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Resistance to quambalaria shoot blight and myrtle rust in *Corymbia calophylla* seedlings

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Abstract

Corymbia calophylla (marri), an endemic keystone tree species in southwest Western Australia, is increasingly impacted by the introduced basidiomycete smut *Quambalaria pitereka*. The basidiomycete rust *Austropuccinia psidii* (myrtle rust), an invasive pathogen recently introduced to Eastern Australia, is expected to spread to the southwest of Western Australia eventually. *Austropuccinia psidii* has similar epidemiology to *Q. pitereka*, and there is concern that *C. calophylla* may be susceptible. Preliminary pathogenicity tests showed significant differences in aggressiveness between twelve *Q. pitereka* isolates, and there was evidence of interactions between isolates and *C. calophylla* provenances. Seedlings from 59 open-pollinated families from 11 provenances covering the natural range of marri were screened for resistance to *Q. pitereka* and *A. psidii* under controlled glasshouse conditions. Resistance of seedlings within provenances to *Q. pitereka* and *A. psidii* differed significantly. There was no significant correlation between resistance to *Q. pitereka* and resistance to *A. psidii*. Seedlings of provenances from wetter regions were more resistant to both pathogens, but the correlation coefficients were insignificant. Seedlings of four families in three provenances (Serpentine, Chidlow, and Kingston) showed 100% resistance to *Q. pitereka*. Narrow-sense heritability estimates were 0.07 for quambalaria shoot blight resistance and 0.34 for myrtle rust resistance. The results indicate the potential to use selected families/individuals resistant to *Q. pitereka* and *A. psidii* for tree improvement programs and adaptive management strategies.

KEYWORDS
Corymbia calophylla, myrtle rust resistance, quambalaria shoot blight resistance

1 | INTRODUCTION

Disease resistance breeding is an effective tool for disease management and improvement programs for coniferous and angiosperm trees growing in plantations, natural forests, or used in amenity plantings (Carson & Carson, 1989; Heimburger, 1962; Sniezko & Koch, 2017). For instance, this approach reduced the damage from blister rust (*Cronartium ribicola*) significantly on white pine and fusiform rust (*Cronartium quercuum*) on southern pines in North America (Sniezko et al., 2014). The breeding program for myrtle rust (*Austropuccinia psidii*) resistance on eucalypts in Brazil has yielded positive results and provided resistant material for use in forests and plantations (Guimarães et al., 2010; Santos et al., 2014). In most cases, the primary selection technique for resistance has

been inoculating seedlings rather than adult trees, reducing time and expense, a method applied to eucalypts (Lee et al., 2015; Pegg et al., 2014; Toda & Kurinobu, 2001), pines (Toda & Kurinobu, 2001; Vivas et al., 2011), acacias (Chi et al., 2019), and melaleucas (Pegg et al., 2018).

Corymbia calophylla (marri) is one of the most distinctive trees in the southwest of Western Australia (SWWA). An endemic, co-dominant with *Eucalyptus diversicolor*, *E. gomphocephala*, *E. marginata*, and *E. patens* in forests, marri is common on roadsides, in remnant urban bushland, and amenity plantings. It has high value as a food source for insects and animals and furniture making. However, since the 1970s, the decline of this species has been of concern, and numerous studies have investigated the causes (Burrows et al., 2010; Croeser et al., 2021; Matusick et al., 2013). The primary causes of the decline are now acknowledged as the diseases caused by the pathogens *Quambalaria* spp. (Paap et al., 2008), *Teratosphaeria* spp. (Taylor et al., 2012), and *Phytophthora* spp. (Paap et al., 2017).

Quambalaria shoot blight (*Quambalaria pitereka*) is a disease resulting in severely damaged seedlings and young trees, poor tree form, and sometimes tree death. It is native to the eastern states of Australia, where its damage has reduced the use of spotted gum as a priority species in the subtropics (Carnegie, 2007; Pegg, Carnegie, et al., 2009). Introduced to Western Australia, *Q. pitereka* is now found on *C. calophylla*, and amenity planted *C. ficifolia* (Paap et al., 2008) and has spread to China, where it occurs in *C. citriodora* and its hybrids (Chen et al., 2017; Zhou et al., 2007). The extent of this pathogen's current and potentially damaging effect on *C. calophylla* necessitates the identification of resistant lines for disease breeding programs to enable reforestation and restoration on severely impacted sites.

Austropuccinia psidii, the causal agent of myrtle rust, is a pathogen native to central and South America, specific to hosts from the *Myrtaceae* (Carnegie & Lidbetter, 2012). Myrtle rust affects new growth, resulting in shoot dieback, stunted growth, and following repeat infections and defoliation, it can cause the death of trees (Carnegie et al., 2016). In recent years, this invasive pathogen has spread to the USA, South Africa, parts of Asia, and the Pacific, affecting many horticultural, agricultural, and ecological species. It was first detected in Australia in 2010 and is now established in various ecosystems along the east coast from southern New South Wales to far north Queensland and west into the Northern Territory and the Tiwi Islands (Westaway, 2016). It has not yet been recorded from southwest WA, where it is expected that numerous plant species belonging to the Myrtaceae will be susceptible. However, there is some debate regarding the potential impact of myrtle rust in Western Australia, as in that state, climatic regions range from unsuitable to highly suitable (Berthon et al., 2018, Kriticos et al., 2013, Narouei-Khandan et al., 2020). This fungus has many common characteristics with *Q. pitereka*. They are primary basidiomycete pathogens, only attacking immature leaves, shoots, flowers, and buds of the Myrtaceae and have similar optimum temperatures for spore growth; for *A. psidii* urediniospores, the optimum temperature is 15–25°C (Kriticos et al., 2013) and 20–30°C for *Q. pitereka* spores (Pegg, Webb, et al., 2009). These similarities suggest that environmental conditions in SWWA are suitable for infection and disease development by *A. psidii*. It is logical to include selection for resistance to this pathogen in any breeding program for a species such as *C. calophylla*, which is likely to be impacted by the myrtle rust disease if (when) *A. psidii* arrives in southwest WA. However, it is only possible to screen for resistance to this pathogen by growing seedlings of Western Australian species in the eastern states and using the pandemic strain of the pathogen from eastern Australia. This pandemic strain or other strains more aggressive to eucalypts could eventually be introduced into WA (Almeida et al., 2021).

This study aimed to identify sources of resistance to quambalaria shoot blight disease and myrtle rust by inoculating seedlings of *C. calophylla* from provenances throughout its geographic range. Since the two pathogens cause similar symptoms in young developing shoots and buds of the Myrtaceae, and both pathogens are obligate biotrophs, a correlation between resistances for the two pathogens was expected. It was hypothesised that the selection for resistance to *Q. pitereka* would simultaneously co-select for resistance to *A. psidii*.

2 | MATERIALS AND METHODS

This study included three experiments. The first tested the pathogenicity of 12 *Q. pitereka* isolates collected in SWWA. The second examined four *Q. pitereka* isolates varying in pathogenicity to four marri provenances. Thirdly, quambalaria shoot blight and myrtle rust screening was conducted to identify potentially resistant *C. calophylla* provenances/families.

2.1 | Experiment 1 The pathogenicity of twelve *Q. pitereka* isolates

The pathogenicity of 12 *Q. pitereka* isolates (ten originating from marri provenances growing in a field trial at Mt Barker and two from different provenances growing in a field at Margaret River) (Ahrens et al., 2019) was tested on *C. calophylla* seedlings. In order to have sufficient seeds from a single seed lot, the *C. calophylla* seedlings used in this experiment were raised from commercial seeds purchased from Nindethana Seed Company (Albany, Western Australia). Seedlings were grown in steam-pasteurized potting mix (Osmocote, Scotts) in 0.5 L free-draining plastic containers and were 3 months old and approximately 20 cm high when inoculated. The method of Pegg et al. (2011) was used to inoculate the seedlings. The spores for each of the twelve *Q. pitereka* isolates were removed from the plates using a fine-haired paintbrush and washed into 100 ml of sterile distilled water (SDW). A concentration of 1×10^6 spores mL^{-1} was used, with two drops of Tween 20 added to each suspension. Using a portable spray gun (Preval Professional DIY), the spore suspension was sprayed onto the seedlings to run off on the adaxial and abaxial leaf surfaces. The control treatment was sprayed with SDW with Tween 20 added. Each spore suspension was sprayed onto a glass slide and checked under a microscope (Olympus BX51) at ten

times magnification to ensure that spores were present. Ten plants from each family were used per isolate. Immediately after inoculation, the seedlings were placed in a completely randomized design inside humid chambers within a temperature-controlled glasshouse to maintain the temperature and humidity conditions after inoculation. Heaters maintained the temperature between 25–28°C in each chamber, and an air humidifier kept humidity above 90%. The seedlings were watered twice daily without wetting the leaves. Seedlings were assessed for disease incidence and severity 18 days after inoculation. The disease incidence was assessed as a percentage of the number of seedlings infected. The disease severity was assessed on a 1 to 5 scale; 1 = no symptoms evident and no yellow/white flecking; 2 = presence of hypersensitive reactions (HR) with brown flecks or necrosis; 3 = small pustules, <0.8 mm diameter, 4 = medium-sized pustules, 0.8–1.6 mm diameter; 5 = large pustules, >1.6 mm diameter.

2.2 | Experiment 2 The pathogenicity of four isolates of *Q. pitereka* on seedlings of four *C. calophylla* provenances

Four isolates of *Q. pitereka*, two highly pathogenic (A129, A149), one moderately (A176), and one non-pathogenic (A122), were selected from experiment 1 and used for pathogenicity screening on seedlings of four *C. calophylla* provenances. The *C. calophylla* provenances used included two scored as resistant (Carey and Plantagenet) and two as susceptible (Chidlow and Peel Inlet) to *Q. pitereka* from the provenance trials at Margaret River and Mt Barker based on field data collected in January 2018 on four-year-old trees (Duong et al., 2022). New seed collections were made from the original provenance locations for this experiment, and each included a mix of approximately ten trees. Seeds were germinated and grown in a glasshouse, and at 3 months old, seedlings were inoculated and maintained as described above. Ten seedlings were used per isolate per provenance. The disease severity was measured by Image Analysis software for disease quantification Assess 2.0 (APS, America), which measures disease severity as a percentage of the total leaf area infected (Bade & Carmona, 2011).

2.3 | Experiment 3 Screening for resistance to quambalaria shoot blight and myrtle rust

2.3.1 | Plant materials

Seeds of 59 families of *C. calophylla* from 11 provenances were collected from natural stands across the SWWA (Table 1). As myrtle rust does not yet occur in WA, the seedling screening was conducted in Queensland. The seeds were sown in 70 ml free-draining polyurethane pots in a glasshouse at Grafton Nursery (Forest Corporation of NSW) using a potting medium consisting of 75% coarse perlite and 25% pine bark fines (0–10 mm) and a mix of 12–14 month slow release Osmocote (N17.9: P0.8: K7.3) fertilizer at a rate of 4 kg m^{-3}, gypsum (1 kg m^{-3}), Micromax (1 kg m^{-3}) and a granular wetting agent Hydroflo 2 (1 kg m^{-3}). Trays of seedlings from different families were randomized in the glasshouse. Seedlings were overhead irrigated for 10 min twice a day. Three-month-old seedlings were transferred to a shade house in Queensland at the Department of Agriculture and Fisheries (DAF) for the screening experiments.

2.3.2 | Experimental design

The experiment comprised two completely randomized trials; one was inoculated with *Q. pitereka* spores and the other with *A. psidii* urediniospores. Each trial contained an equal number of seedlings (n = 1180) belonging to 59 families and 11 different provenances. Initially, 20 seedlings per family were inoculated with each pathogen. However, variable numbers of seedlings within families were

TABLE 1 *Corymbia calophylla* provenances and numbers of families screened for resistance to quambalaria shoot blight disease and myrtle rust

Provenances	Prov	No of families	Latitude	Longitude	Precipitation mm	Max temp°C	1/aridity index
Plantagenet	PLA	6	−34.653	117.499	733	26.7	1.59
Boorara	BOO	5	−34.639	116.124	1159	25.6	0.95
Carey	CAR	6	−34.420	115.821	1106	25.9	1.02
Kingston	KIN	5	−34.081	116.330	820	27.7	1.49
Bramley	BRA	5	−33.916	115.083	1072	26.1	1.04
Peel Inlet	PEE	6	−32.685	115.743	885	30.4	1.49
Lupton	LUP	5	−32.521	116.499	635	31.6	2.22
Serpentine	SER	5	−32.353	116.076	1173	30.5	1.12
Chidlow	CHI	6	−31.868	116.223	900	32.2	1.54
Mogumber	MOG	5	−31.099	116.051	579	33.3	2.56
Hill River	HIL	5	−30.311	115.202	563	31.7	2.56

Geographic and climatic information for each provenance is shown

not in a susceptible growth phase at the treatment time (i.e. they were not actively growing and had no young leaves or buds). None of these plants developed disease symptoms and were removed from the data set, together with any that died from drought or factors other than the experimental treatment. After this adjustment, >15 seedlings were assessed for most families, but only three could be assessed for family CH3 inoculated with *Q. pitereka*.

2.3.3 | Inoculum production and inoculation

The *Q. pitereka* isolate A149 selected from the preliminary pathogenicity test was used in the inoculation experiment. This isolate was cultured on half-strength potato dextrose agar (½ PDA) at 25°C in the dark for 14 days, then spores were removed from the plates using a fine paintbrush and washed into 100 ml sterile distilled water (SDW). A concentration of 1×10^6 spores mL^{-1} SDW with two drops of Tween 20 per 100 ml suspension (Pegg et al., 2011) was used to inoculate the first subset of 1180 seedlings.

Urediniospores of *A. psidii* were collected from a *Syzygium jambos* growing in Chapel Hill, a suburb of Brisbane, Queensland, Australia. Urediniospores were placed in a desiccator for 7 days before storing in Nunc tubes (Thermo Scientific™) at −80°C until required. For inoculation, urediniospores were removed from −80°C storage and allowed to warm to room temperature, then added to SDW with two drops of Tween 20 per 100 ml and stirred to reduce clumping. Urediniospore counts were then conducted using a haemocytometer, and the suspension was adjusted to a concentration of 1×10^5 spores ml^{-1} for use for inoculations of the second subset of 1180 seedlings.

Seedling trays were placed into plastic trays (L-350 × W-295 × Ht-58 mm), and the plants were inoculated with the spore suspension using a fine mist spray (2.9 kP pressure) generated by a compressor-driven spray gun (Iwata Studio series 1/6 hp, gravity spray gun RG3). The adaxial and abaxial leaf surfaces of the seedlings were sprayed until runoff. Twenty seedlings of *C. calophylla* were sprayed with SDW with Tween 20 but with no spores as a negative control, and 20 seedlings of *Melaleuca quinquenervia*, which is highly susceptible to myrtle rust (Carnegie, 2015), were inoculated with the urediniospores of *A. psidii* suspension as a positive control. Immediately after inoculation, hot water (60°C) was poured into trays inside the cabinet to ensure a high humidity level, and seedlings were then covered with plastic sheeting to maintain high humidity. Plants were placed in the dark in a controlled environment room maintained at 18–20°C and 85% RH and kept in this state for 24 h. The plastic was then removed, and plants were transferred to a shade house and watered twice a day for 10 min using an overhead irrigation system.

2.3.4 | Quambalaria shoot blight and myrtle rust assessments

Quambalaria shoot blight and myrtle rust symptoms are similar and include lesions on actively growing leaves and shoots, leading to deformed leaves, heavy leaf loss, stunted growth, and sometimes plant death. The only difference is the colour of the spores on the lesions; these are white powdery masses for quambalaria shoot blight and yellow or orange-yellow for myrtle rust. Therefore, the same disease assessment method was applied to assess the severity of the disease for both pathogens (Junghans et al., 2003). Accordingly, seedlings were assessed 14 days after inoculation for disease severity on new shoots, and expanding leaves were assessed on a 1 to 5 scale as given above for the preliminary trial. Ratings 1–2 indicate resistance, while 3–5 indicate susceptibility.

2.3.5 | Data analysis

All data were checked for normal distribution and homogeneity of variance. Since the criteria of ANOVA were not met, the non-parametric Kruskal-Wallis was used to analyse the variation of variables, followed by the pairwise test for multiple comparisons of mean rank sums (Dunn's-Test) in R (R core team, 2021). The aligned rank transform (ARTool) was used to analyse the variance of isolate, provenance, and interaction between isolate and provenance; and compared within each factor. Pearson's correlation was used to examine the relationship between the resistance to different pathogens and the resistance to each pathogen and environmental factors. Figures 2 and 3 were created in ggplot2 in R.

Heritability for resistance (h^2) was estimated for *Q. pitereka* and *A. psidii* on *C. calophylla*. The variance components for estimating narrow-sense heritability were obtained from restricted maximum likelihood (REML) mixed-model analyses implemented with ASReml 4.1. The narrow-sense open-pollinated heritability was approximated as the ratio of additive genetic variance to the within-provenance phenotypic variance.

$$h^2_{op} = \frac{\sigma 2a}{\sigma^2 a + \sigma 2e}$$

where σ^2_a is the additive genetic variance within the provenance and σ^2_e is the residual variance.

3 | RESULTS

3.1 | The pathogenicity of twelve *Q. pitereka* isolates

The *C. calophylla* seedlings developed symptoms after 2 weeks. Symptoms were characteristic of *Q. pitereka* with white spore masses in leaf spots and, in some cases, lesions and spore masses on young stems and petioles. *Quambalaria pitereka* was successfully re-isolated from symptomatic leaves. There was a broad ($p < .05$) variation in pathogenicity between the twelve isolates. Three isolates caused no disease symptoms, three isolates caused mild symptoms, and six isolates caused severe symptoms to the seedlings. Isolates collected from Margaret River and Mt Barker showed a wide range of pathogenicity (Figure 1).

3.2 | The pathogenicity of four isolates of *Q. pitereka* on seedlings of four *C. calophylla* provenances

Significant differences in disease severity were identified between the *C. calophylla* seedlings of different provenances when inoculated with four *Q. pitereka* isolates (Table 2). The disease severity was measured by Image Analysis software for disease quantification Assess 2.0 (APS, America), which measures disease severity as a percentage of the infected leaf area and the total leaf area. Disease severity was highest when seedlings were inoculated with isolates A129 and A149, while A176 caused far less severe damage, and as mentioned above, A122 caused no disease symptoms. The interaction based on disease severity between isolates and provenances was significant ($p = 0.0028$), with Chidlow being the least affected overall, followed by Carey, Peel, and Plantagenet being the most affected. No seedlings of any provenance were completely resistant.

3.3 | Resistance of *C. calophylla* seedlings from different provenances and families to *Q. pitereka*

Different provenances of *C. calophylla* showed significant ($p < .05$) differences in shoot and quambalaria shoot blight disease ratings. The range of the mean quambalaria shoot blight disease rating of the provenances was 1.7–2.5, and the percentage of resistant seedlings was 46.9%–82.9%. The three most resistant seed provenances were Serpentine, Bramley, and Chidlow, with resistant percentages of 82.9%, 77.3%, and 76.3%, respectively. The Hill River provenance

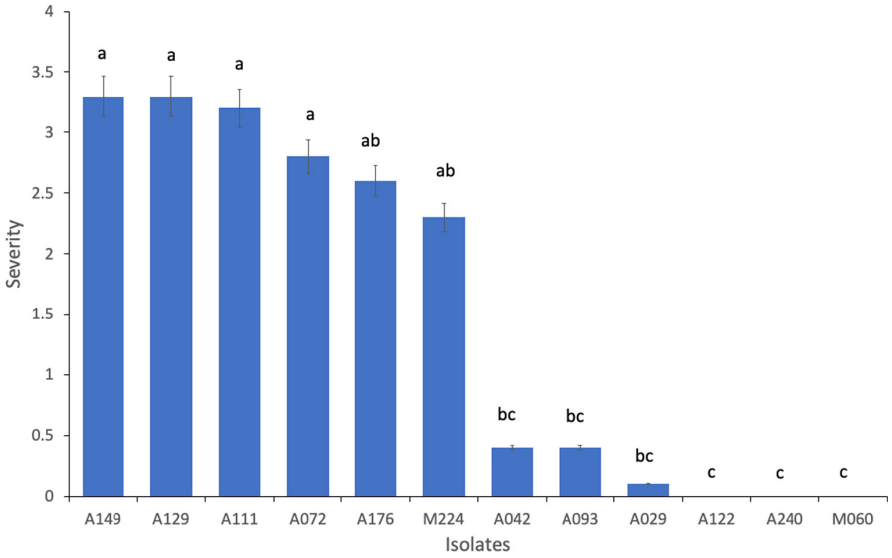

FIGURE 1 Mean disease severity caused by each isolate of *Quambalaria pitereka* on three-month-old *Corymbia calophylla* seedlings. The bars represent the 95% confidence limits. Matching letters designate means that do not differ significantly (Kruskal-Wallis rank-sum test, and pairwise comparisons using Dunn's-test for multiple comparisons $p < .05$)

TABLE 2 Mean percentage disease severity (assessed as % leaf area infected) caused by four *Quambalaria pitereka* isolates on seedlings of four *Corymbia calophylla* provenances and ANOVA table

	Mean disease severity			
	Corymbia calophylla provenances			
***Quambalaria pitereka* isolate**	**Carey[A]**	**Chidlow[B]**	**Peel Inlet[A]**	**Plantagenet[A]**
A122[b]	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
A129[a]	7.4 ±2.0	3.5 ±1.5	9.3 ±3.7	4.9 ±0.8
A149[a]	5.3 ±0.9	1.8 ±1.0	7.3 ±1.7	5.3 ±1.0
A176[b]	2.3 ±1.1	0.0 ±0.0	0.0 ±0.0	0.7 ±0.5
ANOVA				
	d.f	**F**	***p*-value**	
Isolate	3	55.7388	<.001	
Provenance	3	9.9265	<.001	
Isolate * Provenance	9	2.9783	.0028	
Residuals	144			

The disease severity observed in provenances followed by the same upper case superscript was not significantly ($p > .05$) different; similarly the disease severity observed between isolates followed by the same lower case superscript was not significantly different

had the lowest percentage of resistant seedlings, 46.9%, significantly less than the other provenances (Table 3).

There were significant ($p < .05$) differences in the percentage of resistant seedlings between the 59 families in this study. However, except for the Carey provenance, there was no significant difference between families of quambalaria shoot blight ($p > .05$) within a provenance (Table 3). There were four families from provenances in Serpentine (1), Chidlow (2), and Kingston (1), which contained 100% resistant seedlings to *Q. pitereka* (Table 3). However, it is noted that the number of seedlings tested in these families was only 3–10.

3.4 | Resistance of *C. calophylla* seedlings from different provenances and families to *A. psidii*

Resistance to *A. psidii* varied among *C. calophylla* provenances (Table 4), with the mean disease rating of provenances ranging between 1.4–2.7 and the percentage of resistant seedlings from 43.3%–91.9%. Seedlings from the Lupton provenance were the most resistant (91.9%) and were significantly different from the least resistant provenances, Hill River (43.3%), Mogumber (65.3%), Kingston (70.7%), and Chidlow (72.8%) (Table 4).

There were significant ($p < .05$) differences in the percentage of resistant seedlings in families within the provenances from Boorara, Chidlow, Peel Inlet, and Serpentine (Table 4). For the remaining provenances, the differences were not significant.

No seedlings of any families were entirely susceptible to the *A. psidii*, but one from Hill River was 75% susceptible. There were nine families from six provenances (Serpentine (2), Plantagenet (1), Peel Inlet (2), Lupton (2), Chidlow (1), and Carey (1)), in which 100% of seedlings were resistant to myrtle rust disease (with 14–20 seedlings per family assessed). All plants of the susceptible *Melaleuca* species used as a positive control developed severe disease symptoms.

3.5 | Comparison of the resistance to *Q. pitereka* and *A. psidii* in *C. calophylla*

The shoot blight disease caused by *Q. pitereka* was significantly ($p < .05$) more severe than myrtle rust disease caused by *A. psidii* (Table 5). The mean disease rating was similar, but 64.2% of seedlings were rated as resistant to *Q. pitereka*, while 75.6 were resistant to *A. psidii*.

There was little correlation between the severity of quambalaria shoot blight and myrtle rust in the same families; the correlation coefficient was not statistically significant ($r = 0.53$, $p > 0.05$). Despite the non-statistically significant correlation between quambalaria shoot blight resistance and myrtle rust resistance, seedlings of two provenances from the cool, wet area (Bramley and Serpentine) were highly resistant to both pathogens, and seedlings of two provenances from the dry, warm area (Mogumber and Hill River) were the most susceptible to both pathogens. Also, 100% of seedlings from families SP9 and CH4 were resistant to both pathogens.

3.6 | Heritability of resistance to *Q. pitereka* and *A. psidii*

The narrow-sense heritability for quambalaria shoot blight resistance of *C. calophylla* was 0.07 ± 0.05, while the heritability for myrtle rust resistance was 0.34 ± 0.08.

3.7 | Correlations between disease resistance and environmental factors

The correlation coefficients between the quambalaria shoot blight-resistant percentage with environmental factors were not statistically significant ($p > 0.05$). However, trends suggested a moderate positive correlation with 1/aridity index and precipitation (correlation coefficients were 0.42 and 0.52, respectively), but there was no correlation with maximum temperature (−0.08) (Figure 2). The correlation coefficients between the percentage myrtle rust resistance with the 1/aridity index and precipitation were also moderate and positive (0.48 and 0.47), and a slight negative correlation with temperature was observed (− 0.39) (Figure 3).

4 | DISCUSSION

This study estimated the heritability of resistance to the introduced pathogen *Q. pitereka* among the marri provenances across the species' natural range. Narrow sense heritability in the seedlings ($h^2 = 0.07 \pm 0.05$) was similar to that recorded for two-year-old trees of the same provenances growing in a common garden field trial in Margaret River ($h^2 = 0.08 \pm 0.03$) but lower than that recorded for the same provenances growing at Mount Barker ($h^2 = 0.19 \pm 0.04$) in the SWWA (Ahrens et al., 2019). The difference is possible because the trees were taller at Mount Barker (Ahrens et al., 2019); Mount Barker is a drier site than Margaret River and possibly had a different infection load. These values for heritability in seedlings are similar to those reported for the heritability of damage by *Q. pitereka* on *C. citriodora variegata* seedlings (0.08 ± 0.10) (Freeman et al., 2019). In the latter study and others on this pathogen-host combination, much higher heritabilities have been recorded for mature trees (0.31 ± 0.34 to 0.42 ± 0.06) (Brawner et al., 2011; Freeman et al., 2019; Johnson et al., 2009). Marri seedlings originating from provenances in wetter regions were less susceptible to *Q. coyrecup* (an endemic pathogen) than those from drier regions (Hossain, 2020). Although the correlations between resistance to *Q. pitereka* and climate at provenance origin were not significant, there was a trend for higher resistance in seedlings of provenances from wetter regions in the current study and a significant correlation for two-year-old trees in field trials in Mount Barker and Margaret River (Ahrens et al., 2019).

Resistance to *A. psidii*, a pathogen that has not yet reached WA, was high (with a narrow-sense heritability of 0.34 ± 0.08), and there was a higher percentage overall of resistant seedlings (76%) compared with those resistant to *Q. pitereka* (64%). This result

TABLE 3 Resistance of seedlings of 11 *Corymbia calophylla* provenances (bold) and nested families to *Q. pitereka*

Provenances/ families	Mean disease rating	Number of plants/disease severity score[a]					Percentage of resistant seedlings[b]
		1	2	3	4	5	
Serpentine	1.7 ±0.11	23	6	3	3	0	82.9 ^{A}
SP9	1.1 ±0.11	8	1	0	0	0	100.0 ^{a}
SP3	1.4 ±0.38	7	0	0	1	0	87.5 ^{a}
SP7	1.4 ±0.40	4	0	1	0	0	80.0 ^{a}
SP4	2.2 ±0.30	4	5	2	2	0	69.2 ^{a}
Chidlow	**1.8 ±0.09**	**40**	**18**	**14**	**2**	**1**	**77.3** ^{A}
CH3	1.3 ±0.33	2	1	0	0	0	100.0 ^{a}
CH4	1.2 ±0.17	5	1	0	0	0	100.0 ^{a}
CH1	1.7 ±0.19	8	6	3	0	0	82.4 ^{a}
CH6	2.0 ±0.30	3	5	1	1	0	80.0 ^{a}
CH5	1.9 ±0.25	9	5	4	0	1	73.7 ^{a}
CH8	1.8 ±0.20	13	0	6	1	0	65.0 ^{a}
Bramley	**1.7 ±0.09**	**46**	**12**	**12**	**5**	**1**	**76.3** ^{A}
BRA9	1.5 ±0.18	13	5	1	1	0	90.0 ^{a}
BRA2	1.7 ±0.24	4	4	1	0	0	88.9 ^{a}
BRA3	1.6 ±0.26	12	0	4	1	0	70.6 ^{a}
BRA5	1.9 ±0.27	10	3	3	3	0	68.4 ^{a}
BRA1	1.9 ±0.41	7	0	3	0	1	63.6 ^{a}
Kingston	**1.9 ±0.11**	**38**	**14**	**13**	**6**	**1**	**72.2** ^{AB}
K13	1.4 ±0.16	6	4	0	0	0	100.0 ^{a}
K8	1.5 ±0.34	4	1	1	0	0	83.3 ^{a}
K10	1.9 ±0.27	8	4	2	2	0	75.0 ^{a}
K12	1.9 ±0.24	11	3	4	2	0	70.0 ^{a}
K7	2.2 ±0.29	9	2	6	2	1	55.0 ^{a}
Boorara	**2.0 ±0.11**	**35**	**20**	**15**	**8**	**2**	**68.8** ^{AB}
BOO6	1.3 ±0.33	8	0	0	1	0	88.9 ^{a}
BOO1	1.9 ±0.24	10	5	4	0	1	75.0 ^{a}
BOO2	2.0 ±0.29	7	3	3	2	0	66.7 ^{a}
BOO4	2.2 ±0.23	4	6	5	1	0	62.5 ^{a}
BOO3	2.4 ±0.28	6	6	3	4	1	60.0. ^{a}
Lupton	**1.8 ±0.11**	**51**	**9**	**24**	**5**	**1**	**66.7** ^{AB}
LU22	1.6 ±0.24	6	3	2	0	0	81.8 ^{a}
LU17	1.5 ±0.24	16	0	2	2	0	80.0 ^{a}
LU20	1.8 ±0.22	12	1	7	0	0	65.0 ^{a}
LU18	1.9 ±0.22	10	1	8	0	0	57.9 ^{a}
LU21	2.4 ±0.28	7	4	5	3	1	55.0 ^{a}
Plantagenet	**2.2 ±0.12**	**44**	**24**	**25**	**9**	**6**	**63.0** ^{AB}
PLA8	1.7 ±0.20	10	6	2	1	0	84.2 ^{a}
PLA2	1.9 ±0.26	8	3	4	1	0	68.8 ^{a}
PLA7	2.2 ±0.29	6	6	3	2	1	66.7 ^{a}
PLA4	2.1 ±0.25	7	4	5	2	0	61.1 ^{a}
PLA1	2.5 ±0.35	7	3	4	2	3	52.6 ^{a}
PLA6	2.5 ±0.32	6	2	7	1	2	44.4 ^{a}
Peel Inlet	**2.0 ±0.10**	**55**	**14**	**29**	**14**	**0**	**61.6** ^{AB}

(Continues)

TABLE 3 (Continued)

Provenances/ families	Mean disease rating	Number of plants/disease severity score[a]					Percentage of resistant seedlings[b]
		1	2	3	4	5	
PEE3	1.9 ±0.24	8	4	4	1	0	70.6 [a]
PEE1	1.8 ±0.26	12	1	4	2	0	68.4 [a]
PEE7	1.7 ±0.25	12	0	5	1	0	66.7 [a]
PEE8	2.1 ±0.22	8	4	7	1	0	60.0[a]
PEE4	2.1 ±0.30	11	0	4	4	0	57.9 [a]
PEE5	2.6 ±0.26	4	5	5	5	0	47.4 [a]
Mogumber	**2.0 ±0.10**	**48**	**12**	**26**	**12**	**0**	**61.2 [AB]**
MG19	1.6 ±0.18	13	3	4	0	0	80.0 [a]
MG14	2.1 ±0.28	10	3	3	4	0	65.0 [a]
MG13	2.2 ±0.25	8	4	5	3	0	60.0 [a]
MG18	2.2 ±0.27	8	2	6	3	0	52.6 [a]
MG16	2.2 ±0.27	9	0	8	2	0	47.4 [a]
Carey	**2.3 ±0.11**	**40**	**16**	**25**	**18**	**3**	**54.9 [AB]**
CAR5	1.6 ±0.22	11	4	2	1	0	83.3 [a]
CAR6	1.6 ±0.29	6	1	2	0	0	77.8 [ab]
CAR1	2.3 ±0.31	6	4	4	2	1	58.8 [ab]
CAR3	2.5 ±0.31	7	2	3	7	0	47.4 [ab]
CAR7	2.6 ±0.28	5	3	7	3	1	42.1 [ab]
CAR4	2.8 ±0.28	5	2	7	5	1	35.0 [b]
Hill River	**2.5 ±0.13**	**34**	**12**	**29**	**17**	**6**	**46.9 [B]**
HR24	2.3 ±0.29	7	4	3	5	0	57.9 [a]
HR27	2.2 ±0.26	8	3	6	3	0	55.0 [a]
HR19	2.6 ±0.33	8	1	3	7	1	45.0 [a]
HR18	2.6 ±0.28	5	3	9	1	2	40.0 [a]
HR25	2.7 ±0.32	6	1	8	1	3	36.8 [a]

The percentage of resistant seedlings is not significantly ($p > .05$) different between provenances followed by the same uppercase superscripts, or between families within each provenance followed by the same lowercase superscripts

[a]Disease severity score of 1 = highly resistant and 5 = highly susceptible.

[b]Plants with disease severity scores of 1 or 2 are classed as resistant.

suggests that *Q. pitereka* may be a more significant pathogen than *A. psidii*, at least for the pandemic biotype of *A. psidii* currently in the eastern states of Australia. Highly significant differences among eucalypt provenances in susceptibility to *A. psidii* have been reported in many other *Eucalyptus* and *Corymbia* species (Freeman et al., 2019; Pegg et al., 2014; Yong et al., 2019) as well as for other species of the Australian Myrtaceae (Morin et al., 2012). The narrow-sense heritability of resistance to myrtle rust in *Corymbia citriodora* was 0.32 to 0.43 (Pegg et al., 2014), and for seedlings, 0.59–0.63 (Freeman et al., 2019), similar to the heritability in the present study on marri.

In marri, the resistance to *A. psidii* showed a non-significant correlation with aridity index, precipitation of provenance origin, and maximum temperature. Similarly, the study in the eastern states of Australia showed no correlation between climatic conditions at the provenance origin of *C. citriodora* and myrtle rust resistance (Pegg et al., 2014). *A. psidii* has not yet reached the southwest of WA, and *Q. pitereka* arrived 20–25 years ago, so it is unlikely that the slight correlations of seedling resistance for either of these pathogens with climatic variables are a result of pathogen-driven genetic divergence of the long-lived host populations.

Although no individual seedling was tested for resistance to both pathogens, a comparison of correlation coefficients between the resistance of seedlings within families to *Q. pitereka* and *A. psidii* was not statistically significant. The lack of correlation means the hypothesis that the similarities between the pathogens would mean that selection for resistance to one pathogen might also identify individuals resistant to the other, was disproven. Similarly, Freeman et al. (2019) reported no genetic correlation between resistance in *C. citriodora* to *A. psidii* and *Q. pitereka*.

Specific resistance is usually absent or rare when a host and pathogen do not share an evolutionary history (Tobias et al., 2016). However, there are exceptions, including genetic variation for resistance to *A. psidii* in *E. cloeziana* and *C. citriodora* in eastern Australia

TABLE 4 Resistance of seedlings of 11 *Corymbia calophylla* provenances (bold) and nested families to *A. psidii*

Provenances/ families	Mean disease rating	Number of plants/disease severity score[a]					Percentage of resistant seedlings[b]
		1	2	3	4	5	
Lupton	**1.4 ± 0.08**	**72**	**19**	**5**	**2**	**1**	**91.9^{A}**
LU17	1.2 ± 0.09	16	4	0	0	0	100.0^{a}
LU20	1.3 ± 0.10	15	5	0	0	0	100.0^{a}
LU21	1.3 ± 0.13	15	3	1	0	0	94.7^{a}
LU18	1.5 ± 0.24	15	2	2	0	1	85.0^{a}
LU22	1.8 ± 0.23	11	5	2	2	0	80.0^{a}
Bramley	**1.7 ± 0.10**	**51**	**28**	**6**	**4**	**3**	**85.9^{AB}**
BRA9	1.4 ± 0.13	13	6	1	0	0	95.0^{a}
BRA5	1.4 ± 0.22	14	4	0	0	1	94.7^{a}
BRA1	1.6 ± 0.18	8	8	0	1	0	94.1^{a}
BRA2	2.0 ± 0.29	6	7	1	1	1	81.3^{a}
BRA3	2.1 ± 0.29	10	3	4	2	1	65.0^{a}
Carey	**1.6 ± 0.09**	**70**	**25**	**10**	**4**	**3**	**84.8^{AB}**
CAR6	1.4 ± 0.13	10	6	0	0	0	100.0^{a}
CAR4	1.3 ± 0.14	17	1	2	0	0	90.0 ^{a}
CAR1	1.6 ± 0.17	9	6	2	0	0	88.2 ^{a}
CAR5	1.5 ± 0.23	16	1	2	0	1	85.0 ^{a}
CAR3	1.5 ± 0.18	12	4	3	0	0	84.2 ^{a}
CAR7	2.5 ± 0.30	6	7	1	4	2	65.0 ^{a}
Serpentine	**1.7 ± 0.09**	**37**	**20**	**9**	**1**	**1**	**83.8 ^{AB}**
SP3	1.2 ± 0.09	16	4	0	0	0	100.0 ^{a}
SP9	1.1 ± 0.07	14	1	0	0	0	100.0 ^{a}
SP7	1.9 ± 0.41	5	3	1	0	1	80.0 ^{ab}
SP8	2.0 ± 0.32	1	3	1	0	0	80.0 ^{ab}
SP4	2.4 ± 0.17	1	9	7	1	0	55.6 ^{b}
Plantagenet	**1.6 ± 0.09**	**72**	**24**	**14**	**2**	**3**	**83.5 ^{AB}**
PLA4	1.2 ± 0.09	16	3	0	0	0	100.0 ^{a}
PLA6	1.3 ± 0.13	15	4	1	0	0	95.0 ^{a}
PLA2	1.8 ± 0.25	8	7	2	0	1	83.3 ^{a}
PLA7	1.6 ± 0.18	13	3	4	0	0	80.0 ^{a}
PLA8	1.8 ± 0.26	10	4	3	0	1	77.8 ^{a}
PLA1	2.1 ± 0.29	10	3	4	2	1	65.0^{a}
Peel Inlet	**1.9 ± 0.10**	**54**	**35**	**19**	**7**	**3**	**75.4^{AB}**
PEE3	1.7 ± 0.11	7	13	0	0	0	100.0^{a}
PEE4	1.4 ± 0.11	13	7	0	0	0	100.0^{a}
PEE1	1.3 ± 0.13	16	3	1	0	0	95.0^{ab}
PEE8	2.0 ± 0.26	10	3	4	3	0	65.0^{bc}
PEE5	2.3 ± 0.29	7	3	6	2	1	52.6^{c}
PEE7	2.9 ± 0.24	1	6	8	2	2	36.8^{c}
Boorara	**2.0 ± 0.12**	**41**	**26**	**13**	**5**	**5**	**74.4^{AB}**
BOO3	1.5 ± 0.15	10	6	1	0	0	94.1^{a}
BOO2	1.7 ± 0.23	10	5	1	2	0	83.3^{ab}
BOO1	1.8 ± 0.19	8	6	4	0	0	77.8^{ab}
BOO4	2.1 ± 0.30	7	6	3	0	2	72.2^{ab}
BOO6	2.7 ± 0.34	6	3	4	3	3	47.4^{b}

(Continues)

TABLE 4 (Continued)

Provenances/ families	Mean disease rating	Number of plants/disease severity score[a]					Percentage of resistant seedlings[b]
		1	2	3	4	5	
Chidlow	**2.0 ± 0.10**	**41**	**34**	**18**	**7**	**3**	**72.8^{B}**
CH4	1.1 ± 0.07	13	1	0	0	0	100.0^{a}
CH6	1.8 ± 0.23	10	7	2	0	1	85.0^{ab}
CH3	2.0 ± 0.37	5	5	0	1	1	83.3^{ab}
CH1	1.9 ± 0.24	8	6	2	2	0	77.8^{ab}
CH8	2.5 ± 0.23	4	7	5	4	0	55.0^{ab}
CH5	2.6 ± 0.19	1	8	9	0	1	47.4^{b}
Kingston	**2.0 ± 0.13**	**47**	**18**	**13**	**6**	**8**	**70.7^{B}**
K12	1.8 ± 0.28	11	6	0	0	2	89.5^{a}
K8	1.4 ± 0.22	12	2	1	1	0	87.5^{a}
K13	2.1 ± 0.26	8	6	4	1	1	70.0^{a}
K7	2.4 ± 0.34	8	3	4	1	3	57.9^{a}
K10	2.4 ± 0.35	8	1	4	3	2	50.0^{a}
Mogumber	**2.1 ± 0.12**	**39**	**25**	**21**	**8**	**5**	**65.3^{B}**
MG19	1.9 ± 0.22	6	10	2	0	1	84.2^{a}
MG13	1.9 ± 0.28	10	6	2	0	2	80.0^{a}
MG16	2.0 ± 0.27	11	1	7	0	1	60.0^{a}
MG14	2.4 ± 0.32	8	2	4	4	1	52.6^{a}
MG18	2.5 ± 0.24	4	6	6	4	0	50.0^{a}
Hill River	**2.7 ± 0.14**	**30**	**12**	**22**	**23**	**10**	**43.3^{B}**
HR18	2.1 ± 0.34	11	2	1	3	2	68.4^{a}
HR27	2.5 ± 0.33	7	3	3	4	2	52.6^{a}
HR19	2.9 ± 0.31	4	3	6	3	3	36.8^{a}
HR24	3.1 ± 0.32	4	3	3	7	3	35.0^{a}
HR25	2.9 ± 0.29	4	1	9	6	0	25.0^{a}

The percentage of resistant seedlings is not significantly ($p > .05$) different between provenances followed by the same uppercase superscripts, or between families within each provenance followed by the same lowercase superscripts

[a]Disease severity score of 1 = highly resistant and 5 = highly susceptible.

[b]Plants with disease severity scores of 1 or 2 classed as resistant.

TABLE 5 A comparison of disease severity 14 days after inoculation of seedlings of *Corymbia calophylla* with *Quambalaria pitereka* or *Austropucinia psidii*

Pathogen	Mean disease rating	No. of plants in each disease severity class (1:2:3:4:5)	Resistant seedlings* (%)
Q. pitereka	2.0 ± 0.04	445:157:215:99:22	64.2^{a}
A. psidii	1.9 ± 0.03	554:266:149:70:46	75.6 ^{b}

*Different letters indicate significant ($p < .05$) differences.

(Freeman et al., 2019; Lee et al., 2015). In conifers, genetic variation for resistance to the exotic pathogen blister rust in white pine (Kinloch & Dupper, 2002), and cedar root disease (*P. lateralis*) in Port Orford cedar (*Chamaecyparis lawsoniana*) have been reported (Sniezko et al., 2020).

Historical selection due to coevolved pathogens could account for the variation in resistance to *Q. pitereka* in the Australian flora. Marri has coevolved with the pathogen *Q. coyrecup*, and although disease expression in *Q. pitereka* and *Q. coyrecup* is very different, it will be interesting to determine if there is a similarity in provenance/family resistance to these two pathogens. However, the resistance mechanism against *Q. pitereka* is not so general that it is equally effective against *A. psidii*.

The results suggest an opportunity to select for resistance to both pathogens. Four provenances with above 70% resistance to *Q. pitereka* and four families with 100% resistant seedlings. For

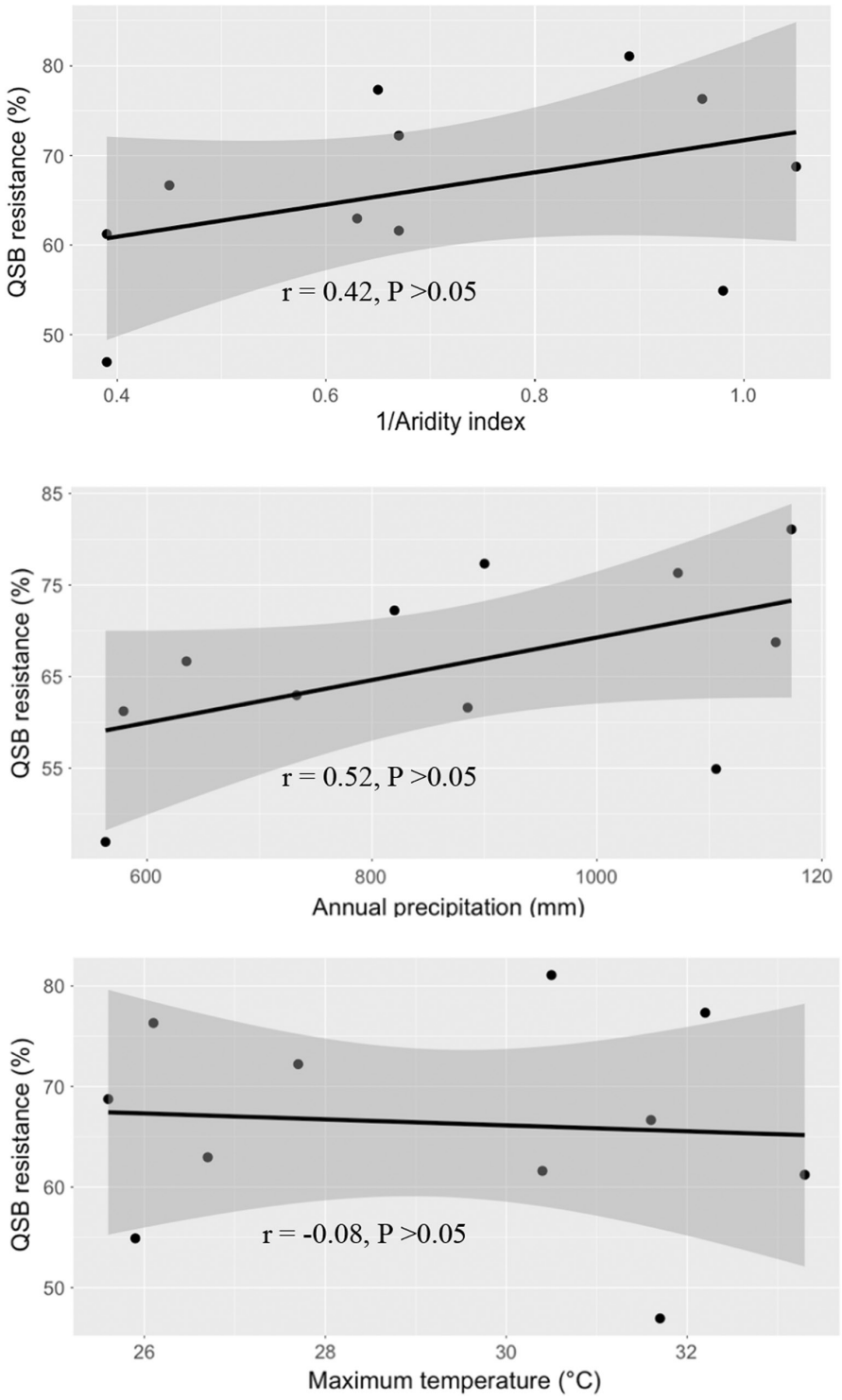

FIGURE 2 Correlation between Quambalararia shoot blight (QSB) resistance with environmental factors at the origin of each provenance, the grey band demonstrates the confidence interval. Aridity index data were from http://www.cgiar.sci.org/

A. psidii, five provenances showed above 80% resistance, and in nine families, all seedlings were resistant. Further investigation of seedlings from these provenances is required. In particular, the expression of disease resistance in adult trees grown from seedlings shown to be resistant is necessary, given the negligible correlation between rankings of resistance in seedlings and two-year-old trees of the same provenance. Resistant families from provenances growing in the most northern locations (such as Serpentine or Chidlow) are likely to be of higher value than those from cooler, wetter regions (such as Bramley and Kingston) as climate change is expected to increase temperatures and aridity in northern areas which are already the most severely impacted by *Q. pitereka*. It is also desirable to assess as many other traits as possible when selecting material for the best-performance seed orchards. Information on resistance to other diseases, particularly *Q. coyrecup*, *Teratosphaeria* spp., *Phytophthora* spp., and insect pests, could be considered. As the resistant trees will be used for natural vegetation rather than plantations or horticulture, a wide range of forms

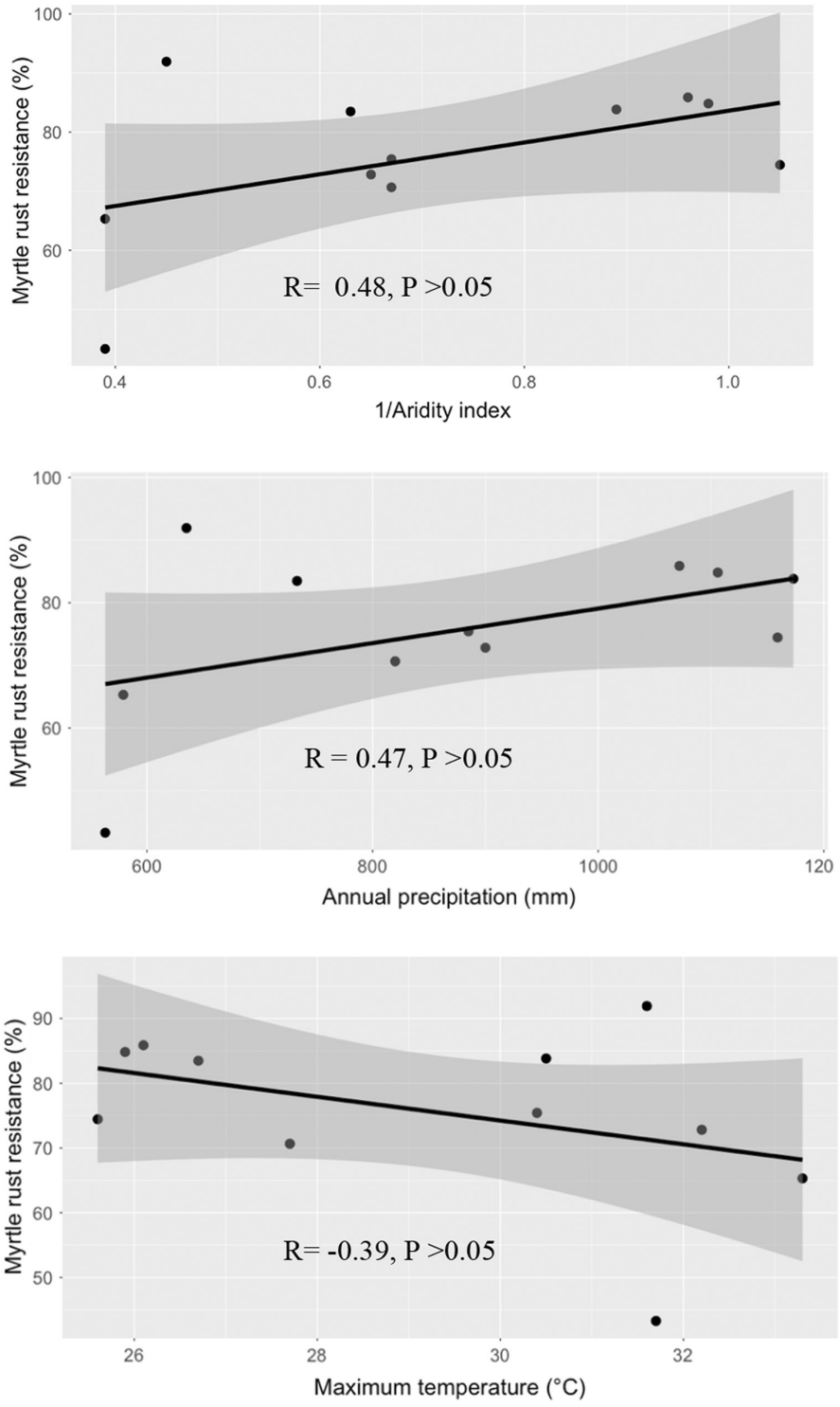

FIGURE 3 Correlation between myrtle rust resistance with environmental factors at the origin of each provenance, the grey band demonstrates the confidence interval

could be acceptable. Cloning of elite genotypes may also be possible to increase the numbers of elite trees in seed orchards. The tissue culture of *C. calophylla* is complex (McComb & Bennett, 1986), but grafting may be an option as it is used commercially to produce flower colour variants of the closely related species *C. ficifolia* for horticultural purposes (Beardsell, 2005).

This investigation of the resistance to *Q. pitereka* and *A. psidii* in marri seedlings from provenances across marri's range showed a level of heritable resistance that would enable opportunities to use selective breeding and adaptive management practices to develop resistant lines of marri to restore areas impacted by *Q. pitereka* and increase resilience to this disease in the face of climate change. In SWWA, the level of resistance of marri to *A. psidii* is already higher than that for *Q. pitereka*. As the potential impact of myrtle rust in Western Australia could be significant, despite the difficulties of resistance breeding against a pathogen not yet present in an area, the selection of lines resistant to both *Q. pitereka* and *A. psidii* is highly desirable.

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DATA AVAILABILITY STATEMENT

Data available on request from the authors

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