Plant Pathology (2011) **60**, 1107–1117 Doi: 10.1111/j.1365-3059.2011.02478.x

Variability in aggressiveness of *Quambalaria pitereka* isolates

G. S. Pegg[ab]*, L. S. Shuey[a], A. J. Carnegie[c], M. J. Wingfield[d] and A. Drenth[b]

[a]*Horticulture and Forestry Science, Agri-Science Queensland, GPO Box 46, Brisbane, Qld 4068;* [b]*Tree Pathology Centre, The University of Queensland/Primary Industries and Fisheries, Qld 4068;* [c]*Forest Science Centre, Industry & Investment NSW, NSW 2119, Australia; and* [d]*Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa*

Quambalaria shoot blight, caused by the fungal pathogen *Quambalaria pitereka*, is a serious disease of eucalypt plantations in Australia. The aggressiveness of four *Q. pitereka* isolates was compared on a range of host genera, species, provenances and clones. Isolates differed substantially in their aggressiveness, with two consistently showing higher levels of aggressiveness based on incidence and severity of disease and lesion size. Isolates derived from *Corymbia citriodora* subsp. *variegata* (Ccv) and *C. torelliana* were shown to have a relatively restricted host range, with lesions but no sporulation found on *Eucalyptus* species, *Angophora* species other than *A. costata* and *Corymbia* species other than Ccv, the host of origin. The level of aggressiveness toward the different provenances of spotted gum and *C. torelliana* varied between isolates and there was evidence of some isolate × host interaction within provenances of Ccv. The two methods of inoculation used in this study, spray and spot inoculation, gave similar results. However, the fact that the spot inoculation method was labour-intensive was a disadvantage limiting the numbers of isolates and hosts that can be tested.

Keywords: Australia, *Corymbia*, *Eucalyptus*, fungal disease, plantation forestry, spotted gum

Introduction

Quambalaria shoot blight, caused by the fungal pathogen *Quambalaria pitereka*, is a serious disease affecting the expanding eucalypt plantation estate in subtropical and tropical eastern Australia (Simpson, 2000; Self *et al.*, 2002; Pegg *et al.*, 2005; Carnegie, 2007). The pathogen is described as being endemic to the coastal forests of eastern Australia, where seedlings and young trees of *Corymbia* species can be severely damaged (Old, 1990). The implementation of hardwood plantation programmes in subtropical regions of eastern Australia saw the widespread use of spotted gum, primarily using provenances of *C. citriodora* subsp. *variegata* (Ccv) (Lee, 2007) and *C. maculata*, but also including *Corymbia citriodora* subsp. *citriodora*, (Ccc) and *C. henryi* for high-value solid-wood products. Quambalaria shoot blight was infrequently observed during the first few years of plantation development but was soon after found to be widespread and causing significant damage infecting young foliage and growing shoots (Self *et al.*, 2002; Carnegie, 2007; Pegg *et al.*, 2009a). Severe damage resulting in poor tree form, and in some cases tree death, resulted in the reduction in the use of spotted gum as a priority species in the subtropics (Carnegie, 2007; Pegg *et al.*, 2009a). More recently *Q. pitereka* was discovered outside Australia on spotted gum plantations in China (Zhou *et al.*, 2007).

Only an asexual state (anamorph) is known for *Q. pitereka* and this is also true for other *Quambalaria* spp. (Simpson, 2000; Pegg *et al.*, 2009b). However, variability has been noted amongst isolates of *Q. pitereka* at the DNA level, with 10 haplotypes identified when comparing DNA sequences of isolates of the fungus collected from different locations and *Corymbia* species in northern New South Wales and Queensland (Pegg *et al.*, 2008). Isolates representing the different haplotypes were found to occur within different geographic regions, and multiple haplotypes were identified from within a single plantation. The significance of this variability in relation to host range and pathogen aggressiveness has not been studied.

Variability in levels of susceptibility to *Q. pitereka* within species, provenances and families of spotted gum has been identified by a number of authors (Stone *et al.*, 1998; Self *et al.*, 2002; Dickinson *et al.*, 2004; Johnson *et al.*, 2009; Pegg *et al.*, 2011). Spotted gum provenances have been selected as sources of seed for use in commercial plantations as well as development of seed orchards. These selections were based on field trial assessments focused on tree performance (growth rate, *Q. pitereka* tolerance) under conditions of natural infection by *Q. pitereka*. However, little attention has been afforded to the pathogen and the possibility that variability in its

*E-mail: Geoff.Pegg@deedi.qld.gov.au

Published online 8 June 2011

population might influence disease development or disease severity within spotted gum trials and commercial plantings.

Trials in subtropical and tropical Australia to select for spotted gum provenance material for use in plantation and seed orchard development are found in diverse locations where *Q. pitereka* disease levels are often inconsistent, primarily as a result of variable climatic conditions. The development and implementation of an artificial screening programme has been recommended (Pegg *et al.*, 2011) to make selections of *Q. pitereka*-tolerant genotypes more consistent and reliable. This would enhance the future development of hardwood plantations using spotted gum by reducing damage by quambalaria shoot blight. In order to establish such an artificial screening procedure, investigations into the variability in host–pathogen interactions will be required. The aim of this study was, therefore, to determine whether there are differences in host range of *Q. pitereka* isolates and if there is variability in aggressiveness or specificity of these isolates to different host species, provenances and clones of spotted gum.

Materials and methods

Quambalaria pitereka isolates

Isolates of *Q. pitereka* were selected from three different regions, including far-north Queensland, southern Queensland and northern New South Wales (Table 1). A series of preliminary studies was conducted using isolates from these regions, as well as those representing different haplotypes identified from DNA sequencing (Pegg *et al.*, 2008) collected from a single property. Isolates showing higher levels of aggression were selected for use in this study. All isolates used in the study were inoculated onto spotted gum or *Corymbia torelliana* plants and re-isolated onto potato dextrose agar (PDA) (Difco – Bacto Laboratories Pty Ltd) and incubated at 25°C in the dark for 2–3 days before being subcultured onto PDA and incubated for a further 2 weeks to ensure culture age was uniform.

Table 1 Isolates of *Quambalaria pitereka* used in host range and pathogenicity testing

Quambalaria isolate number	Origin	Isolate's original host taxon
Q107	Mareeba, north Queensland	*Corymbia torelliana* × *Corymbia citriodora* ssp. *citriodora*
Q152	Beaudesert, south east Queensland	*C. citriodora* ssp. *variegata*
Q298	Beaudesert, south east Queensland	*C. citriodora* ssp. *variegata*
Q322	Grafton, northern New South Wales	*C. citriodora* ssp. *variegata*

Inoculation methods

Two methods of inoculation were used in this study. These included spraying the inoculum onto plants or inoculating leaves in a defined spot. Spores were removed from plates using a fine-haired paint brush and washed into 100 mL sterile distilled water (SDW). A concentration of 1×10^6 spores mL^{-1} was used for all inoculations with two drops of Tween 20 added to each suspension. The spore concentration used in this study was based on preliminary studies conducted to identify the optimum spore concentration for symptom development (G. S. Pegg, unpublished data).

For both inoculation methods, seedlings were germinated and grown in steam-sterilized soil mix and fertilized with slow release Osmocote® (Native Trees) (N 17·9: P 0·8: K 7·3) as required. Plants were irrigated twice a day for 10 min each time using overhead spray irrigation. Glasshouse temperatures were maintained at 28–30°C during the day and 22–24°C overnight. Following inoculation, seedlings were covered with plastic bags for 48 h to maintain high humidity levels and leaf wetness and placed on benches in a complete random design. Previous studies (Pegg *et al.*, 2009b) identified that spore germination occurred optimally at humidity levels above 90% RH and penetration of the host was within 24 h. Uninoculated controls were treated in the same way as inoculated seedlings. Subsamples of the spore suspension applied to the trees were placed onto PDA and incubated at 25°C for 48 h to ensure that the spores were viable.

Spray inoculation

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), to the upper and lower leaf surfaces of the seedlings until runoff was achieved. Uninoculated controls were sprayed with SDW with Tween 20 added as per spore suspensions. Disease incidence (I) was assessed as a percentage of leaves infected out of the total number of leaves present. Disease severity (S) was a subjective assessment of the percentage of the total area of infected foliage on diseased leaves only. Seedlings were assessed for disease incidence and severity 14 days after inoculation.

Spot inoculation

Preliminary studies on detached leaves were used to compare inoculation of leaves on adaxial and abaxial leaf surfaces. No lesion development occurred on leaves inoculated on the adaxial leaf surface and for this reason all further studies were conducted on abaxial leaf surfaces. A plastic tube (5 mm in diameter) with a cotton applicator placed inside was dipped into the spore suspension prior to application and then inoculated onto the abaxial leaf surfaces. On each leaf, isolates of *Q. pitereka* were inoculated onto one side of the midrib either at the apex, middle or base of the leaf (Fig. 1). The positioning of each isolate was rotated for each leaf with each isolate inoculated onto the leaf apex, middle or base, once per

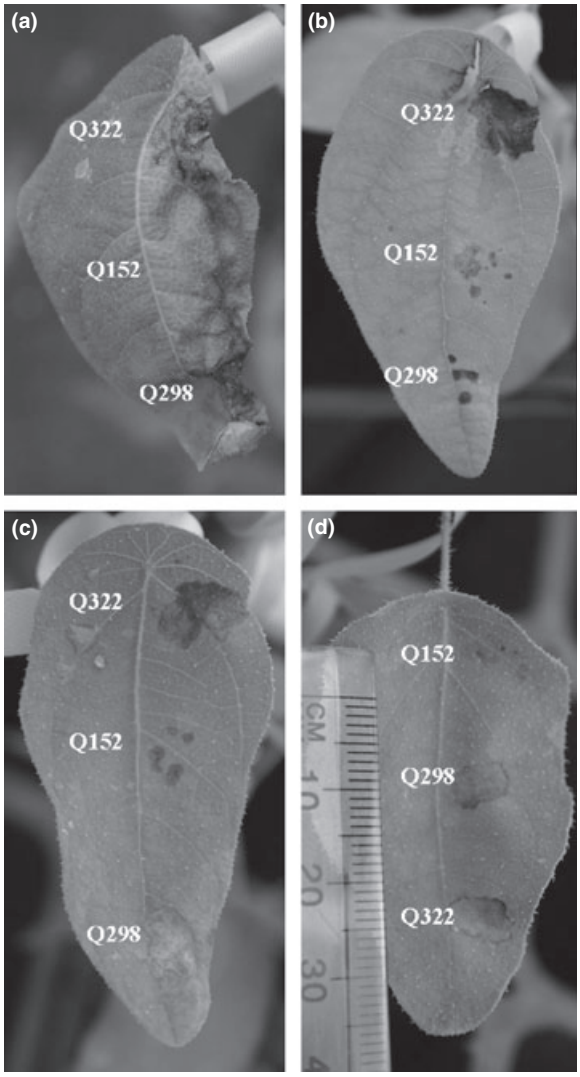

Figure 1 Lesion development 14 days after inoculation using the spot inoculation method with three isolates of *Quambalaria pitereka* onto *Corymbia citriodora* subsp. *variegata* provenance Presho (a) and *C. henryi* provenance Myrtle Creek (b–d).

seedling. The youngest fully expanded foliage was selected for inoculation. Control seedlings were inoculated with SDW and Tween 20 solution. Disease levels were measured as a percentage of the area inoculated that was necrotic 14 days after inoculation.

The aggressiveness of *Q. pitereka* isolates was compared on increasing 'resolution' of genotypes from species to provenances to clones. The aggressiveness of isolates was compared on *Corymbia* species known to be susceptible to *Q. pitereka* as well as on *Angophora*, *Corymbia* and *Eucalyptus* species on which *Q. pitereka* has not been identified. Variability of isolate aggression was also compared on provenances and clones of spotted gum. For the purpose of this study the term 'aggression' refers to the severity of disease caused by each isolate (Pariaud *et al.*, 2009).

Comparisons of isolate host range

To determine the extent of variation in the host range within isolates of *Q. pitereka*, three isolates of *Q. pitereka* (Q107, Q298 and Q322) were inoculated onto *Corymbia* species not known to be susceptible to the pathogen (i.e. those other than spotted gum and *C. torelliana*) using the spot inoculation method. Species used were *C. intermedia* (seedlot 197), *C. ptychocarpa* (seedlot 2772) and *C. tessellaris* (seedlot 5915). A range of *Angophora* species were also tested: *A. costata* (seedlot unknown), *A. leiocarpa* (seedlot 3294) and *A. melanoxylon* (seedlot 2182). Two *Eucalyptus* species were also tested: *E. drepanophylla* (seedlot 95) and *E. melanophloia* (seedlot 2677). Ccv (Presho provenance seedlot 4928) was also included as a known host. Six replicates were used per isolate per host and placed in a complete random design in the glasshouse.

Comparisons of isolate aggressiveness on *Corymbia* species

The variability in aggressiveness of isolates of *Q. pitereka* towards Ccc, *C. henryi*, Ccv and *C. torelliana* (Table 2) was examined. Using the spray method, four isolates (Q107, Q152, Q298 and Q322) were inoculated onto *Corymbia* hybrids grown from seed (Table 2). Ten replicates were used per isolate per host and placed in a complete random design in the glasshouse. Ten control seedlings from each provenance were inoculated with SDW and Tween 20 solution.

Comparisons of isolate aggressiveness on *Corymbia* hybrids

The variability in aggressiveness of isolates of *Q. pitereka* towards *Corymbia* hybrids (Table 2) was examined. Using the spray method, four isolates (Q107, Q152, Q298 and Q322) were inoculated onto *Corymbia* species grown from seed (Table 2) as mentioned above. Ten control seedlings from each provenance were inoculated with SDW and Tween 20 solution.

Comparisons of isolate aggressiveness on provenances of *Corymbia* species

The variability in aggressiveness of isolates of *Q. pitereka* towards provenances of *Corymbia* species (Table 2) was examined. Using the spray method, four isolates (Q107, Q152, Q298 and Q322) were inoculated onto seedlings of 17 provenances within *Corymbia* species Ccc, *C. henryi*, Ccv and *C. torelliana* grown from seed (Table 2). Ten replicates were used per isolate per host and placed in a complete random design in the glasshouse. Ten control seedlings from each provenance were inoculated with SDW and Tween 20 solution.

As a comparison, and to reduce the influence of host variability, a spot inoculation technique was also used to investigate the interaction between isolates and

Table 2 Origin and species of spotted gum and *Corymbia torelliana* provenances and *Corymbia* hybrids

Species[a]	Provenance	Origin	Seedlot	Latitude (S)	Longitude (E)	MAR[b] (mm)
Ccc	Barron	Northern Queensland	5609	17°15′	145°30′	1200
Ccc	Cheviot Hills	Northern Queensland	3506	19°38′	144°12′	673
Ccc	Kirrama	Northern Queensland	5298	18°12′	145°46′	2000
Ccc	Yeppoon	Central Queensland	11246	23°6′	150°44′	1325
Corymbia henryi	Myrtle Creek	Northern New South Wales	5554	29°9′	152°59′	1100
C. henryi	Nerang	South east Queensland	10257	27°59′	153°19′	1200
Ccv	Brooyar	South east Queensland	10248	26°10′	152°30′	1143
Ccv	Curra	South east Queensland	K6035	26°4′	152°39′	1138
Ccv	Grange	Northern New South Wales	CVS90062	29°31′	152°32′	1212
Ccv	Home	South east Queensland	10235	26°5′	152°43′	1143
Ccv	Mt McEuan	South east Queensland	12805	26°14′	151°39′	1200
Ccv	Presho	Central Queensland	4928	25°3′	149°13′	675
Ccv	Richmond Range	Northern New South Wales	19469a	28°38′	152°48′	1267
Ccv	Wondai	South east Queensland	10253	26°22′	151°49′	800
Ccv	Woondum	South east Queensland	11185-096	26°15′	152°49′	1600
C. torelliana	Cairns	Northern Queensland	3552	16°57′	145°45′	1992
C. torelliana	Helensvale	Northern Queensland	K6340			
C. torelliana × Ccc			X213			
C. torelliana × *C. henryi*			X135			
C. torelliana × Ccv			X72			

[a]Ccc, *Corymbia citriodora* subsp. *citriodora*; Ccv, *C. citriodora* subsp. *variegata*.
[b]MAR, mean annual rainfall.

provenances (Presho, Home, Yeppoon, Mt McEuan, Richmond Range and Myrtle Creek). Three isolates of *Q. pitereka* were compared (Q152, Q298 and Q322) on six spotted gum provenances. Ten trees were used per spotted gum provenance with controls treated with SDW and Tween 20 solution.

Comparisons of isolate aggressiveness on Ccv clones

Four *Q. pitereka* isolates (Q107, Q152, Q298 and Q322) were compared on seven Ccv clones (Forests NSW) using the spot inoculation technique. Plants for each clone were produced from micropropagation of archived cultures developed by Forests NSW (Lan *et al.*, 2011). Ten replicates per clone per isolate were inoculated as described above and placed in a complete random design in the glasshouse.

Analysis of data

Normality of data was checked using an equality of variance *F*-test. All proportion data was arcsine square root transformed prior to analysis using ANOVA and compared using Fisher's PLSD *post hoc* test (StatView®). Back-transformed data were used in all graphed and tabulated data.

Results

Inoculation methods

Both methods of inoculation were effective in producing symptoms typical of *Q. pitereka* infection within 14 days after inoculation. Using the spray inoculation method allowed for the testing of a large number of host plants in comparison to the spot inoculation method, which was much more time consuming. Leaf size limited the number of isolates that could be tested on a single leaf using the spot inoculation method.

Comparisons of isolate host range

Small lesions developed on all species tested when inoculated with three isolates of *Q. pitereka* (Q107, Q298 and Q322), but *A. costata* and Ccv were most susceptible (Fig. 2). However, the presence of sporulation on lesions was recorded on Ccv and *A. costata* only. No disease symptoms were present on uninoculated controls. Significant differences were found between isolates (two-way ANOVA $F_{2,297} = 3{\cdot}5$, $P = 0{\cdot}03$). Based on lesion size, isolate Q322 showed the highest level of aggressiveness on *A. costata*, *A. leiocarpa*, *A. melanoxylon*, Ccv, *C. ptychocarpa, E. drepanophylla* and *E. melanophloia* and showed a significantly higher level of aggressiveness than Q107 only. Isolate Q298 showed a higher level of aggressiveness on *C. tessellaris* and *C. intermedia*. There was a significant difference in host susceptibility ($F_{8,297} = 19{\cdot}6$, $P < 0{\cdot}0001$) but no significant host × isolate interaction ($F_{16,297} = 5{\cdot}8, P = 0{\cdot}5$).

Comparisons of isolate aggressiveness on *Corymbia* species

Using spray inoculations significant differences in disease incidence and severity occurred when spotted gum species and *C. torelliana* (Table 3) were inoculated with *Q. pitereka* isolates. Isolate Q322 showed the highest level of aggressiveness on Ccv, *C. henryi* and *C. torelliana*

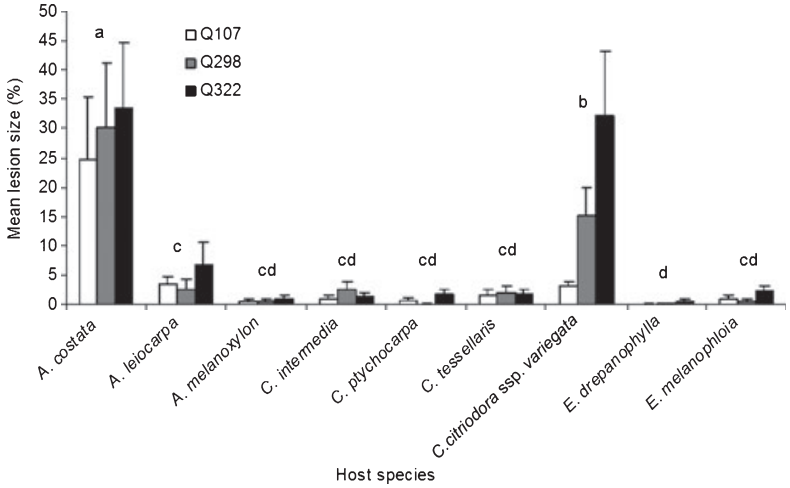

Figure 2 Comparison of host range of three *Quambalaria pitereka* isolates on species of *Angophora*, *Corymbia* and *Eucalyptus* showing mean lesion size (+1 standard error). Matching letters designate means that do not differ significantly (Fisher's PLSD test $P < 0{\cdot}0001$).

Table 3 Comparison of mean incidence (a) and severity (b) of infection by isolates of *Quambalaria pitereka* 14 days after inoculation using the spray inoculation method onto species of spotted gum and *Corymbia torelliana*. Matching letters designate means that do not differ significantly

(a) *Q. pitereka* isolate				*Corymbia citriodora* ssp. *citriodora* A	*Corymbia citriodora* ssp. *variegata* B	*Corymbia henryi* B	*Corymbia torelliana* B
Q107 a				51·7±5·4	47·4±3·2	28·3±7·2	33·8±6·6
Q152 b				56·7±4·4	57·3±3·1	40·9±7·9	23·7±4·6
Q298 c				80·6±4·4	70·2±2·8	52±7·4	48·6±5·7
Q322 c				75·4±4·8	71·3±3·4	58·6±7·4	55±6·8
	d.f.	*F*	*P*				
Isolate	3	18·8	<0·0001				
Host	3	18·1	<0·0001				
Isolate*Host	9	0·7	0·7				
Residual	635						

(b) *Q. pitereka* isolate				*C. citriodora* ssp. *citriodora* A	*C. citriodora* ssp. *variegata* B	*C. henryi* BC	*C. torelliana* C
Q107 a				14·7 ± 2·4	18·3 ± 2·1	6 ± 1·6	5·5 ± 1·1
Q152 a				13·2 ± 1·9	23 ± 2·2	11·7 ± 3·3	4·2 ± 0·7
Q298 b				18 ± 2	21·9 ± 1·6	17·2 ± 4	11·2 ± 2·1
Q322 b				17·6 ± 2·2	27·5 ± 2·4	21·3 ± 4·7	12·2 ± 1·8
	d.f.	*F*	*P*				
Isolate	3	9	<0·0001				
Host	3	19·8	<0·0001				
Isolate*Host	9	0·8	0·6				
Residual	635						

based on disease incidence and all spotted gum species and *C. torelliana* based on disease severity. Isolate Q322 was not significantly different from isolate Q298. Isolates Q107 and Q152 showed the lowest level of aggressiveness. No symptoms were present on control seedlings.

Significant differences in susceptibility were identified between host species (Table 3). Disease incidence levels were lowest on *C. torelliana* for all isolates except isolate Q107. Disease severity levels were lowest on *C. torelliana* for all isolates. There was no significant host × isolate interaction (Table 3).

Comparisons of isolate aggressiveness on *Corymbia* hybrids

Using spray inoculations significant differences in disease incidence and severity occurred when *Corymbia* hybrids (Table 4) were inoculated with *Q. pitereka* isolates. Isolate Q298 showed the highest level of aggressiveness, but not significantly different from isolate Q322. Isolates Q152 and Q107 showed the lowest level of aggressiveness.

Disease incidence and severity levels were significantly lower on the *C. torelliana* × *C. henryi* hybrids. There was

Table 4 Comparison of mean incidence (a) and severity (b) of infection by isolates of *Quambalaria pitereka* 14 days after inoculation using the spray inoculation method onto *Corymbia* hybrids. Matching letters designate means that do not differ significantly

(a) *Q. pitereka* isolate				*Corymbia torelliana* × *Corymbia citriodora* ssp. *citriodora* A	*C. torelliana* × *Corymbia henryi* B	*C. torelliana* × *C. citriodora* ssp. *variegata* C
Q107 a				52·2 ± 7·2	8·1 ± 3·8	39·4 ± 12·8
Q152 a				28 ± 9·9	34·3 ± 11·6	63·9 ± 16·3
Q298 b				66·5 ± 8	40·9 ± 9·3	85·8 ± 5·3
Q322 b				64·5 ± 9·7	20·5 ± 10·1	75·8 ± 7·1
	d.f.	*F*	*P*			
Isolate	3	6	0·001			
Host	2	14·3	<0·0001			
Isolate*Host	6	2·1	0·06			
Residual	635					

(b) *Q. pitereka* isolate				*C. torelliana* × *C. citriodora* ssp. *citriodora* A	*C. torelliana* × *C. henryi* B	*C. torelliana* × *C. citriodora* ssp. *variegata* A
Q107 a				10 ± 1·7	2·1 ± 1	6·7 ± 2·5
Q152 a				5 ± 1·7	6·4 ± 2·1	12·5 ± 5
Q298 b				11 ± 1·2	7·9 ± 1·5	26·4 ± 5
Q322 b				21·5 ± 9	7·1 ± 4·1	15 ± 1·5
	d.f.	*F*	*P*			
Isolate	3	43	0·007			
Host	2	5·9	0·004			
Isolate*Host	6	1·8	0·1			
Residual	82					

no significant interaction between hybrids and isolates of *Q. pitereka* (Table 4). No disease was found on control seedlings.

Comparisons of isolate aggressiveness on provenances of *Corymbia* species

Corymbia citriodora subsp. *citriodora* (Ccc)

Using spray inoculations, significant differences in disease incidence were identified on Ccc provenances when inoculated with *Q. pitereka* isolates (Table 5). Disease severity levels were not significantly different. No single isolate showed a consistently higher level of aggressiveness towards all hosts, but Q298 and Q322 caused significantly higher levels of disease incidence on provenances of Ccc than isolates Q107 and Q152. Isolates Q298 and Q322 caused higher disease severity levels on all provenances, but differences from isolate Q107 and Q152 were not significant.

Disease levels were significantly different on Ccc provenances with Yeppoon and Cheviot Hills most susceptible (Table 5). There was no significant host × isolate interaction for disease incidence, but for disease severity the host × isolate interaction was significant. No disease was found on control seedlings.

Corymbia henryi

Using spray inoculations, significant differences in disease incidence and disease severity were identified on *C. henryi* provenances when inoculated with *Q. pitereka* isolates (Table 6). Isolates Q298 and Q322 showed a significantly higher level of aggressiveness than isolate Q107. Isolates Q298 and Q322 were not significantly different from each other or Q152. Isolate Q152 was not significantly different from isolate Q107. Myrtle Creek provenance was significantly less susceptible than Nerang. There was no significant host × isolate interaction and no disease identified on control seedlings (Table 6).

Corymbia citriodora subsp. *variegata* (Ccv)

Using spray inoculations, significant differences in disease incidence and disease severity were identified on Ccv provenances when inoculated with *Q. pitereka* isolates (Table 7). No single isolate showed a higher level of aggressiveness on all hosts with a significant isolate × host interaction (Table 7). Isolates Q298 and Q322 showed a significantly higher level of aggressiveness than Q107 and Q152, causing higher disease incidence levels on seven of the nine provenances tested. Isolates Q298 and Q322 also caused higher disease severity levels on six of the nine provenances, but there was no significant difference between these isolates and Q152 and Q107. Only isolate Q322 caused significantly higher disease severity levels than Q107.

Significant differences in provenance susceptibility were also identified with disease incidence levels lowest on Richmond Range, Grange and Curra provenances (Table 7). Disease severity levels were lowest on Brooyar, Curra and Grange, with Presho and Woondum being most susceptible. No disease symptoms were detected on control seedlings.

Table 5 Comparison of mean incidence (a) and severity (b) of infection by isolates of *Quambalaria pitereka* 14 days after inoculation using the spray inoculation method onto *Corymbia citriodora* subsp. *citriodora* provenances. Matching letters designate means that do not differ significantly

(a)

Q. pitereka isolate	Barron A	Kirrima AB	Yeppoon BC	Cheviot Hills C
Q107 a	30·2 ± 10·9	45·2 ± 6·6	64·5 ± 11·5	65 ± 10·9
Q152 a	46·8 ± 8·9	44·6 ± 8·9	75 ± 7	60·2 ± 8·3
Q298 b	82·1 ± 8·7	81·4 ± 7·9	72·7 ± 12·4	86·3 ± 5·5
Q322 b	54 ± 11·8	67·4 ± 8·9	82·3 ± 5·8	95 ± 7·4

	d.f.	*F*	*P*
Isolate	3	10·1	<0·0001
Host	3	4·0	0·009
Isolate*Host	9	1·4	0·2
Residual	139		

(b)

Q. pitereka isolate	Barron A	Kirrima A	Yeppoon B	Cheviot Hills B
Q107 a	7·2 ± 2·5	8 ± 61·3	20·5 ± 6	22·5 ± 5·6
Q152 a	9·5 ± 1·9	8 ± 1·9	21 ± 5·7	14·5 ± 3·1
Q298 a	16 ± 4	16 ± 4·3	16 ± 4·1	24 ± 3·3
Q322 a	7·2 ± 1·9	10 ± 1·8	32·5 ± 4·8	19 ± 2·2

	d.f.	*F*	*P*
Isolate	3	2·0	0·12
Host	3	10·1	<0·0001
Isolate*Host	9	2	0·04
Residual	139		

Table 6 Comparison of mean incidence (a) and severity (b) of infection by isolates of *Quambalaria pitereka* 14 days after inoculation using the spray inoculation method onto *Corymbia henryi* provenances. Matching letters designate means that do not differ significantly

(a)

Q. pitereka isolate	Myrtle Creek A	Nerang B
Q107 a	14·2 ± 6·9	42·5 ± 11·2
Q152 ab	17·7 ± 8	64·2 ± 9
Q298 b	33·7 ± 9	70·3 ± 7·7
Q322 b	47·6 ± 11·2	70·9 ± 8·3

	d.f.	*F*	*P*
Isolate	3	3·8	0·005
Host	1	27·6	<0·0001
Isolate*Host	3	0·7	0·5
Residual	67		

(b)

Q. pitereka isolate	Myrtle Creek A	Nerang B
Q107 a	3 ± 1·5	9 ± 2·6
Q152 ab	5 ± 2·2	18·5 ± 5·6
Q298 bc	6·9 ± 1·9	27·5 ± 5·8
Q322 c	19 ± 6·9	24 ± 6·7

	d.f.	*F*	*P*
Isolate	3	5·2	0·003
Host	1	17·8	<0·0001
Isolate*Host	3	0·98	0·4
Residual	67		

Corymbia torelliana

Using spray inoculations, significant differences in disease incidence and disease severity were identified on *C. torelliana* provenances when inoculated with *Q. pitereka* isolates (Table 8). Disease incidence was highest when provenances were inoculated with isolates Q298 and Q322, significantly higher than with Q152. Disease incidence levels were also significantly higher on seedlings inoculated with Q322 than on those inoculated with Q107. Disease severity levels were significantly higher on seedlings inoculated with isolates Q298 and Q322 than isolates Q107 and Q152. Isolate Q152 showed the lowest level of aggressiveness, but not significantly different from Q107.

There was no significant difference in disease incidence and severity levels on the two provenances and there was no significant isolate × host interaction (Table 8). No disease symptoms were detected on control seedlings.

Using spot inoculations (Fig. 3), significant differences were identified between isolates of *Q. pitereka* on Presho (one-way ANOVA $F_{2,87} = 90{\cdot}6$; $P < 0{\cdot}0001$), Home ($F_{2,87} = 14{\cdot}9$; $P < 0{\cdot}0001$), Yeppoon ($F_{2,87} = 19$; $P < 0{\cdot}0001$), Richmond Range ($F_{2,61} = 15{\cdot}6$; $P < 0{\cdot}0001$) and Myrtle Creek ($F_{2,66} = 20{\cdot}4$; $P < 0{\cdot}0001$). There was no significant difference when isolates were inoculated onto Mt McEuan seedlings ($F_{2,87} = 1{\cdot}7$; $P = 0{\cdot}2$). Isolate Q298 showed a higher level of aggressiveness on Presho, Home and Yeppoon, whereas isolate Q322 showed a higher level of aggressiveness on Mt McEuan, Richmond Range and Myrtle Creek. Apart from the result for Yeppoon this matched the results from the spray inoculation study. Isolate Q152 showed the lowest level of aggressiveness of the three isolates. Isolate Q298 and Q322 did not show significantly different levels of aggressiveness on any of

Table 7 Comparison of mean incidence (a) and severity (b) of infection by isolates of *Quambalaria pitereka* 14 days after inoculation using the spray inoculation method onto *Corymbia citriodora* subsp. *variegata* provenances. Matching letters designate means that do not differ significantly

(a) *Q. pitereka* isolate	Richmond Range A	Grange A	Curra A	Wondai B	Mt McEuan B	Woondum B	Home B	Brooyar B	Presho B
Q107 a	24·5 ± 10·5	24·4 ± 8·1	59·5 ± 7	27·8 ± 11·3	41·8 ± 8·6	65·2 ± 6·9	66·1 ± 6·5	52·5 ± 11·1	56·8 ± 7
Q152 b	26·6 ± 10·9	50·8 ± 7·7	41·2 ± 8·1	72·1 ± 9·8	66·1 ± 7·8	49·2 ± 6·3	78·3 ± 10·3	71·6 ± 6·8	65·7 ± 7·1
Q298 c	57·7 ± 7·4	47·9 ± 5·5	53·3 ± 11·3	74·8 ± 9·6	78·3 ± 5·4	71·6 ± 6·5	71·9 ± 6·9	75·9 ± 8·1	96·7 ± 3·3
Q322 c	71·8 ± 9·7	57·7 ± 14·4	52·3 ± 10·2	81·7 ± 10	68·1 ± 9·8	79·8 ± 5·5	60·4 ± 13·4	78·8 ± 5·3	92·1 ± 4·2

	d.f.	*F*	*P*
Isolate	3	16·0	<0·0001
Host	8	7·6	<0·0001
Isolate*Host	24	2·0	0·004
Residual	310		

(b) *Q. pitereka* isolate	Richmond Range A	Wondai AB	Curra ABC	Grange ABC	Home ABC	Mt McEuan BCD	Brooyar CD	Woondum CD	Presho D
Q107 a	17·5 ± 10·3	5 ± 1·8	26 ± 7·7	8 ± 2·8	20 ± 4·1	12·5 ± 3·7	17 ± 4·3	21·5 ± 5·2	32 ± 8·2
Q152 ab	5 ± 2·1	26·4 ± 7·8	20·5 ± 7	15 ± 3·6	30·5 ± 7·4	30·5 ± 8	29 ± 6·5	20 ± 7	31·5 ± 5·4
Q298 ab	12·5 ± 2·8	12·8 ± 2·2	16 ± 3·9	20 ± 4·3	19 ± 2·5	17·5 ± 3·8	26·5 ± 4	33 ± 6·6	37 ± 6·1
Q322 b	21 ± 6·3	22·5 ± 5·3	18 ± 4·1	40 ± 13·1	13 ± 4	39·4 ± 5·2	34 ± 8·9	31·1 ± 6·9	30 ± 5

	d.f.	*F*	*P*
Isolate	3	4·3	0·005
Host	8	4·2	<0·0001
Isolate*Host	24	1·8	0·01
Residual	310		

Table 8 Comparison of mean incidence (a) and severity (b) of infection by isolates of *Quambalaria pitereka* 14 days after inoculation using the spray inoculation method onto *Corymbia torelliana* provenances. Matching letters designate means that do not differ significantly

(a) *Q. pitereka* isolate	Cairns A	Helensvale A
Q107 ab	23·5 ± 8·2	43 ± 9·6
Q152 a	21·3 ± 6·2	26 ± 6·9
Q298 bc	52·1 ± 9·3	45·1 ± 7·2
Q322 c	48 ± 12·2	62 ± 5·9

	d.f.	*F*	*P*
Isolate	3	5·3	0·003
Host	1	1·7	0·2
Isolate*Host	3	0·7	0·6
Residual	67		

(b) *Q. pitereka* isolate	Cairns A	Helensvale A
Q107 a	6·1 ± 1·8	5 ± 1·3
Q152 a	4 ± 1	4·5 ± 1·2
Q298 b	11·9 ± 3	10·6 ± 3·2
Q322 b	12·5 ± 3·1	12 ± 1·9

	d.f.	*F*	*P*
Isolate	3	6·2	0·0009
Host	1	0·03	0·8
Isolate*Host	3	0·2	0·9
Residual	67		

the host provenances. Both Q298 and Q322 caused larger lesions than Q152 on all spotted gum provenances. No lesion development occurred on controls inoculated with SDW and Tween 20.

Comparisons of isolate aggressiveness on Ccv clones

Significant differences in isolate aggressiveness were identified when assessed on seven different spotted gum clones using spot inoculation with spores of *Q. pitereka* (two-way ANOVA $F_{3,291} = 13{\cdot}6$; $P < 0{\cdot}0001$) (Fig. 4). No single isolate showed a different level of aggressiveness on all clones with a significant isolate × host interaction ($F_{18,291} = 1{\cdot}9$; $P = 0{\cdot}02$). Isolate Q322 showed a higher level of aggressiveness on four of the seven clones assessed and caused significantly higher disease levels than isolates Q107 and Q152. Isolate Q152 showed the lowest level of aggressiveness, significantly different from all other isolates. Isolate Q298 showed the highest level of aggressiveness on two of the seven clones and was significantly different from Q107.

Significant differences in clone susceptibility were also identified ($F_{6,291} = 42{\cdot}8$; $P < 0{\cdot}0001$) with disease levels greatest on clone 2. No lesion development was detected on control seedlings.

Discussion

Results of this study are the first to consider possible variability in the important leaf and shoot pathogen *Q. pitereka*. The results show conclusively that isolates differ substantially in their aggressiveness to host species, provenances and clones of spotted gum, as well as *Corymbia* hybrids. While the number of isolates examined in this study was relatively limited, the differences in isolate aggressiveness shown will have significant implications for future breeding programmes. Interesting questions can also be raised regarding the source of the

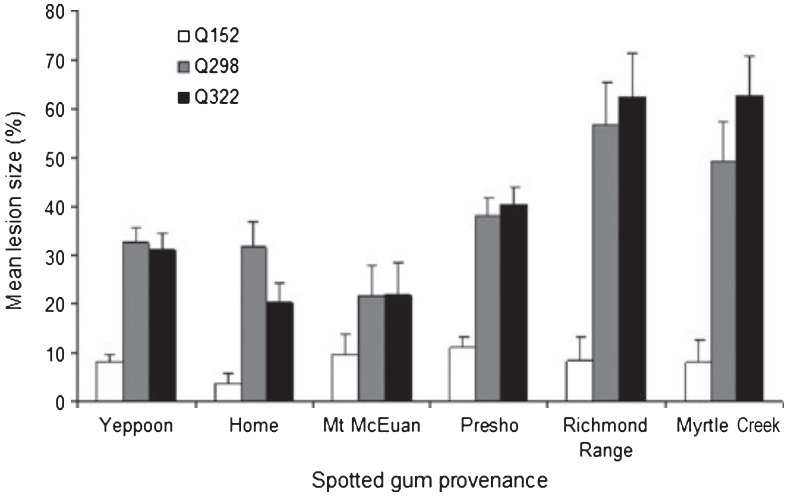

Figure 3 Comparison of mean lesion size (+1 standard error) on different provenances of spotted gum species 14 days after inoculation using the spot inoculation method with three isolates of *Quambalaria pitereka*.

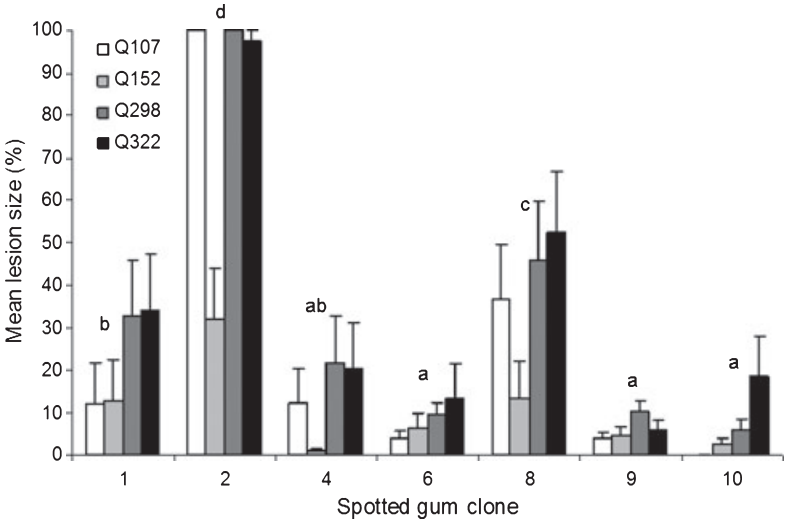

Figure 4 Comparison of *Quambalaria pitereka* isolate aggressiveness on seven spotted gum clones using the spot inoculation method showing mean lesion size (+1 standard error). Matching letters designate means that do not differ significantly (Fisher's PLSD test $P < 0{\cdot}0001$).

observed variability, particularly given that only the anamorph of *Q. pitereka* is known.

Results of this study indicate that *Q. pitereka* isolates derived from spotted gum and *C. torelliana* have a relatively restricted host range. While lesions developed on all host species, they were small and much less severe than those on Ccv. The lack of sporulation on these lesions could also be an indication of a noncompatible host–pathogen interaction, with the pathogen unable to complete its life cycle. Interestingly, significant lesion development was observed on *A. costata*. Why this species is so susceptible in comparison to other *Angophora* species and, more to the point, other closely related *Corymbia* species, is unknown. Previous studies (Walker & Bertus, 1971) suggest that *Q. pitereka* has a wider host range than that identified here, with infection following artificial inoculation occurring on *C. ficifolia*, *C. exima* and *C. maculata*. Simpson (2000) also determined that *Q. pitereka* infected species of *Angophora* and *Corymbia* and was responsible for shoot dieback of ghost gums, *Blakella*, in central Australia. Species of *Eucalyptus* in this study produced nonsporulating lesions, suggesting a noncompatible host–pathogen interaction.

Analysis of collections of *Q. pitereka* samples deposited in herbarium collections in Queensland (BRIP) and New South Wales (DAR) indicate that *Q. pitereka* has been identified on a very wide range of hosts, including numerous *Corymbia* species (*C. ficifolia*, *C. trachyphloia*, *C. tessellaris*, *C. calophylla*, *C. intermedia*, *C. polycarpa*, *C. watsoniana*, *C. zygophylla*, *C. bloxsomei*, *C. peltata*, *C. haematoxylon*, *C. gummifera*, *C. ferruginea*, *C. papuana* and *C. nesophila*), several *Angophora* species (*A. cordifolia*, *A. melanoxylon* and *A. floribunda*) and two *Eucalyptus* species (*Eucalyptus crebra* and *E. grandis*) (G. S. Pegg, unpublished data). The holotype specimen used to describe the species was collected from *C. exima* (Simpson, 2000) and when examined along with isolates from spotted gum plantations using DNA studies, it resided within a common haplotype (Pegg

et al., 2008). This would suggest that the host range for *Q. pitereka* is wide, although generally restricted to *Corymbia* spp. However, many of these specimens collected were identified prior to the use of molecular tools in fungal taxonomy, the identification of other *Quambalaria* species, and prior to the genus being reclassified (Simpson, 2000; de Beer *et al.*, 2006). Therefore, records for *Eucalyptus* spp. could represent *Q. eucalypti*, recently found in Australia (Pegg *et al.*, 2008). The fact that the present study showed *Q. pitereka* isolates from spotted gum and *C. torelliana* to have a relatively restricted host range suggests that more detailed population and host-range studies are required.

Of the four isolates in this study, two showed consistently higher levels of aggressiveness on spotted gum species, *C. torelliana* and *Corymbia* hybrids, but there was no evidence of host specificity. Isolates collected from spotted gum and *C. torelliana* were able to infect both host species in reciprocal transfer experiments. The fact that isolates selected from spotted gum in southern Queensland and northern New South Wales caused disease symptoms on *C. torelliana* in the glasshouse raises some interesting questions. *Quambalaria pitereka* has been found on *C. torelliana* in north Queensland (Pegg *et al.*, 2008), but has not been detected on this host in south east Queensland or northern New South Wales, despite being planted widely as an amenity tree (Lee, 2007; Pegg *et al.*, 2008). However, isolates collected from spotted gum in these regions showed a significantly higher level of aggressiveness on *C. torelliana* than those collected from *C. torelliana* in north Queensland. Conversely, the isolate originating from *C. torelliana* caused disease symptoms on spotted gum species. This is in contrast to a previous study (Pegg *et al.*, 2008) where isolates collected from *C. torelliana* and *Corymbia* hybrids in north Queensland were limited to a haplotype specific to north Queensland and these taxa. Isolates from spotted gum within the same plantation did not share this haplotype nor did isolates taken from *Corymbia* hybrids in southern Queensland.

Isolates were different in their aggressiveness to *Corymbia* provenances, and the provenances displayed differences in susceptibility, tested both with spot and spray inoculation. While two isolates showed higher levels of aggressiveness in general, the level of aggressiveness toward the different provenances varied and there was evidence of some isolate × host interaction within provenances of Ccv. It was unclear from the results whether there was any definitive pattern of host specificity such as has been found for various other tree pathogens. For example, Powers & Matthews (1980) found that pine seedlings from different geographic sources were most susceptible to infection by their respective local fusiform rust fungal isolates, indicating host specificity. Likewise, Thompson & Burdon (1992) also suggested that locally derived pathogen isolates are more likely to be virulent in a given host population than those more distantly derived. Whether this phenomenon applies to *Q. pitereka* has not clearly emerged from the present study.

While variability in resistance to *Q. pitereka* has been identified at provenance and family level, no trials have been conducted using seeds derived from sources close to the trial sites and then replicated across various regions. However, spotted gum species and provenance trials have not as yet shown evidence of a region × pathogen interaction. In fact, the opposite was shown with recent trials in northern New South Wales (Pegg *et al.*, 2011), where spotted gum provenances from northern Queensland were more resistant to infection by *Q. pitereka* at the trial site than provenances collected from regions closer to the trial site. This may reflect a host species difference rather than a level of host–pathogen interaction.

The two methods of inoculation used in this study gave similar results, particularly for the inoculation of provenances. However, the spot inoculation method reduced the influence of host variability and generally gave more consistent results. A disadvantage of this method is that it is labour-intensive and thus limits the numbers of isolates and hosts that can be tested. Leaf size can also limit the number of isolates that can be compared at any one time. The spray method in contrast provides an effective means to expose large numbers of individual hosts to a larger number of isolates. This method may be improved by limiting the assessment for disease levels to the first two newly developing leaves. In the present study, all leaves were assessed for disease and variable rates of leaf development may influence disease development and thus affect the accuracy of comparisons between seedlings. Sporulation and timing of spore production may also be a useful indicator to measure components of host–pathogen interaction in future assessments of both isolate variability and host susceptibility. Other quantitative traits that could be used in addition to lesion size and sporulation include infection efficiency, latent period and rate of lesion development.

While this study focused on a small number of isolates collected from only three regions, variability in aggressiveness was shown and this knowledge will be important when developing screening procedures. Identifying the level and extent of variation in relation to isolate aggression and host susceptibility is crucial in the development of a disease screening assay for plantation development using spotted gum. Detailed population studies are required to enhance the knowledge of *Q. pitereka* and aid in improving future evaluation of susceptibility of *Corymbia* germplasm for commercial development of spotted gum as a plantation species. Current breeding has identified variability in host susceptibility within spotted gum (Dickinson *et al.*, 2004; Johnson *et al.*, 2009) but has not considered the variability in pathogen aggression. Much of the selection is currently focused on quantitative traits, such as growth (Lee *et al.*, 2009). The significance of the variability in isolate aggression, particularly in relation to disease development within plantation spotted gum, is unknown, as is the level of variability within plantations and native environments. The influence of the proximity and density of native spotted gum species to plantations in relation to disease development and the

makeup of the pathogen population must also be considered. This is crucial for the development of the hardwood industry in Australia, where *Corymbia* species could play an important role in the future of timber production.

Acknowledgements

We thank Queensland Department of Primary Industries Innovation and Biosecurity Program Investment, Forest Plantations Queensland, Elders Forestry (formerly Integrated Tree Cropping) and Forest Enterprises Australia for providing the necessary funding for this research. We also thank Forests NSW for providing spotted gum clones.

References

de Beer WZ, Begerow D, Bauer R, Pegg GS, Crous PW, Wingfield MJ, 2006. Phylogeny of *Quambalariaceae* fam. nov. including important *Eucalyptus* pathogens in South Africa and Australia. *Studies in Mycology* **55**, 289–98.

Carnegie AJ, 2007. Forest health condition in New South Wales, Australia, 1996–2005. II. Fungal damage recorded from eucalypt plantations during forest health surveys and their management. *Australasian Plant Pathology* **36**, 225–39.

Dickinson GR, Lee DJ, Huth JR, 2004. Early plantation growth and tolerance to ramularia shoot blight of provenances of spotted gums (*Corymbia citriodora* subsp. *citriodora*, *C. citriodora* subsp. *variegata* and *C. henryi*), grown on a range of sites. *Australian Forestry* **67**, 122–30.

Johnson IG, Carnegie AJ, Henson M, 2009. Growth, form and quambalaria shoot blight tolerance of spotted gum provenances and families in north-eastern New South Wales, Australia. *Silvae Genetica* **58**, 180–91.

Lan J, Raymond CA, Smith HJ *et al.*, 2011. Variation in growth and *Quambalaria* tolerance of clones of *Corymbia citriodora* subsp. *variegata* planted on four contrasting sites in north eastern NSW. *Australian Forestry* **74** (in press).

Lee DJ, 2007. Development of *Corymbia* species and hybrids for plantations in eastern Australia. *Australian Forestry* **70**, 11–6.

Lee DJ, Huth JR, Brawner JT, Dickinson GR, 2009. Comparative performance of *Corymbia* hybrids and parental species in subtropical Queensland and implications for breeding and deployment. *Silvae Genetica* **58**, 205–12.

Old KM, 1990. Diseases caused by fungi. In: Cremer KW, ed. *Trees for Rural Australia*. Melbourne, Australia: Inkata Press, 210–6.

Pariaud B, Ravigne V, Halkett H, Goyeau H, Carlier J, Lannou C, 2009. Aggressiveness and its role in the adaptation of plant pathogens. *Plant Pathology* **58**, 409–24.

Pegg GS, Carnegie AJ, Drenth A, Wingfield MJ, 2005. *Quambalaria pitereka* on spotted gum plantations in Queensland and northern New South Wales, Australia. *The International Forestry Review* **7**, 337.

Pegg GS, O'Dwyer C, Carnegie AJ, Wingfield MJ, Drenth A, 2008. *Quambalaria* species associated with plantation and native eucalypts in Australia. *Plant Pathology* **57**, 702–14.

Pegg GS, Carnegie AJ, Wingfield MJ, Drenth A, 2009a. *Quambalaria* species: increasing threat to eucalypt plantations in Australia. *Southern Forests* **71**, 111–4.

Pegg GS, Webb RI, Carnegie AJ, Wingfield MJ, Drenth A, 2009b. Infection and disease development of *Quambalaria* spp. on *Corymbia* and *Eucalyptus* species. *Plant Pathology* **58**, 642–54.

Pegg GS, Carnegie AJ, Wingfield MJ, Drenth A, 2011. Variable resistance to *Quambalaria pitereka* in spotted gum reveal opportunities for disease screening. *Australasian Plant Pathology* **40**, 76–86.

Powers Jr HR, Matthews FR, 1980. Comparison of six geographic sources of loblolly pine for fusiform rust resistance. *Phytopathology* **70**, 1141–3.

Self NM, Aitken EAB, Dale MD, 2002. Susceptibility of provenances of spotted gums to Ramularia shoot blight. *New Zealand Plant Protection* **55**, 68–72.

Simpson JA, 2000. *Quambalaria*, a new genus of eucalypt pathogens. *Australasian Mycologist* **19**, 57–62.

Stone C, Simpson JA, Eldridge RH, 1998. Insect and fungal damage to young eucalypt trial plantings in northern New South Wales. *Australian Forestry* **61**, 7–20.

Thompson JN, Burdon JJ, 1992. Gene-for-gene coevolution between plants and parasites. *Nature* **360**, 121–5.

Walker J, Bertus AL, 1971. Shoot blight of *Eucalyptus* spp. caused by an undescribed species of *Ramularia*. *Proceedings of the Linnean Society of New South Wales* **96**, 108–15.

Zhou X, de Beer W, Xie Y, Pegg GS, Wingfield MJ, 2007. DNA-based identification of *Quambalaria pitereka* causing severe leaf blight of *Corymbia citriodora* in China. *Fungal Diversity* **25**, 245–54.