab
		11246-1	1:2:3:7:2	3·5 ± 0·3	40 b
		11246-10	0:2:4:5:4	3·7 ± 0·3	40 b
		11246-9	0:0:5:1:9	4·3 ± 0·2	33 b
CCV	Brooyar	10248-8	1:6:7:0:1	2·6 ± 0·2	93 a
		10248-4	4:5:4:1:1	2·3 ± 0·3	87 ab
		10248-5	3:7:1:2:2	2·5 ± 0·3	73 ab
		10248-6	2:4:5:3:1	2·8 ± 0·3	73 ab
		10248-7	1:2:8:4:0	3·0 ± 0·2	73 ab
		10248-2	1:5:3:2:3	3·1 ± 0·3	71 abc
		10248-9	0:4:6:3:2	3·2 ± 0·3	67 abcd
		10248-10	1:1:7:4:2	3·3 ± 0·3	60 bcd
		10248-3	1:1:3:3:6	3·9 ± 0·3	36 cd
		10248-1	0:2:3:5:5	3·9 ± 0·3	33 d
CCV	Mt McEuan	12805-23	6:7:1:1:0	1·8 ± 0·2	93 a
		12805-27	3:9:1:2:0	2·1 ± 0·2	87 ab
		12805-18	3:5:5:1:1	2·5 ± 0·3	87 ab
		12805-26	2:6:5:1:1	2·5 ± 0·3	87 ab
		12805-22	0:6:6:2:1	2·9 ± 0·2	80 ab
		12805-19	0:6:4:2:2	3·0 ± 0·3	79 ab
		12805-24	1:7:3:2:2	2·8 ± 0·3	73 ab
		12805-25	2:3:5:2:3	3·1 ± 0·3	73 ab
		12805-21	2:6:2:0:5	3·0 ± 0·4	67 ab
		12805-20	1:4:4:1:5	3·3 ± 0·4	60 b
CCV	Woondum	11185-112	3:6:5:0:1	2·3 ± 0·3	93 a
		11185-111	1:10:1:2:1	2·5 ± 0·3	80 ab
		11185-115	2:4:5:2:2	2·9 ± 0·3	80 ab
		11185-114	0:4:7:4:0	3·0 ± 0·2	73 ab
		11185-120	1:3:7:3:1	3·0 ± 0·3	73 ab
		11185-119	1:2:6:1:4	3·6 ± 0·3	64 b
		11185-117	2:4:3:5:1	2·9 ± 0·3	60 b
		11185-110	1:3:5:5:1	3·1 ± 0·3	60 b
		11185-116	0:5:4:2:4	3·3 ± 0·3	60 b
		11185-113	0:4:3:6:1	3·3 ± 0·3	50 b
		11185-118	0:1:1:6:7	4·3 ± 0·2	13 c
CCV	Seed orchard	X7375	1:6:6:2:0	2·6 ± 0·2	87 a
		X7376	5:3:3:2:2	2·5 ± 0·4	73 ab
		X7371	3:4:4:1:3	2·8 ± 0·4	73 ab
		X7377	0:3:8:2:2	3·2 ± 0·2	73 ab
		X7378	2:6:1:4:2	2·9 ± 0·3	60 abc
		X7374	0:4:5:3:3	3·3 ± 0·3	60 abc
		X7370	1:1:6:1:5	3·6 ± 0·3	57 abc
		X7373	1:2:4:4:2	3·3 ± 0·3	54 bc
		X7379	0:3:3:5:4	3·7 ± 0·3	40 bc
		X7372	0:1:4:6:4	3·9 ± 0·2	33 c

Table 4 (Continued)

Species	Provenance	Family	Number of plants/severity class (1:2:3:4:5)	Disease rating	Resistant seedlings (%)[a]
CH	Lockyer	10250-3	5:7:2:1:0	1·9 ± 0·2	93 a
		10250-2	5:5:4:1:0	2·1 ± 0·2	93 a
		10250-6	6:4:0:3:2	2·0 ± 0·3	87 ab
		10250-7	4:6:3:0:2	2·3 ± 0·3	87 ab
		10250-10	4:6:1:2:1	2·3 ± 0·3	79 ab
		10250-9	2:2:7:2:2	3·0 ± 0·3	73 ab
		10250-4	6:4:0:3:2	2·4 ± 0·4	67 ab
		10250-1	0:5:4:0:6	3·5 ± 0·3	60 bc
		10250-5	0:3:1:4:7	4·0 ± 0·3	27 cd
		10250-8	1:1:1:6:6	4·0 ± 0·3	20 d
CH	Nerang	10257-9	10:2:1:1:1	1·7 ± 0·3	87 a
		10257-3	5:2:4:3:1	2·5 ± 0·3	73 ab
		10257-10	1:9:1:0:4	2·8 ± 0·4	73 ab
		10257-5	5:0:5:3:2	2·8 ± 0·4	67 ab
		10257-8	4:3:2:5:1	2·7 ± 0·4	60 abc
		10257-4	6:0:3:3:3	2·8 ± 0·4	60 abc
		10257-1	5:0:3:3:4	3·1 ± 0·4	53 bc
		10257-7	2:2:3:2:6	3·5 ± 0·4	47 bc
		10257-6	1:2:3:6:2	3·4 ± 0·3	43 bc
		10257-2	1:2:1:5:6	3·9 ± 0·3	26 c
CH	Seed orchard	X6092	8:1:2:3:1	2·2 ± 0·4	73 a
		X6096	8:2:1:2:2	2·2 ± 0·4	73 a
		X6093	1:1:0:1:0	2·3 ± 0·9	67 ab
		X6094	7:2:1:1:4	2·5 ± 0·5	67 ab
		X6090	3:3:2:3:2	2·8 ± 0·4	61 ab
		X6091	2:4:3:4:2	3·0 ± 0·3	60 abc
		X6089	2:0:1:2:2	3·3 ± 0·6	42 abc
		X6095	1:4:1:3:6	3·6 ± 0·4	40 abc
		X6088	0:0:3:5:2	3·9 ± 0·2	30 bc
		X6097	0:0:0:1:2	4·7 ± 0·3	0 c

[a]Resistant seedling percentage followed by same letters shows means that do not differ significantly (pairwise comparison $P < 0·05$).

Table 5 Susceptibility of *Corymbia torelliana* and *Corymbia* hybrid families to *Puccinia psidii* ranked least to most susceptible

Species	Seed lot	Number of plants/severity class (1:2:3:4:5)	Disease rating	Resistant seedlings (%)[a]
C. torelliana	X168	3:5:6:1:0	2·3 ± 0·2	93 a
	X164	0:10:4:1:0	2·4 ± 0·2	93 a
	X169	0:7:6:1:1	2·7 ± 0·2	87 a
	X15	1:6:3:3:2	2·9 ± 0·3	67 ab
	X34	1:2:5:4:3	3·4 ± 0·3	53 b
Corymbia hybrid clones	CTVA-022	11:3:0:0:0	1·2 ± 0·1	100 a
	CTVA-011	10:5:0:0:0	1·3 ± 0·1	100 a
	CTVA-018	11:4:0:0:0	1·3 ± 0·1	100 a
	CTVA-001	9:6:0:0:0	1·4 ± 0·1	100 a
	CTVA-008	3:3:0:0:0	1·5 ± 0·2	100 a
	CTVA-006	2:4:0:0:0	1·7 ± 0·2	100 a
	CTVA-013	2:4:0:0:0	1·7 ± 0·2	100 a
	CTVA-039	5:10:0:0:0	1·7 ± 0·1	100 a
Corymbia hybrid	X161	0:3:0:0:0	2·0 ± 0	100 a
	X159	1:10:4:0:0	2·2 ± 0·1	100 a
	X196	1:8:6:0:0	2·3 ± 0·2	100 a
	X135	1:6:6:2:0	2·6 ± 0·2	87 ab
	X136	0:6:2:2:0	2·6 ± 0·3	80 ab
	X137	2:5:3:3:2	2·9 ± 0·3	67 bc
	X194	1:1:3:6:4	3·7 ± 0·3	33 c

[a]Resistant seedlings percentage followed by same letters shows means that do not differ significantly (pairwise comparison $P < 0·05$).

Table 6 Comparison for differences between experiments when retesting selected CCV families for susceptibility to myrtle rust (*Corymbia* spp.) using chi-square analysis

Provenance	Family	Resistant seedlings (%) Experiment 1	Resistant seedlings (%) Experiment 2	χ^2	*P* value
Brooyar	10248-8	93	80	1·15	0·3
	10248-4	87	92	0·2	0·6
	10248-5	73	87	0·83	0·4
	10248-6	73	50	1·67	0·2
	10248-7	73	60	0·6	0·4
	10248-2	71	64	0·16	0·2
	10248-9	67	80	0·68	0·4
	10248-10	60	67	0·14	0·2
	10248-3	36	53	0·9	0·3
	10248-1	33	33	0	1·0
Mt McEuan	12805-23	93	67	3·33	0·06
	12805-27	87	87	0	1
	12805-18	87	87	0·03	1
	12805-26	87	73	0·83	0·4
	12805-22	80	87	0·24	0·6
	12805-19	79	67	0·51	0·5
	12805-24	73	73	0	1·0
	12805-25	73	58	0·67	0·4
	12805-21	67	47	1·22	0·3
	12805-20	60	27	3·39	0·06
Woondum	11185-112	93	87	0·37	0·5
	11185-111	80	53	2·4	0·1
	11185-115	80	43	4·24	0·03
	11185-114	73	71	0·16	0·7
	11185-120	73	80	0·19	0·7
	11185-119	64	53	0·4	0·5
	11185-117	60	73	0·6	0·4
	11185-110	60	60	0	1
	11185-116	60	33	2·14	0·1
	11185-113	50	27	1·67	0·2
	11185-118	13	13	0	1

provenance and provenance by screening interaction effects were insignificant across the two trials.

Genetic parameters for rust resistance in CCC, CCV & CH

The genetic parameter estimates (Table 7) indicate the proportion of phenotypic variation that is attributable to differences among provenances (P^2) and differences among families within provenances (narrow sense heritability, h^2). When data were analysed either one species at a time or across all species, there was clear evidence from all three assessment methods that there was little variation among provenances compared to among families for all three assessment variables.

It should be noted again that analyses to generate the genetic parameter estimates used data that excluded all hybrids and CT in an attempt to obtain parameter estimates that were applicable to the closely related species within the *Corymbia* complex. The small sample of provenances included for each taxon adds uncertainty to the variance component estimates as evidenced by the large standard errors relative to the estimate. Nevertheless, there is a consistent pattern across the three species and across the three traits with differences among provenances being minimal relative to differences among families. Comparisons among the three taxa at the provenance level indicate variation among CCV provenances is numerically, but not significantly, greater than the other two taxa for most assessment methods (Table 7). Correlations between assessment methods at the provenance level are high but again poorly estimated (large standard errors), indicating all three scoring methods are evaluating similar resistance mechanisms.

The heritability estimates for all three assessment variables are moderate to high and indicate a significant level of additive genetic variance for rust resistance within the populations. Of the three assessments, heritability estimates for severity and rating were highest, with the assessment using disease rating consistently greater than the other two assessment methods. Given that the greatest differentiation among genetic entries was found using the subjective rating, a tentative recommendation of using this scale for further screening may be made. Comparisons among taxa indicate there is greater additive variation within the CCC and CH populations than there is within CCV, and selection of rust resistant populations would require a greater sampling intensity within CCV to achieve similar levels of improvement to what may be achievable in the other taxa.

Discussion

This is the first study to characterize rust resistance within CCC, CCV, CH, CT and *Corymbia* hybrids to *P. psidii*. Inter- and intraspecific variability was observed among *Corymbia* species and the results clearly identify the potential to select for resistance at the family level within species and provenances tested. Relative to selection among families within provenances, selection at the provenance level alone would provide less genetic improvement for rust resistance. However, the results also suggest the potential for *P. psidii* to impact on young plantations developed using unimproved seed from all provenances studied, including some commonly used in plantation development in Queensland. For example, >30% of the seedlings from Woondum, the most commonly used provenance in hardwood plantations in Queensland, were given a rating of 4 or 5 and deemed highly susceptible to rust infection.

When comparisons were made between all species, including the *Corymbia* hybrids (seedling and clones), CCC was the most susceptible to *P. psidii* and the vegetatively propagated hybrid clones were the least susceptible. The difference between infection in hybrid clones (zero) and the seven full-sib hybrid families is non-intuitive, as neither had been selected for rust resistance and both sources of the hybrids had similar selection strategies for growth and form traits. A hypothesis that

Table 7 Genetic parameter estimates for the proportion of provenance variation (P^2) and narrow sense heritability (h^2) from univariate analyses of each taxon (single taxa) presented above parameter estimates for all taxa analysed jointly, where P^2 and h^2 estimates are presented along the diagonal (italics) with type-A correlations at the provenance and family level below the diagonal

			Incidence	Severity	Disease rating
P^2	Single taxa	CCC	0·02 ± 0·05	0 ± 0	0 ± 0
		CCV	0·02 ± 0·03	0·03 ± 0·04	0·02 ± 0·03
		C. henryi	0 ± 0	0 ± 0	0 ± 0
	All taxa	Incidence	*0·003 ± 0·003*		
		Severity	0·5 ± 0·77	*0·003 ± 0·004*	
		Rating	0·93 ± 0·54	0·78 ± 0·48	*0 ± 0*
h^2	Single taxa	CCC	0·38 ± 0·17	0·45 ± 0·18	0·54 ± 0·19
		CCV	0·18 ± 0·09	0·24 ± 0·11	0·26 ± 0·11
		C. henryi	0·43 ± 0·15	0·46 ± 0·15	0·47 ± 0·15
	All taxa	Incidence	*0·32 ± 0·08*		
		Severity	0·93 ± 0·04	*0·42 ± 0·09*	
		Rating	0·98 ± 0·02	0·98 ± 0·02	*0·43 ± 0·09*

different foliage characteristics between the vegetatively propagated clonal hybrids and the seedling family hybrids may lead to differences in infection could be plausible in this case, even though the clones and the seedlings were acclimated together for 4 weeks prior to inoculation. Various inoculation studies using the same system as that employed in this study have demonstrated an influence of leaf age on infection after artificial inoculation (authors' unpublished data). Given the differences in the propagation history and clear preference of *P. psidii* to infect juvenile foliage, further inoculation studies of vegetatively propagated and seedling hybrids must be undertaken to verify the resistance of the hybrid clones.

The *Corymbia* hybrid families ranged from 33 to 100% resistant to *P. psidii*, with three of the seven hybrid families tested rated as 100% resistant. None of 95 families of the parental taxa had families rated as 100% resistant to the disease. This may indicate that the hybrids have benefited from polygenic or additive gene action controlling resistance to the disease. This needs further investigation, but supports findings by Alves *et al.* (2012) that additive genetic variation from the parental species is an important contributor to rust resistance of eucalypt hybrids.

There was no apparent relationship between climatic conditions at the provenance origin and disease resistance. While the three provenances ranked as most resistant, Mt McEuan, Lockyer and Presho, are from drier inland areas (703, 892 and 675 mm mean annual rainfall (MAR), respectively), the three most susceptible provenances included one higher rainfall provenance, Nerang (1439 mm MAR) and also the two drier inland provenances of Ballon and Mt Garnet (656 and 832 mm MAR), respectively. Woondum (MAR 1536 mm), a provenance used commonly in plantation development in Queensland, showed moderate levels of resistance to rust with only six out of the 11 families tested having a percentage of resistant seedlings >60%.

Woondum shows higher disease tolerance levels to another basidiomycete fungus endemic to Australia, *Quambalaria pitereka* (Lee, 2007). However, similar to the results obtained in this study with *P. psidii*, resistance to *Q. pitereka* can be identified within all provenances (Pegg *et al.*, 2010; Brawner *et al.*, 2011). While it is not clear if resistance to one pathogen implies resistance to the other, there is some evidence that different resistance mechanisms may be at work. Correlations between breeding value predictions for rust resistance from this study and *Quambalaria* breeding values from two other field trials (451D and 451H; Brawner *et al.*, 2011) were not significantly different than zero, implying little to no relationship between resistance mechanisms. Given the biology of these fungi, this is not surprising. *Quambalaria pitereka* only enters via stomata but never grows intercellularly in comparison to *P. psidii,* which penetrates directly through the cuticle, entering the cell and producing haustoria (Xavier *et al.*, 2001; Pegg *et al.*, 2009). Interestingly, Presho provenance, identified as being highly susceptible to *Q. pitereka* (Lee, 2007), shows similar levels of resistance to *P. psidii* as Woondum.

The fact that seed orchard material (CCV and CH), which has been field selected for resistance to *Q. pitereka*, showed similar resistance levels to 'unimproved' families further supports differences in resistance mechanisms for the two pathogens. However, it could also reflect the fact that selection for resistance was based solely on performance in field trials, the result of which may be influenced by variable climatic conditions and non-uniform exposure to inoculum (Pegg *et al.*, 2010).

Previous studies have identified that *Q. pitereka*, a co-evolved pathogen, is quite variable based on DNA sequencing (Pegg *et al.*, 2009) and isolate aggressiveness (Pegg *et al.*, 2010). It therefore must also be considered that the level of natural inoculum and variation in virulence may have influenced field selection, and further screening under controlled conditions is required to compare resistance to both *Q. pitereka* and *P. psidii*. Given this, the level of variation in rust resistance of families from the seed orchard should allow selection of good

growth form and rust resistance for future breeding and deployment. The high rust resistance of the CT and *Corymbia* hybrid families may indicate that CT has higher levels of resistance to the strain of rust in Australia. This needs further study.

There was good correspondence between the two screenings, with significantly different results ($P < 0{\cdot}05$) being obtained for only one of 31 families (11185-115). This finding supports the idea that the artificial inoculation process used to screen seedlings is robust between screenings. While the very high genetic correlation between the two artificial screening procedures demonstrates repeatability in this study, greater certainty that this process will be useful in identifying germplasm that will express resistance requires further optimization, examining influence of seedling age and nutrient status in addition to testing under field conditions.

Comparisons of genetic parameters estimated for each species separately indicate a lower level of genetic control in CCV, which may require a greater screening effort to achieve similar levels of genetic improvement relative to CCC and CH. However, similarities in the ratio of the amount of variation explained by provenance to variation explained by families within provenances indicate resistance is present in most populations of the species examined. This provides guidance to tree improvement programmes so that they may focus on selecting individuals from a wide range of populations rather than focusing on a single population to provide resistance. Studies by Ochieng *et al.* (2010) and Shepherd *et al.* (2008) indicate that there is weak genetic structuring between populations within the northern spotted gums (CCC, CCV and CH), with an isolation-by-distance model occurring where proximal populations were more similar than more distant ones. This weak genetic structuring may be caused by long distance gene flow associated with two important pollinator groups of the spotted gums, fruit bats (e.g. grey headed flying foxes) and nectar feeding birds (Southerton *et al.*, 2004; Bacles *et al.*, 2009). This weak genetic structure and large interpopulation gene flow is probably the reason that resistance genes are found in all populations and may explain why it is better to select for resistance at the family rather than population level.

The heritability of traits indicates that giving individual seedlings a rating provides a better means of discriminating between resistant and susceptible material than using incidence or average severity assessments. Nevertheless, the genetic correlations near to one suggest all three assessment methods are likely to provide similar rankings of families for rust resistance, and the method applied for future resistance screening work could therefore be based upon the ease of use and the speed of assessment. Given the limited range of provenances sampled in this study, it will be essential to screen additional material to verify the repeatability of these genetic parameter estimates.

Based solely upon clear visual differences in the pattern and extent of damage within families and populations, there appear to be multiple resistance mechanisms acting within the spotted gums. For example, obvious differences were noted in the location and type of pustule formation with individuals demonstrating either numerous, small pustules or few very large pustules. Differences within the hypersensitive rating class were also noted (data not presented). These findings may indicate that rust resistance shown here has a complex mode of inheritance regulated by multiple genes rather than a single resistance gene. Alves *et al.* (2012) found that additive (major gene) and non-additive effects were equally as important in rust resistance. The present data may fit this model. While this complicates selection for disease resistance, it means that some individuals may use major resistance alleles or a range of mechanisms to achieve resistance to rust. Further work on the molecular genetics of rust resistance in the genetically diverse plant populations that the rust is currently spreading through is clearly required.

This study demonstrates the feasibility of identifying spotted gum germplasm that is resistant to *P. psidii* using an artificial screening method. However, there is a need to determine if the resistance to the single rust biotype identified in Australia confers resistance to different biotypes present in other countries. In addition to this, including a range of isolates of *P. psidii* collected from different hosts and locations over time in Australia will be important, accompanied by development of a host differential allowing for rapid testing for differences in pathogenicity between different isolates. Genetic parameter estimates indicate that the variation that is available within provenances and families is sufficient to develop rust resistant breeds of spotted gum. Infected individuals were identified within most families, indicating either that a single resistance gene is not typically dominant in the maternal population or that many genes are interacting to provide disease resistance within this genus. Larger scale studies with a greater number of provenances and sources from *Corymbia* seed orchards, as well as families within provenances, should be effective at identifying *P. psidii* resistant selections for breeding populations.

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