Australasian Plant Pathol. (2011) 40:76–86
DOI 10.1007/s13313-010-0016-8

Variable resistance to *Quambalaria pitereka* in spotted gum reveal opportunities for disease screening

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Received: 31 May 2010 / Accepted: 19 October 2010 / Published online: 2 November 2010

Abstract Quambalaria shoot blight, caused by the fungus *Quambalaria pitereka*, is a serious disease affecting the development of spotted gum (*Corymbia citriodora* subsp. *citriodora*, *C. citriodora* subsp. *variegata, C. henryi* and *C. maculata*) plantations in subtropical and tropical Australia. Incorporation of screening for resistance to *Q. pitereka* into current breeding programs is essential for the future development of plantations using spotted gum and *Corymbia* hybrids. The aim of this study was to determine whether there is variability in resistance among and within different species provenances and families of spotted gum to infection by *Q. pitereka*. A secondary aim was to consider whether the origin of seed source is a significant indicator of resistance to *Q. pitereka*. Assessments were conducted in trials consisting of spotted gum provenances, families and clones, all at the same site with high levels of disease pressure and with optimum climatic conditions for disease development. While all species and provenances of spotted gum could be infected by *Q. pitereka*, results showed that there are high levels of variability in resistance between and within species, provenances and families, indicating the potential to select for disease resistance. Provenance was shown to be an unreliable indicator of resistance to *Q. pitereka*.

Keywords Shoot blight · Provenance · Quambalaria

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Introduction

Quambalaria shoot blight, caused by the fungus *Quambalaria pitereka*, is a serious disease affecting the expanding eucalypt plantation estate in subtropical and tropical eastern Australia (Simpson 2000; Self et al. 2002; Carnegie 2007; Pegg et al. 2008). In these plantations the pathogen infects foliage, stems and woody tissue of species of the genera *Corymbia*, *Blakella* and *Angophora* (Walker and Bertus 1971; Bertus and Walker 1974; Simpson 2000, Pegg et al. 2008). Old (1990) described *Q. pitereka* as being endemic to the coastal forests of eastern Australia, where seedlings and young trees of *Corymbia* species can be severely affected.

Since the inception of spotted gum (*Corymbia citriodora* subsp. *citriodora* (hereafter referred to as *C. citriodora*), *C. citriodora* subsp. *variegata* (hereafter referred to as *C. variegata*), *C. henryi* and *C. maculata*) as a plantation species in Queensland and New South Wales (NSW), trials have been established to select individuals for future seed production and as sources of select trees for establishment of grafted seed orchards and breeding populations (Lee 2007; Lee et al. 2009; Smith et al. 2007). The aim has been to provide genetically improved planting stock for pulp-

and solid-wood production as well as information regarding genetic variation for traits such as growth, form, pest and disease resistance and adaptability across a range of sites. Spotted gum tree improvement programs in NSW and Queensland are focused on *C. variegata* and on hybrids between *C. torelliana* and species of spotted gum specifically in Queensland (Lee 2007; Smith et al. 2007; Lee et al. 2009). Many of these trials have included assessments for resistance to *Q. pitereka* (Stone et al. 1998; Self et al. 2002; Dickinson et al. 2004; Smith et al. 2007; Johnson et al. 2009; Lee et al. 2009, Lan et al. 2010).

Identification and incorporation of resistance in spotted gum is important in efforts to effectively control Quambalaria shoot blight. A common strategy used in breeding programs is to identify resistant parents within the centre of origin of the tree. Stone et al. (1998) observed variation in susceptibility of spotted gum to *Q. pitereka* with a provenance originating from Warwick, an inland region of Queensland, suffering two to four times more damage by *Q. pitereka* than a *C. variegata* provenance from a more coastal location near Coffs Harbour in NSW. It was suggested that seed provenance may be an indicator for resistance (Stone et al. 1998). Dickinson et al. (2004) reported on the assessment of 28 different provenances of *C. citriodora*, *C. variegata* and *C. henryi* across 22 sites located in areas with mean average rainfall (MAR) ranging from 690 to 1,320 mm per year. The incidence of *Q. pitereka* increased as the MAR levels increased, with disease severity highest at sites receiving more than 800 mm per year. The *C. variegata* provenance from Woondum, Queensland, was found to have consistently lower susceptibility across all sites and was recommended as the best provenance for plantation development on sites where MAR exceeds 800 mm.

Self et al. (2002) assessed the resistance to *Q. pitereka* in a range of spotted gum provenances including five of *C. variegata*, two of *C. citriodora*, one of *C. henryi* and one of *C. torelliana*. Differences in resistance were noted between the seed provenances, with Yeppoon, Queensland (*C. citriodora*) and Richmond Range, NSW (*C. variegata*) showing higher levels of disease resistance. In this trial, the Woondum provenance showed a relatively high level of susceptibility to *Q. pitereka* and the inland *C. variegata* provenances of Presho and Leyburn, both in Queensland, displayed the highest levels of disease (Self et al. 2002).

Johnson et al. (2009) assessed a spotted gum provenance and progeny trial in NSW for growth, form and resistance to *Q. pitereka*. The trial included a wide range of germplasm, consisting mainly of *C. variegata* provenances and families. They identified four coastal Queensland provenances and one coastal NSW provenance as having a high proportion of families that had low QSB damage, including Home, Wolvi, Woondum (Queensland) and Newry (NSW). Individual families showing high levels of resistance to *Q. pitereka* and superior growth and form were identified for their use in future breeding programs. Families within provenances from the southern boundaries of *C. variegata*, which possibly contain *C. maculata* or intergrades of *C. variegata*, were generally highly susceptible to *Q. pitereka* (Johnson et al. 2009).

Lee et al. (2009) reported results of growth, and genetic parameters, from six trials containing *Corymbia* hybrids and pure species, and Quambalaria shoot blight from two of these trials, from a range of sites across Queensland. They reported that select *Corymbia* hybrid families had better growth than open-pollinated seedlots of the pure species. Unfortunately, there was a low level of disease in the trials and so no quantitative information on the relative resistance of the *Corymbia* hybrids, compared to the parent species, could be obtained. While various authors (Self et al. 2002; Dickinson et al. 2004; Johnson et al. 2009) have identified variability in resistance to *Q. pitereka* within families of spotted gum provenances, the influence of provenance varied. Dickinson et al. (2004) speculated that MAR at provenance of origin was a good indicator of susceptibility to *Q. pitereka*. However, both Self et al. (2002) and Johnson et al. (2009) found no correlation between MAR at origin and susceptibility to *Q. pitereka*. In contrast, Johnson et al. (2009) found a positive correlation between provenance latitude and susceptibility to *Q. pitereka*, with southern provenances being more susceptible.

Due to restricted seed availability, growers need to source seed for commercial plantations from a relatively restricted pool of spotted gum seed provenances. These provenances are selected primarily on growth and availability of seed but also based on knowledge of their relative resistance to *Q. pitereka* gained from field-based selection trials (Self et al. 2002; Dickinson et al. 2004. However, various authors (Stone et al. 1998; Self et al. 2002; Dickinson et al. 2004); Smith et al. 2007; Johnson et al. 2009) have shown that there is a wide variation in resistance to *Q. pitereka* within these selected provenances, which is reflected in the significant variability in QSB damage within current commercial plantations (Carnegie 2007).

The incorporation of screening for resistance to *Q. pitereka* in the current breeding programs is essential for the future development of plantations using spotted gum and *Corymbia* hybrids. Accurate evaluation of previous trials is seriously hampered due to the use of different assessment methods for resistance and the lack of comparative genotypes between trials, as well as a highly variable disease pressure over time and at the different trial localities. Furthermore, variation between and within provenances (Johnson et al. 2009) illustrates that evaluation of performance at the provenance level is an unreliable indicator of disease resistance. Yet, this is all that is currently available for commercial growers.

From a disease resistance breeding perspective, it is important to understand the level of variability in susceptibility to *Q. pitereka*. It is paramount that susceptibility can be assessed reliably and selected through the breeding process. Therefore, the overall objective of this study was to test whether there are differences in resistance between (i) different *Corymbia* species, (ii) provenances of these species, (iii) families within provenances and (iv) clones within families of spotted gum. In addition, we sought to rigorously test whether the origin of the seed source can be used as a predictor for resistance to the disease.

Methods

Resistance to *Q. pitereka* was assessed under conditions optimal for repeat infection and high inoculum levels. Trials were established in close proximity to native spotted gum forests during a period of above average rainfall. Resistance was assessed in absence of growth assessments.

Resistance of spotted gum species and provenances

In order to determine the variability in resistance to *Q. pitereka* of spotted gum species, and provenances, a mixed species and provenance trial was assessed. The site, located at Grafton, northern NSW, owned and developed by Forests NSW, consisted of a replicated mixed planting of provenances of *C. citriodora* (5 provenances), *C. variegata* (24 provenances) and *C. henryi* (2 provenances) (Table 2). The trial, established in 2006, consisted of 20 replicates of five-tree line plots for each seed-lot, planted in an incomplete block design with spacing of 4.0×2.5 m. The trial site was surrounded by high density stands of native *C. henryi*. Prior to establishment of the trial, *Q. pitereka* symptoms were identified on mature trees and seedlings in the understory of the surrounding native spotted gum trees. The first seven replicates of the trial were used in assessment for resistance to *Q. pitereka*.

The trial was assessed 6 and 18 months after planting. Uniformity of *Q. pitereka* infection was determined prior to assessment by comparing disease incidence and severity at 10 m intervals along a transect through the full length of the trial. Uniformity of disease was later confirmed by plotting the disease levels within each replicate and comparing levels across the site (data not shown). At six months of age, disease incidence and severity were assessed, based on the method of Johnson et al. (2009). Incidence (I) was calculated as a percentage of total new shoots and immature, expanding foliage showing visible signs of Quambalaria shoot blight. Severity (S) was calculated as a percentage of total area of infected new shoots and immature, expanding foliage. A Quambalaria Shoot Blight (QSB) score was then calculated {(I x S)/100} where a score of 0 indicated no disease and a score of 100 reflected that all new shoots and immature, expanding foliage was severely infected by *Q. pitereka*.

At 18 months of age, the trial was assessed for impact of *Q. pitereka* based on a tree form rating where 1 = apically dominant, no evidence of stem death and no or limited evidence of *Q. pitereka* symptoms; 2 = double leader as a result of infection by *Q. pitereka*; 3 = multiple leaders as a result of infection by *Q. pitereka*; 4 = shrub like appearance due to infection of all stems by *Q. pitereka*, no current infection obvious; 5 = shrub like appearance due to infection of all stems by *Q. pitereka* with all stems currently heavily infected and 6 = tree killed by *Q. pitereka* (Fig. 1). Samples of infected leaf and shoot material were collected at both assessment times and examined microscopically to confirm that *Q. pitereka* was the causal agent.

Resistance of families within spotted gum provenances

To determine if there was variability in resistance among and within families within provenances of *C. citriodora* and *C. variegata*, the families represented in the mixed provenance trial at Grafton and a *C. citriodora* progeny trial planting were assessed 6 and 18 months after planting. The *C. citriodora* progeny trial, established within the same area as described previously for the mixed *Corymbia* provenance trial, was made up of 20 replicates of five *C. citriodora* provenances with a total of 24 families in single tree plots in a row-column design. Disease assessments were as described for the mixed provenance trial.

Variability in susceptibility of *C. variegata* clones

To determine the level of variability among clones within families of *C. variegata*, a trial consisting of nine *C. variegata* clones originating from three different provenances was assessed at 6 and 18 months. The 2006 trial was established at Grafton, using a 25 tree random block design with four replicates per treatment. These nine clones were derived from 33 clones selected for growth and resistance to *Q. pitereka* in four trials established in NSW in 2003. These in turn were a subset of the original 69 clones from nine selected families from five NSW provenances planted at four sites in NSW (Smith et al. 2007). The original clones had been selected from previous spotted gum provenances and family trials in northern NSW (Smith et al. 2007; Lan et al. 2010). Trees were assessed as previously mentioned.

Data analysis

Normality of data was checked using an equality of variance *F* test. All proportion data were Arcsine square

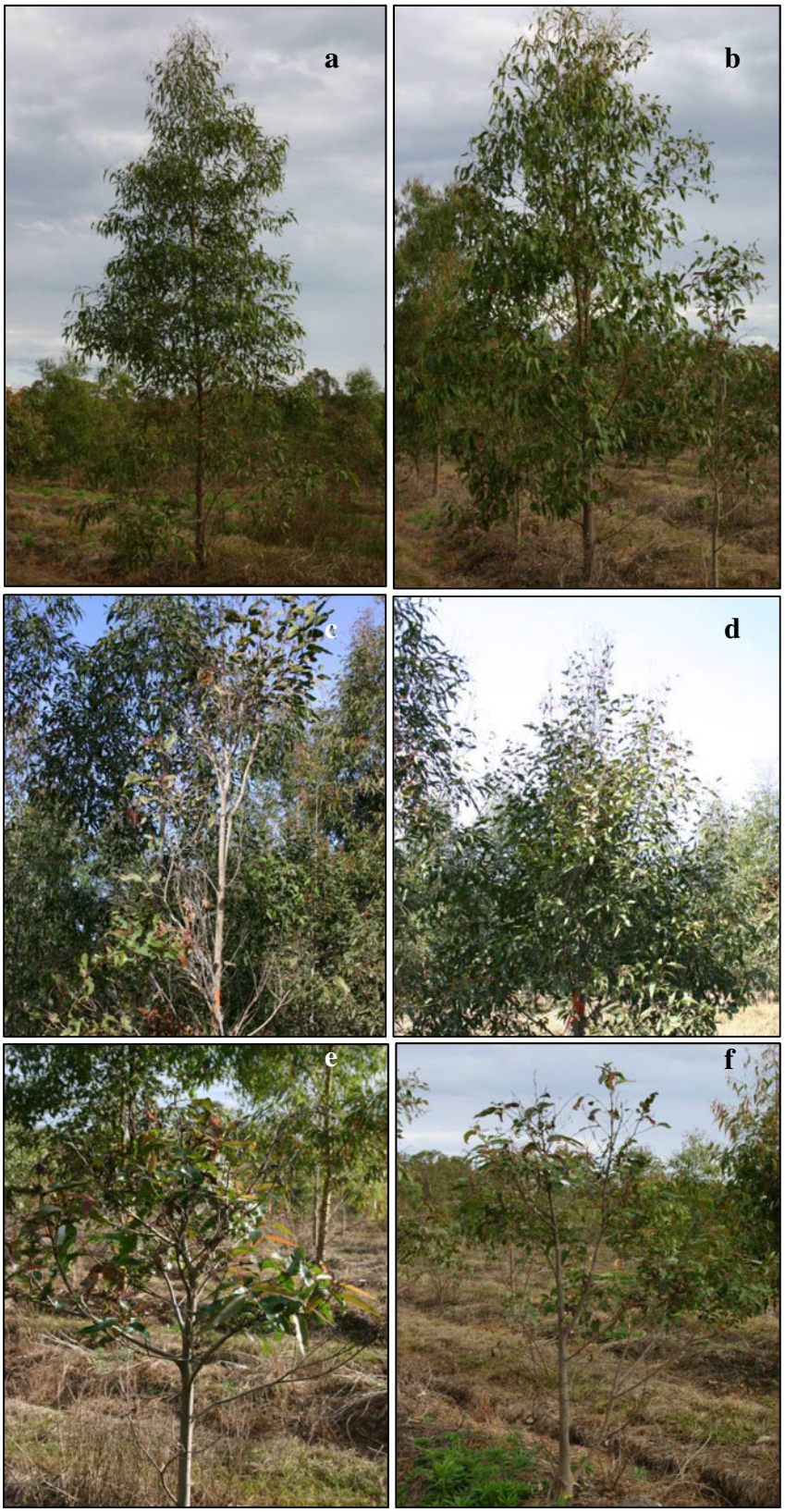

Fig. 1 Quambalaria form ratings based on the impact of *Quambalaria pitereka* **a** Rating 1—apically dominant; **b** Rating 2—double leader; **c** Rating 3—multiple apical leaders; **d** Rating 4—no apical dominance and shrub like growth; **e**, **f** Rating 5—no apical dominance and all new shoots infected or dead

root transformed prior to analysis using ANOVA and compared using Fishers PLD post hoc test (Statview®).

Results

Spotted gum species resistance

Symptoms of infection by *Q. pitereka* were observed on all species of spotted gum. Based on the mean of all provenances assessed, *C. citriodora* was significantly more resistant to *Q. pitereka* than *C. variegata* and *C. henryi*, both 6 and 18 months after planting (Table 1) (One Way ANOVA, QSB Score $F_{2,1467}$=46, P<0.0001; P=0.0003; Form Rating $F_{2,1466}$=101, P<0.0001; P=0.0007). *Corymbia henryi* and *C. variegata* were not significantly different from each other in resistance to the pathogen.

Susceptibility of provenances of spotted gum species

All provenances, based on means of families, showed symptoms of infection by *Q. pitereka*. Of the 31 provenances assessed, only one, Mt Garnet, had a mean QSB score less than 10 (Table 2, Fig. 2). Two other provenances (Barron and Kirrama) had mean QSB scores of less than 30 and another seven (Windsor, Neerdie, Woondum, Monto, and Yeppoon) less than 50. All other provenances had mean QSB scores above 50 and were considered highly susceptible to *Q. pitereka*. Richmond Range, one of the main provenances used in commercial plantation development, had a mean QSB score of 60.1 (Table 2).

Eighteen months after planting, Mt Garnet provenance had a mean QSB form rating of 1.2, Barron 1.5 and Kirrama 1.8 (Table 2). Only two other provenances (Woondum and Windsor) had a mean QSB form rating score of 3 or less. A tree with a rating of 3 or more would be considered undesirable for plantation development with loss of apical dominance and the presence of multiple leaders.

Resistance of families within spotted gum provenances

Resistance to *Q. pitereka* varied among and within families in all spotted gum provenances (Table 2, Fig. 3). Significant differences in susceptibility were observed among numerous families, particularly the *C. variegata* provenances of Neerdie ($F_{16,78}$=4.89; P<0.0001), Woondum ($F_{12,61}$=2.56; P=0.0082), Richmond Range ($F_{16,78}$=1.78; P=0.12), St John D'Aguilar ($F_{47,211}$=2.025; P=0.0004), Lockyer ($F_{17,65}$=1.97; P=0.0265) (Fig. 4, Table 2), Cherry Tree ($F_{19,79}$=2.022; P=0.016) and Paddys Land ($F_{28,92}$=2.087; P=0.0047) (Table 2). Within Woondum and Neerdie provenances, family mean resistance levels, as indicated by the QSB score, ranged from less than 5 to in excess of 80 (Table 2, Fig. 3). Individual trees within families also varied in levels of resistance to *Q. pitereka*.

When assessing the *C. citriodora* progeny trial, significant differences in resistance to *Q. pitereka* were identified between Mt Garnet ($F_{5,\ 103}$=3.96; P=0.0025) and Kirrama ($F_{5,\ 95}$=2.44; P=0.04) families (Fig. 4). There were no significant differences between families from the Barron ($F_{3,\ 63}$=0.33; P=0.81), Yeppoon ($F_{4,\ 86}$= 1.88; P=0.121) or Windsor ($F_{3,\ 53}$=1.266; P=0.29) provenances (Fig. 4). However, in all families, within each provenance examined, individual trees showed differing levels of resistance to *Q. pitereka* (Fig. 4). At 18 months of age, significant differences in resistance based on tree form were only recorded for families from Mt Garnet ($F_{5,\ 103}$=1.88; P=0.053) and Yeppoon ($F_{4,86}$= 3.63; P=0.0088) provenances (Table 3).

Variability in resistance of *C. variegata* clones

Significant differences in resistance among clones of *C. variegata* selected from previous trials located in NSW was identified (QSB Score $F_{8,27}$=12.01; P<0.0001: Quambalaria form rating $F_{8,815}$=82.68; P<0.0001). Clone 6 was significantly more resistant to *Q. pitereka* than all other clones with a mean QSB score of 6.32 and mean QSB form rating of 1.27 (Table 4). Only one other clone, clone 3, had a QSB score of less than 30 and QSB form rating of less than 3 (Table 4). All other clones were moderately to highly susceptible to *Q. pitereka*. No significant differences were found between replicates of the same clone.

Discussion

Results of this study showed that, while all species and provenances of spotted gum can be infected by *Q. pitereka*, there is significant variability in resistance among and within provenances as well as families within provenances. The data clearly indicates that selection for higher levels of resistance is possible. However, due to wide variation within provenances (and within families), selecting for resistance to *Q. pitereka* based solely at the provenance level is likely to give variable results.

Table 1 Susceptibility of spotted gum species, *Corymbia citriodora*, *C. variegata* and *C. henryi*, to *Quambalaria pitereka* (based on provenance means) in field based trials conducted in northern New South Wales

Spotted gum species	Mean QSB score	Mean QSB form rating
C. citriodora	25.2^{a}±2.58	2.3^{a}±0.12
C. variegata	56.1^{b}±1.10	4.1^{b}±0.04
C. henryi	83.3^{b}±16.7	4.5^{b}±0.76

Table 2 Susceptibility of spotted gum provenances to *Quambalaria pitereka* in a mixed provenance trial. Provenances listed from least to most susceptible to *Quambalaria pitereka*

Spotted gum species	Spotted gum provenance	State of Origin	Latitude (Deg Min)	Longitude (Deg Min)	Altitude (MAS)	Mean Annual Rainfall (mm)	Number of families	Number of individuals	Mean (provenance) QSB Score 6 Months	Mean (provenance) QSB Form rating 18 Months	Family Range QSB Scores	Family Range QSB Form Ratings
C. citriodora	Mt Garnet	Qld	18 0	145 11	700	832	6	31	4.4±1.9 ^{a}	1.2±0.1 ^{a}	0–10.3	1–1.8
C. citriodora	Barron SF	Qld	17 15	145 30	830	1,200	6	35	23.2±6.0 ^{b}	1.5±0.2 ^{a}	1.3–33.9	1–2
C. citriodora	Kirrama SF	Qld	18 12	145 46	560	2,000	6	35	25.6±5.7 ^{b}	1.8±0.2 ^{a}	0.9–57.5	1.2–3.5
C. citriodora	Windsor SF	Qld	16 13	144 58	1,160	1,250	6	31	31.9±6.1 ^{b}	2.8±0.2 ^{b}	11.8–50.0	2.4–3
C. variegata	Neerdie	Qld	26 0	152 39	123	1,138	17	99	35.8±4.0 ^{b}	3.2±0.2 ^{b}	1.1–80.4	1.2–4.8
C. variegata	Woondum SF	Qld	26 17	152 17	120	1,600	13	81	36.6 ±4.5 ^{b}	3±0.2 ^{b}	8–87.7	1.5–5.5
C. variegata	Monto SF	Qld	24 49	150 56	475	780	11	64	38 ±4.4 ^{b}	3.3±0.2 ^{b}	19–62.5	1.7–4.4
C. citriodora	Yeppoon	Qld	23 6	150 44	30	1,325	6	32	40.6 ±6.0 ^{bc}	3.9±0.2 ^{c}	23.8–63.7	3.6–4.4
C. variegata	St John D'Aguilar SF	Qld	27 15	152 42	540	1,600	48	264	51.8±2.5 ^{c}	3.9±0.10 ^{c}	1.3–96.4	2–5.8
C. variegata	Dalby	Qld	27 13	150 52	380	660	4	25	52.5±9.1 ^{cd}	4.3±0.3 ^{c}	32.5–100	3.6–5
C. variegata	Esk SF	Qld	27 18	152 18	300	1,020	7	39	53±5.1 ^{cd}	4.3±0.2 ^{c}	27–72.5	3.2–5.2
C. variegata	Lockyer SF	Qld	27 28	152 28	150	840	18	85	54.2±4.1 ^{c}	4.2±0.2 ^{c}	13.5–100	2.8–6
C. variegata	Ingalba SF	NSW	30 53	152 48	115	1,344	2	4	56.2±18.8 ^{cd}	4±0 ^{bc}	31.7–75	4
C. variegata	Neil Range	Qld	25 47	151 28	300	1,200	17	98	57.2±4.2 ^{cd}	4±0.2 ^{bc}	32.3–100	2.2–6
C. variegata	Cherry Tree SF	NSW	28 56	152 45	350	1,104	19	100	58.3±4.3 ^{cd}	4.1±0.2 ^{c}	21.14–100	2.7–5.8
C. variegata	Paddys Land SF	NSW	30 6	150 56	1,100	931	29	127	58.9±3.7 ^{cd}	4.4±0.1 ^{cd}	1.25–100	2.3–6
C. variegata	Richmond Range	NSW	28 38	152 48	350	1,267	9	39	60.1±7.2 ^{cd}	4.2±0.3 ^{cd}	30.1–100	1–5.7
C. variegata	Curra SF	Qld	26 4	152 39	160	1,138	1	7	63.6±13.9 ^{cd}	3.5±0.7 ^{bc}	–	–
C. variegata	Dalmorton SF	NSW	29 49	152 23	690	1,016	1	2	65.6±9.4 ^{cd}	5±1 ^{cd}	–	–
C. henryi	Candole SF	NSW	29 44	153 14	40	1,359	1	3	66.7±33.3 ^{cd}	3.7±1.3 ^{bcd}	–	–
C. variegata	Grange SF	NSW	29 31	152 32	690	1,016	1	4	66.7±22.1 ^{cd}	4.3±1.7 ^{bcd}	–	–
C. variegata	Murphy Range	Qld	25 17	149 11	420	720	9	51	67.8±4.8 ^{d}	4.8±0.12 ^{d}	42.8–89.3	4–5.4
C. variegata	Leyburn SF	Qld	28 2	151 37	500	660	9	40	68.6±6.1 ^{d}	4.5±0.24 ^{cd}	31.5–98.3	3.8–5.4
C. variegata	Chaelundi SF	NSW	29 56	152 23	720	1,027	2	8	68.7±14.4 ^{d}	4.7±0.4 ^{cd}	64.6–71.9	4.3–5.3
C. variegata	Saddlers Springs	Qld	25 6	148 4	700	720	13	63	69.9±4.3 ^{d}	5±0.11 ^{d}	46.3–100	4–6
C. variegata	Kangaroo River SF	NSW	30 5	152 52	370	1,268	2	10	73±14.2 ^{d}	5.1±0.6 ^{d}	67.9–83.3	4.7–6
C. variegata	Barakula SF	Qld	26 16	150 32	300	660	11	62	73.8±4.4 ^{d}	5.1±0.1 ^{d}	43.6–100	4.3–5.8
C. variegata	Mt Hutton	Qld	25 52	148 16	650	660	10	38	78±4.6 ^{d}	5.3±0.1 ^{d}	51.4–91.7	5.0–5.8
C. variegata	Mt Moffat NP	Qld	24 53	147 59	1,000	780	10	48	79.2±4.4 ^{d}	5.2±0.1 ^{d}	59.3–100	4.4–6
C. variegata	Boonanghi SF	NSW	31 3	152 32	405	1,332	1	1	100±0 ^{d}	6±0 ^{cd}	–	–
C. henryi	Braemer SF	NSW	29 3	152 59	55	1,008	2	3	100±0 ^{d}	5.3±0.7 ^{cd}	100	5.3

Qld Queensland; *NSW* New South Wales; *SF* State Forest

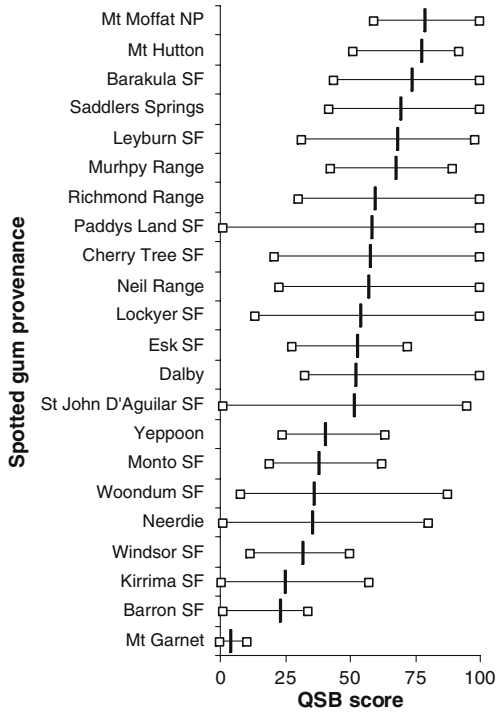

Fig. 2 Variability in susceptibility within provenances (means of families) of spotted gum to *Q. pitereka* showing the range (□ = upper and lower QSB scores) and means (—). The provenances are ranked, based on mean QSB scores, from least susceptible at the bottom to most susceptible at the top. (*SF* State Forest)

When examining results at the species level, *C. citriodora* was shown to have significantly higher levels of resistance to *Q. pitereka* than *C. variegata*, with *C. henryi* being the most susceptible. It is, however, necessary to be cautious when defining one species as being more or less susceptible to *Q. pitereka* using results from a single field site and where species numbers are unevenly represented. In addition, there also is a continuing debate regarding the taxonomic classification of spotted gum species. Recently, Shepherd et al. (2008) suggested that there was insufficient distinction between the northern taxa, including *C. henryi*, *C. variegata* and *C. citriodora*, to justify their treatment as a single species and that they were better viewed as two or three sub-species or races.

The results of this study revealed substantial variation in resistance levels between provenances of spotted gum species. Provenances of *C. citriodora* were identified as being the most resistant to *Q. pitereka*. *Corymbia variegata* provenances Woondum and Richmond Range, both of which have been recommended for commercial plantation development (Self et al. 2002; Dickinson et al. 2004), were rated as moderately and highly susceptible respectively. Differences in observed levels of resistance, have been reported previously. Dickinson et al. (2004) and Johnson et al. (2009) identified Woondum provenance as one of the more resistant to *Q. pitereka* and Richmond Range as being moderately susceptible (Johnson et al. 2009). However, Self et al. (2002) found Richmond Range to be superior and Woondum to be moderately susceptible. One possible reason for this contradiction, in addition to genetic variability present in provenances, is the influence of variation in disease levels and frequency of infection at different sites.

It is evident from this and previous studies (Johnson et al. 2009; Lee et al. 2009) that there are also significant differences between and within families. Disease levels of individual trees within families ranged from very high levels of susceptibility to trees with minor or no evidence of infection. This is also one of the likely sources of variability when comparing provenance resistance levels in different trials. This level of variability in resistance within provenances and families in a pathosystem is not unique to spotted gum and Quambalaria shoot blight. Thus, similarities can be drawn to studies on Loblolly and Slash pine resistance to Fusiform rust (Kuhlman et al. 1995; Kinloch and Stonecypher 1969) and investigations into resistance of families and hybrids to pitch canker (Roux et al. 2007). In both cases, significant differences in resistance were identified between and within provenances and families.

The level of variability observed in this study would suggest that it is risky to try and distinguish their level of resistance to *Q. pitereka* at the provenance level. A single family of seed is derived from a maternal native open pollinated tree. This is an arbitrary process and unlikely to be based on any perceived levels of susceptibility to *Q. pitereka* or the potential disease pressures. The selection of individuals with high resistance to *Q. pitereka* from a range of seed provenances, also recommended by Johnson et al. (2009), would be beneficial in the development of a more effective breeding strategy, particularly if the pathogen population is variable.

When studying relative resistance of loblolly and slash pine to Fusiform rust, Kuhlman et al. (1995) found a significant family x location effect indicating that the relevant performance of families is affected by the variation in virulence of the rust pathogen at different locations. This finding supported the hypothesis that mixes of resistant families would limit Fusiform rust infections more effectively than single family plantings. Interactions between seed source and rust fungal collections were also found to be highly significant when studied by Powers and Matthews (1980). This indicated that seed from several seed sources could be used to reduce the impact of Fusiform rust. This has yet to be determined for *Q. pitereka* and spotted gum.

Our study clearly indicates that determining the source of seed for plantation establishment based on provenance is a poor predictor with regards to the level of resistance to *Q. pitereka*. Previous studies have identified a link with

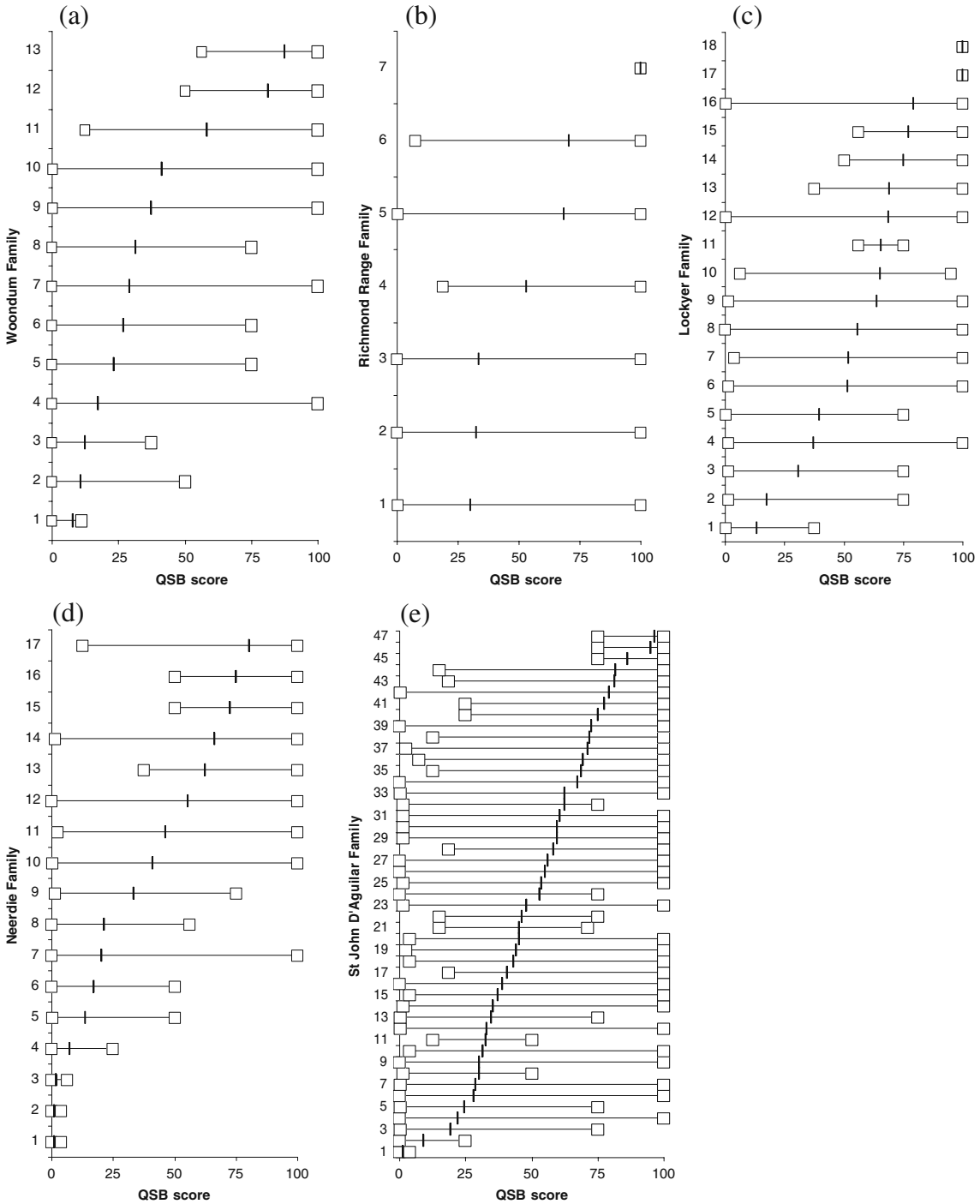

Fig. 3 Variability in susceptibility within families of selected spotted gum provenances to *Q. pitereka* showing the range (□ = upper and lower QSB scores) and means (▬): **a** Woondum provenance, **b** Richmond Range **c** Lockyer **d** Neerdie and **e** St John D'Aguilar. The families are ranked, based on mean QSB scores, from least susceptible at the bottom to most susceptible at the top

provenance resistance and climate or location. Dickinson et al. (2004) suggested a correlation between increasing MAR and increased disease resistance. Johnson et al. (2009) found a positive correlation between provenance latitude and susceptibility to *Q. pitereka*. It is likely that disease levels at origin have a significant influence on host resistance. Goddard et al. (1975) when evaluating progenies of slash pine from high and low Fusiform rust selection pressure areas found that the primary factor affecting genetic gain was the amount of rust in the original population. However, no studies of the spotted gum x *Q. pitereka* system have specifically investigated this.

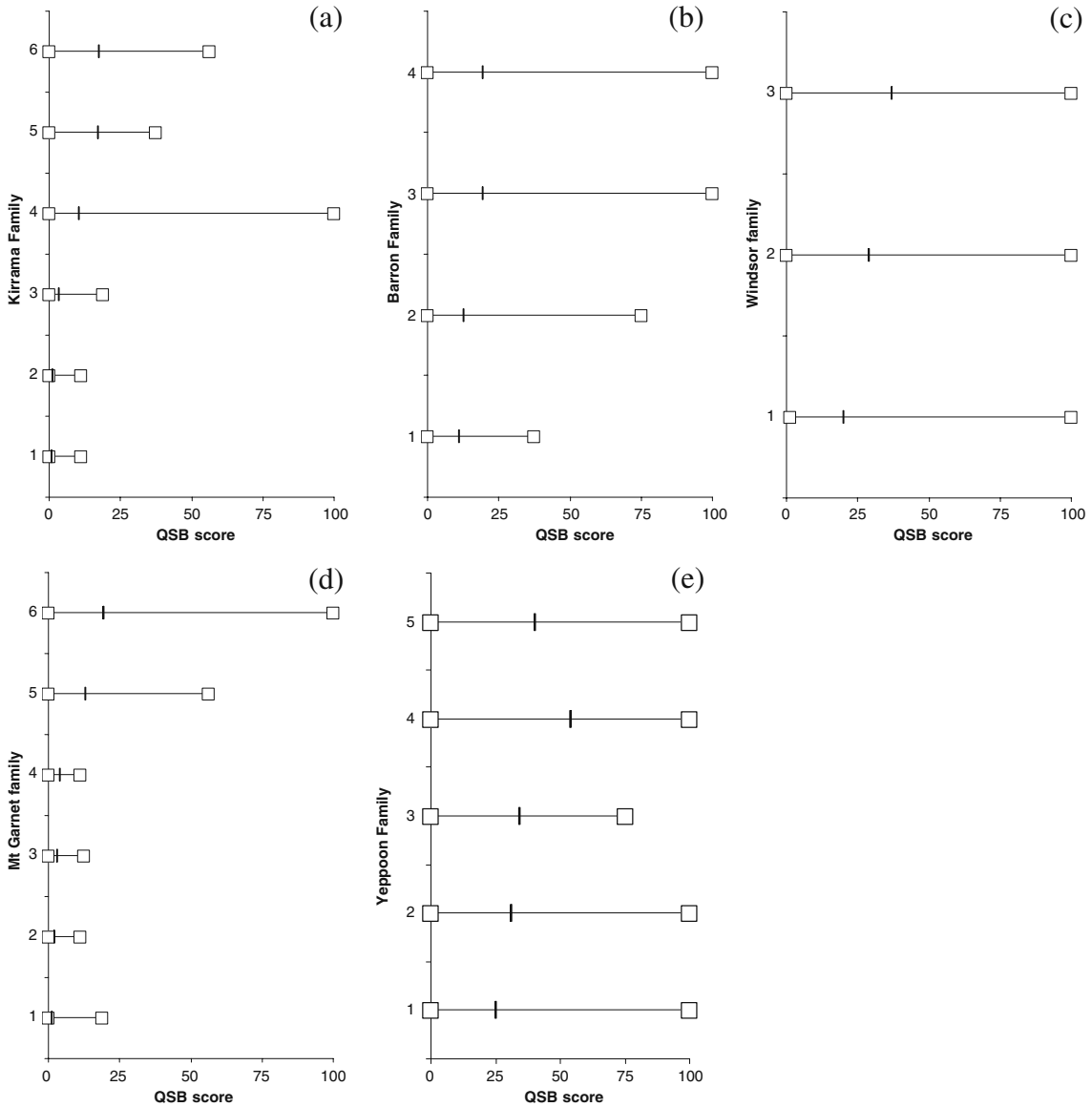

Fig. 4 Variability within families of *Corymbia citriodora* provenances in a progeny trial assessed 6 months after planting for susceptibility to *Quambalaria pitereka* **a** Kirrama, **b** Barron, **c** Windsor, **d** Mt Garnet and **e** Yeppoon. (□ = upper and lower QSB scores; ▬ = mean QSB score)

Nine clones, previously identified from a series of field trials as having high resistance to *Q. pitereka* and superior growth traits (Lan et al. 2010), were assessed as part of our study. However, in our study, only one clone had high levels of disease resistance with three others rated as being highly susceptible. The clones assessed by Lan et al. (2010) were originally selected from trials conducted at four sites in NSW during a period when rainfall levels were recorded as extremely low. As such, only a relatively small proportion of trees had significant disease (Lan et al. 2010). In contrast, rainfall levels at the time of our study were well above average and more conducive to repeat infection and more rapid build up of disease in the plantation, illustrated by the high proportion of trees with significant disease. Our study is therefore likely to more accurately discriminate amongst different disease levels and more accurately identify resistant clones. This raises a number of questions of how to optimise selection for resistance and the risks associated with relying on field assessments alone. Drought is likely to have an adverse effect on the build up of disease and the frequency of infection within spotted gum trial plantings potentially giving inaccurate levels of resistance.

It must also be considered that the level of natural *Q. pitereka* inoculum and variation in virulence may differ from site to site. Kuhlman et al. (1995) found a significant interaction effect of family by location when studying Fusiform rust resistance in loblolly and slash pine. They

Table 3 Family variability within *Corymbia citriodora* provenances in a progeny trial assessed 18 months after planting for susceptibility to *Quambalaria pitereka*

Corymbia citriodora Provenance	Family	Mean QSB Form 18 Months
Kirrama	20007	1.47±0.2a
	20008	1.8±0.3a
	20009	2.05±0.3a
	20010	2.06±0.4a
	20011	1.4±0.2a
	20012	1.89±0.2a
Barron	20002	2.12±0.4a
	20003	1.89±0.3a
	20004	2.05±0.4a
	20005	2.15±0.4a
Windsor	20013	3.2±0.3a
	20016	3.8±0.3a
	20018	3.06±0.4a
Mt Garnet	20019	1.44±0.2ab
	20020	1.05±0.1ab
	20021	1.81±0.3a
	20022	1.55±0.3ab
	20023	2.00±0.3a
	20024	2.06±0.3a
Yeppoon	20026	3.05±0.3a
	20027	3.6±0.4 ac
	20028	3.17±0.4 ac
	20029	4.71±0.3b
	20030	4.06±0.3bc

identified that the relative performance of families is affected by the variation in virulence of the rust pathogen at different locations. Powers and Matthews (1980) found that pine seedlings from seed source areas were most susceptible to infection by their respective local rust fungal isolates. To date all screening for *Q. pitereka* resistance has preceded detailed knowledge of site factors and fungal variability.

Without a better understanding of the host-pathogen interaction, and factors influencing disease development, the current tree improvement programs have a reduced chance of success to produce spotted gum with high levels of resistance to *Q. pitereka*. Field trials suffer from the disadvantage of being exposed to variable climatic conditions and a lack of uniformity of inoculum. An alternative approach would be to consider the effectiveness of an artificial inoculation and screening protocol such as those used for various other tree diseases (Kuhlman et al. 1995; Roux et al. 2007; Hodge and Dvorak 2000). The development and implementation of artificial disease screening protocols is an important first step to eliminate the climatic factors influencing field trial assessments. Here it is also important to consider variation in the aggressiveness of strains and regional pathotypes to be used in artificial

Table 4 Susceptibility of selected spotted gum clones to *Quambalaria pitereka* 6 and 18 months after planting. Clones are ranked from least to most susceptible

Spotted gum clone	Mean QSB score	Ranking	Mean QSB form rating
6	6.32±1.9a	1	1.27±0.1a
3	24.78±2.7b	2	2.57±0.1b
1	36.94±6.9bc	3	3.73±0.2c
2	38.75±2.5bc	4	4.35±0.1d
8	42.98±5.0c	5	4.54±0.2d
10	45.07±8.7c	6	3.43±0.1c
5	62±7.1d	7	4.33±0.1d
4	66.63±7.8d	8	4.89±0.1e
9	67.39±5.2d	9	4.9±0.1e

inoculations. Pegg et al. (2008) have previously identified variability when studying a limited number of isolates of *Q. pitereka* from regions in Queensland and NSW. What is not known is the importance of pathogen variability in relation to aggressiveness and its role in disease development.

Irrespective of the ease with which artificial inoculations might be made, it still remains important to verify the results under field conditions where the level of inoculum and degree of pathogen variability is likely to be higher. Selecting the appropriate site, which favours disease development, to conduct such tests will be imperative to the development of an effective disease screening program. These findings need to be considered where planting programmes seek to increase the level of resistance in spotted gum from the current breeding program. Failure to do so will hinder the further development of spotted gum as a plantation species in Australia.

Acknowledgments We thank Queensland Department Primary Industries Innovation and Biosecurity Program Investment, Forest Plantations Queensland, Integrated Tree Cropping, Forest Enterprises Australia and Forests NSW for providing the necessary funding for this research. We also thank Dr Helen Nahrung for assistance with statistical analysis of field and glasshouse data. Forests NSW, in particular Michael Henson and Helen Smith, allowed access to their spotted gum trials for which we are most grateful.

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