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Spread and development of quambalaria shoot blight in spotted gum plantations

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The aim of this study was to examine the disease development of quambalaria shoot blight, caused by the fungal pathogen *Quambalaria pitereka*, in plantation-grown spotted gum (*Corymbia citriodora* subsp. *citriodora*, *C. citriodora* subsp. *variegata*, *C. henryi* and *C. maculata*) in south-east Queensland, Australia. The results showed that native spotted gums are a primary source of inoculum followed rapidly by the production of secondary inoculum from infected trees in the plantation. The rate of spread and development of *Q. pitereka* within plantations increased exponentially over time as additional trees became infected and produced secondary inoculum. Spore concentration was shown to play an important role in disease development, with disease severity increasing with increasing disease incidence on individual trees and incidence across the plantation.

Keywords: Australia, *Corymbia*, disease incidence, disease severity, myrtaceae

Introduction

In subtropical and tropical areas of Australia, plantations of spotted gum (*Corymbia citriodora* subsp. *citriodora*, *C. citriodora* subsp. *variegata*, *C. henryi* and *C. maculata*) have been damaged significantly (Pegg *et al.*, 2005, 2008; Carnegie, 2007a) by the disease commonly known as quambalaria shoot blight (QSB) caused by the basidiomycete pathogen *Quambalaria pitereka* (Simpson, 2000; de Beer *et al.*, 2006). The genus *Quambalaria* includes five species, *Q. pitereka*, *Q. eucalypti*, *Q. cyanescens*, *Q. coyrecup* and *Q. pusilla*, all of which have been identified from eucalypts (de Beer *et al.*, 2006). At present only the asexual stages of these fungi have been identified (Pegg *et al.*, 2009).

Corymbia citriodora subsp. *variegata* is the most widely planted taxon for saw log production, making up 70% of the plantings in Queensland (Dickinson *et al.*, 2004). In the mid 1990s, several failures of spotted gum in plantations were recorded in southeast Queensland (Self *et al.*, 2002) and northern New South Wales (Stone *et al.*, 1998). Despite the effort to identify provenances with higher levels of resistance (Dickinson *et al.*, 2004; Lee, 2007; Johnson *et al.*, 2009), *Q. pitereka* has continued to impact negatively on plantation establishment and growth (Carnegie, 2007b; Pegg *et al.*, 2008). Based on forecast planting regimes in 2006, spotted gum plantations were set to expand from 12 000 ha to around 100 000 ha by the year 2016. This target has now been drastically reduced because of the severe impact of *Q. pitereka* in 2008 and again in 2009 when climatic conditions were favourable for disease development.

Quambalaria pitereka has been detected in all plantations and trial plantings of spotted gum in Queensland and northern New South Wales (Self *et al.*, 2002; Pegg *et al.*, 2005; Carnegie, 2007b). Incidence of *Q. pitereka* varies among *Corymbia* taxa trials located throughout the natural range of spotted gum (Dickinson *et al.*, 2004), with recorded disease levels low in regions having a mean average rainfall (MAR) of <750 mm and increasing as MAR increases. On sites where MAR exceeded 800 mm, widespread, severe damage and some tree deaths have occurred. Records of *Q. pitereka* from native forest are less common, but it has been identified from mature trees and natural regeneration in a relatively wide geographical range in Queensland and New South Wales (Walker & Bertus, 1971; Old, 1990; Simpson, 2000; Self *et al.*, 2002; Pegg *et al.*, 2008).

Development of quambalaria shoot blight in spotted gum plantations typically occurs 3–6 months after planting (Self *et al.*, 2002). Lesions begin to develop on immature leaves and stems typically 5 days after infection, becoming visible as discrete chlorotic spots with necrotic centres (Pegg *et al.*, 2005, 2009). These lesions can develop into large, sporulating lesions 10–14 days after infection under favourable conditions. Masses of white conidia are produced on conidiophores arising from sto-

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matal cavities on young leaves, shoots and young stem tissue (Pegg *et al.*, 2008). Conidiophores are produced within the area of necrosis on the upper and lower leaf surfaces (Pegg *et al.*, 2009). It is unclear how long individual lesions remain as sources of viable fungal inoculum for re-infection of adjacent trees. Infected trees are typically reduced in height and show a shrub-like appearance as a result of the death of young shoots giving rise to the loss of apical dominance (Carnegie, 2007a; Pegg *et al.*, 2008).

Quambalaria pitereka has been identified in spotted gum trees surrounding plantations, on amenity plantings and in native forests, causing foliage spots, leaf and shoot blight and stem death (Pegg *et al.*, 2008). Pegg *et al.* (2008) also identified *Q. pitereka* from woody stem cankers and in association with hail wounds on young plantation-grown spotted gum. Simpson (2000) consistently isolated *Q. pitereka* from symptomless young woody shoots and stems on various spotted gum species. However, detailed studies of these potential sources of inoculum that could initiate and enhance the development and spread of disease within spotted gum plantations have not been conducted.

Selection of spotted gum to be used in commercial plantation development and tree breeding programmes is based on levels of resistance observed under field conditions. However, this strategy has preceded any detailed knowledge of factors influencing *Q. pitereka* disease development. The overall aim of this study was, therefore, to examine disease development within plantation-grown spotted gum in an effort to gain an understanding of the epidemiology of *Q. pitereka*. Specifically, it investigated the rate and patterns of disease spread within spotted gum plantations and the environmental factors that influenced disease development. The potential sources of primary inoculum and the role and source of secondary inoculum in disease development were studied and the progress of disease incidence and severity on individual trees, as well as within the plantation, was considered. Furthermore, the possibility of a correlation between disease incidence at a plantation level and within-host incidence and severity was investigated. Such knowledge would help facilitate the identification of optimal sites to establish disease screening trials.

Materials and methods

Rate and pattern of spread within spotted gum plantations

Study sites

To determine the pattern and rate of spread of *Q. pitereka* within plantations, two recently established spotted gum plantations in south-east Queensland were selected. Both plantations had been established using the *C. citriodora* subsp. *variegata* seed provenance Richmond Range. Trees were between 6 and 9 months old during the assessment period.

Site 1, located near Beaudesert in south-east Queensland (27°97′S, 152°99′E), was planted in May 2005 at a stocking density of 1250 trees ha^{-1} and consisted of two adjoining blocks totalling 5·25 ha (Fig. 1). Remnant, mature spotted gum trees, all in excess of 20 m in height, occurred within and at the south-western edge of the plantation. Native spotted gum was also a common species on properties surrounding the plantation, which consisted of semicleared horse- and cattle-grazing paddocks.

Site 2, located near Traveston Crossing in south-east Queensland (26°20′S, 152°42′E), was planted in May 2008 within a production trial including various *Eucalyptus* species as well as spotted gum. This site was 2·22 ha stocked at a rate of 1100 trees ha^{-1}. Unlike Site 1, there were no spotted gum or other *Corymbia* species within or at the immediate edge of the plantation but there was a single spotted gum amenity tree located 125 m to the south-west of the plantation.

Surveys were conducted at both sites to detect symptoms of *Q. pitereka* infection within the plantation prior to intensive and quantitative assessments. Structured disease surveys were initiated at both sites after *Q. pitereka* infection was first detected on a single tree. Assessments were conducted every 7–15 days for 3 months, depending on accessibility to the sites during periods of heavy rainfall. Infected trees were tagged using coloured flagging tape to allow easy identification in subsequent surveys and their positions were recorded using a global positioning system (GPS; Garmin 76 Series).

Pattern of spread

To determine the distribution of *Q. pitereka* infections within plantations, spatial patterns of disease incidence within the plantation were examined based on the GPS survey data collected. The patterns of spread were studied using GPS data downloaded onto digital maps using MapSource® in ArcGIS. Two blocks (1 and 2) at site 1 and the single block at site 2 were subdivided into 10- × 10-m plots and the number of infected trees within each block was determined. The distribution of infection was determined using a goodness-of-fit to Poisson random distribution (Zar, 1999) four times throughout the sampling period. A Bonferonni adjustment was made ($P < 0{\cdot}004$) to control for these multiple comparisons.

To gain an understanding of the importance of native spotted gum and their proximity to plantation spotted gum trees, as well as changes in spread pattern over time, disease gradients were examined. At site 1, the percentage of the total number of infected trees was plotted for block 1 from a potential source of inoculum (a mature spotted gum tree >20 m tall) 25 m from the south-western edge of the plantation at time of initial infection and then compared to disease spread 42 days after initial infection (Fig. 1). The distance was measured from this tree, in a north to north-easterly direction, using GPS data collected during surveys and the percentage of the total number of infected trees at 10-m intervals was plotted 5 and 42 days after the first detection of disease within

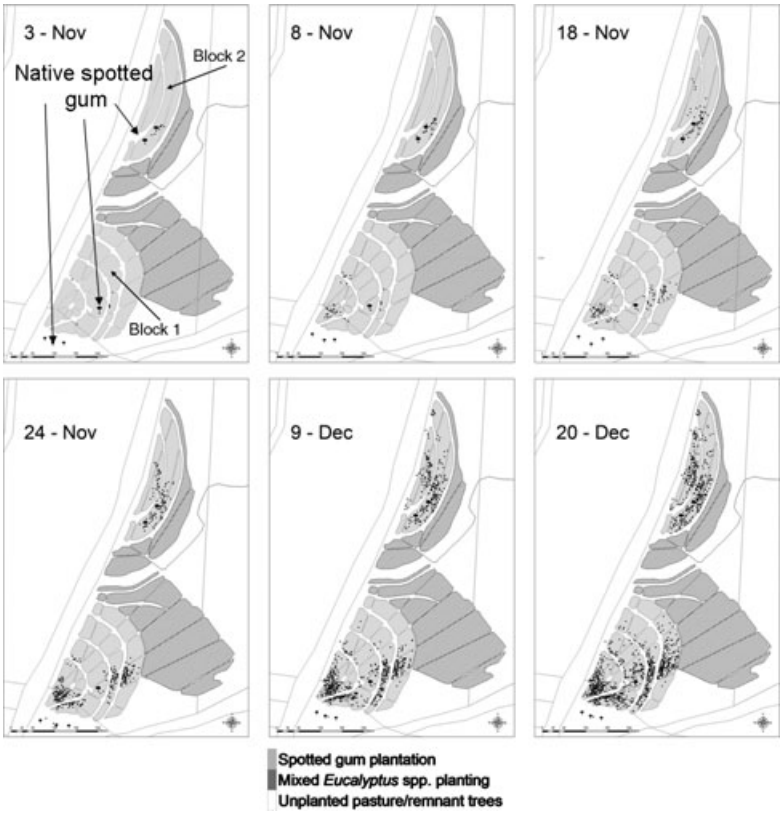

Figure 1 Spread of *Quambalaria pitereka* in plantation spotted gum at site 1 (blocks 1 and 2), south-east Queensland in 2005.

the plantation. A second mature spotted gum tree (>20 m tall) was located within the plantation 148 m from the first spotted gum. A similar assessment was made in block 2, but measurements were taken radially from a point between two mature spotted gum trees, which were also >20 m tall, growing close to each other within the plantation.

At site 2, which was free of native spotted gum trees within the plantation, similar measurements were made to site 1 at the time when the disease first appeared and 58 days after symptoms were first observed. Disease gradient was measured based on the number of infected trees at 10-m intervals from the midpoint of the south-western edge of the plantation and plotted as a percentage of the total number of infected trees. This was based on the observation of the pattern of spread at site 1 and the presence of the closest spotted gum tree 125 m to the south-west of the plantation.

Rate of increase in disease incidence

To determine the rate of increase of *Q. pitereka*, disease incidence within the plantation was assessed over time. Using GPS data, the number of trees infected at site 1 was determined at the time of initial detection of disease and 7, 14, 24, 30, 45, 56 and 71 days after the disease was first detected. A similar assessment was conducted at site 2 at the time of initial infection and 13, 28, 40, 60, 76 and 87 days after the disease was first found. Disease incidence within the plantation was determined as a proportion of trees infected and changes over time in the number of infections occurring per day, calculated based on the number of new infections occurring between assessment periods.

Disease incidence and severity

To determine the influence of disease incidence within the plantation on disease development on individual trees, incidence and severity of *Q. pitereka* infection was assessed. At site 1, a total of 304 trees centred on points of infection within the plantation were assessed fortnightly, apart from the final assessment which was completed 1 month after the previous assessment. Incidence of diseased trees within the trial was determined as a proportion of the total number of trees. Disease incidence and severity levels were also determined for each tree. Incidence of disease on individual trees was calculated as the percentage of total new shoots and immature, expanding foliage showing visible signs of infection. Severity was calculated as the percentage of the total area of infected new shoots and immature, expanding foliage (Johnson *et al.*, 2009; Pegg *et al.*, 2010). Data were analysed for differences in incidence and severity levels and compared at different times using ANOVA. Relationships between incidence of disease within the trial and incidence and severity of infection on individual trees were also compared.

At site 2 the entire 800-tree plot was assessed for the incidence of *Q. pitereka* disease every 10–14 days for 3 months. Disease incidence was also recorded for each tree on a 0–4 scale, where 0 = no disease present; 1 = 1–25% of total new shoots and immature, expanding foliage infected; 2 = 26–50% infection; 3 = 51–75% infection and 4 = 76–100% infection. Disease severity was not assessed at site 2.

Sources of inoculum

Infected foliage

To determine the importance of infected foliage as a source of secondary inoculum for disease spread and development within a plantation, infected foliage was assessed for spore production over time. Seven expanding leaves on a spotted gum tree with similar-sized lesions and stage of development were labelled and assessed for spore production over 4 weeks. Using a fine–bristled paint brush, spores were collected weekly from the upper and lower surfaces of the lesions and washed into a plastic vial containing 10 mL sterile distilled water (SDW). Preliminary studies (G. S. Pegg, unpublished data) had shown no difference in the level of spore production when comparing the upper and lower leaf surfaces. These vials were then placed in a cool container and transported back to the laboratory.

Spore germination rates were assessed for each vial by placing 100-μL droplets onto the surface of a cleaned and sterilized glass slide and incubating in the dark at 25°C in a sealed container with SDW added to maintain 100% relative humidity (RH) (Pegg *et al.*, 2009). Six slides, with four droplets of spore suspension on each slide, were incubated and 100 conidia were counted from each slide after 6 h. Spores were recorded as having germinated if a germ tube was clearly visible at ×400 magnification. Spore concentrations were assessed using a haemocytometer.

Senesced leaves

To determine the importance of prematurely senesced leaves infected with *Q. pitereka* as a source of inoculum, spore production and germination rates were tested from fallen leaves and compared to those of infected leaves and stems still attached to trees. Five treatments were assessed: (i) infected leaves removed from the tree and air-dried in a paper bag for 10 days in the laboratory, (ii) dried (brown in colour) infected leaves collected from under trees within plantation spotted gum, (iii) fallen infected leaves still green in colour collected from under spotted gum trees, (iv) infected leaves still attached to trees, and (v) infected stem material still attached to trees.

Spores were removed from lesions on all leaf/stem types using a fine-bristled paint brush and placed into 10 mL SDW. A 100-μL droplet of spore suspension was placed onto a sterilized glass microscope slide and allowed to air-dry in a laminar flow cabinet for 15 min or until the slide was free of any visible moisture. Slides were placed on wire stands suspended 5 cm above the level of SDW in containers. SDW was used to achieve 100% RH following the method of Sheridan (1968), with four replicates per treatment. All containers were sealed to ensure airtightness and placed in the dark at 25°C. Spore germination was assessed after 6 h by determining the number of germinated spores out of 100. Spores were recorded as having germinated if a germ tube was clearly visible at ×400 magnification.

The viability of spores on senesced leaves was further tested using a method to determine the length of time lesions continue to produce viable spores after senescence. Eight *Q. pitereka*-infected new shoots, expanding leaves, fully expanded leaves and green stems were removed from trees and placed into a wire basket at the base of a spotted gum tree. After 2 and 4 weeks, four of each leaf type and stem tissue were removed and transported back to the laboratory, where spores were washed from the lesions and germination rates assessed as above.

Spore concentration and disease development

To determine the importance of spore concentration on disease development, disease incidence and severity levels on spotted gum seedlings inoculated with a range of spore concentrations were assessed in glasshouse studies. *Corymbia citriodora* subsp. *variegata* seedlings were grown in steam-sterilized soil mix, fertilized with slow release Osmocote® (Native Trees) as required and irrigated twice a day for 10 min each time using overhead sprinklers. *Quambalaria pitereka* was grown on potato dextrose agar (PDA) for 2 weeks in the dark at 25°C. Spores were removed from plates using a fine-bristled paint brush and washed into SDW. In the first experiment, a dilution series was used to achieve spore concentrations of 1×10^6, $2{\cdot}5 \times 10^5$, $1{\cdot}5 \times 10^5$, 1×10^5 spores mL^{-1} SDW. Two drops of Tween-20 were added to each spore suspension. Six trees were inoculated per treatment and placed in a complete random design within the glasshouse after inoculation, along with six uninoculated controls. Each control was sprayed with a SDW and Tween-20 solution.

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. Following inoculation, seedlings were covered with plastic bags for 48 h to maintain high humidity levels and leaf wetness. Control plants were treated in exactly the same manner as inoculated seedlings. Glasshouse temperatures were maintained at 28–30°C during the day and 22–24°C overnight. 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. Seedlings were assessed for disease incidence and severity 7 and 14 days after inoculation.

Because there were no significant differences in disease incidence and severity at the concentrations tested above, a second inoculation experiment was conducted using the spore concentrations of 1×10^6, 1×10^3 and

1×10^2 spores mL^{-1} distilled water. Inoculation and assessment were the same as in the first inoculation trial, but assessment was conducted only once, 14 days after inoculation. Six trees were used per treatment and compared to six uninoculated controls. The controls were treated as mentioned above and placed in a complete random design in the glasshouse along with treated trees.

For both inoculation trials, disease incidence (I) was assessed as a percentage of total number of leaves infected. Disease severity (S) was assessed as a percentage score of the total area of infected foliage on diseased leaves only. As seedlings were used in these studies, all foliage was considered immature and thus susceptible to infection. Incidence and severity levels were compared between treatments.

Statistical analysis

Unless otherwise described, normality of the data was assessed using an equality of variance *F*-test. All proportion data were subjected to arcsine square root transformation prior to analysis using ANOVA and compared using Fisher's PLSD *post hoc* test (StatView®). Back-converted data were used to present data graphically. Correlations were used to determine the interdependence of disease levels using GenStat® v11·1.

Results

Rate and pattern of spread within spotted gum plantations

Pattern of spread

At site 1, the first trees detected with symptoms of *Q. pitereka* infection were found in close proximity to mature spotted gum trees occurring within or on the south-western edge of the plantation (Fig. 1). Spread of disease appeared to be focused around these trees, only becoming more widespread at the time of the last two assessments in December. A final assessment conducted in January 2006 identified *Q. pitereka* on most trees within the plantation. Mapping of this was not possible because of the inordinately large number of trees to mark with GPS. Infection data deviated significantly from random (Poisson) distribution, beginning and remaining strongly aggregated throughout the season (goodness of fit to Poisson distribution, block 1: χ^2 = 708–50 146, $P < 0{\cdot}0001$; block 2: χ^2 = 393–7614, $P < 0{\cdot}0001$; Fig. 2).

The first infected trees detected at site 2 were focused around three points, becoming more widespread 2 months after initial detection (Fig. 3). Initially, a number of trees were found to be infected near a single focal point and a few additional trees scattered in the plantation. By the final assessment, disease symptoms were detected across the area of assessment. Infection data deviated significantly from random (Poisson) distribution, beginning and remaining strongly aggregated throughout the season (goodness of fit to Poisson distribution, χ^2 = 165–5575, $P < 0{\cdot}0001$; Fig. 3).

In block 1 of site 1, there were two distinct peaks of infection with steep gradients (Fig. 4). The first peak occurred 70–80 m from the mature spotted gum >20 m tall occurring 25 m outside the edge of the plantation. The second peak occurred 160–170 m from this same point but only 12 m from a second mature spotted gum located within the plantation. Diseased trees were more evenly distributed 42 days later and this was reflected in the flatter gradient (Fig. 4).

In block 2 of site 1, there was also a steep gradient of infection in the early stages of disease development, with the highest proportion of infected trees occurring within 20–30 m of a mature spotted gum tree within the centre of the plantation. As was observed in block 1, this gradient reduced over time and the influence of the mature spotted gum was no longer evident.

Despite there being no native spotted gum in close proximity to the plantation at site 2, a focal point of infection was present at the north-eastern corner of the plantation. This distribution changed over time, with a higher proportion of infected trees occurring in the south-western part of the plantation (Fig. 4).

Rate of increase in disease incidence

Even though plantation establishment occurred in May 2005 at site 1, *Q. pitereka* symptoms did not become apparent until November of the same year. Disease development over time within the plantation was initially slow, with <1% of the plantation infected at 14 days after initial detection of disease, 5% infection at 45 days and 19% at 56 days. Over 50% of the plantation was infected with *Q. pitereka* within 70 days of the disease first being detected. Based on infection rate per day, increase in disease spread within the plantation was exponential ($y = 1{\cdot}9e^{0{\cdot}061x}$; $R^2 = 0{\cdot}97$; Fig. 5).

At the first assessment in site 2, 2% of the trees were infected. Disease levels exceeded 6% of the trees planted 28 days after initial infections were detected and 20% by 60 days. After 87 days, disease levels within the plantation exceeded 60%. Based on number of trees infected per day disease development was exponential over time ($y = 0{\cdot}24e^{0{\cdot}05x}$; $R^2 = 0{\cdot}8$; Fig. 5).

Disease incidence and severity

At site 1, disease development was initially slow with the incidence of trees infected within the plots not exceeding 50% until 28 days after disease was first detected. Within 80 days of first detection, more than 90% of the trees showed symptoms of *Q. pitereka* infection (Fig. 6). Average disease incidence and severity levels on trees within the plots increased significantly over time (incidence $F_{6,2121} = 288{\cdot}7$, $P < 0{\cdot}0001$; severity $F_{6,2121} = 240$, $P < 0{\cdot}0001$; Fig. 6). Average disease incidence levels on trees were initially <10%, but exceeded an average of 70% by 81 days after initial infection was detected (Fig. 6). There was a strong correlation between incidence of disease within the trial and disease incidence ($\rho = 0{\cdot}95$; $P < 0{\cdot}0001$) and severity ($\rho = 0{\cdot}86$; $P < 0{\cdot}0001$) on individual trees. Incidence and severity levels were

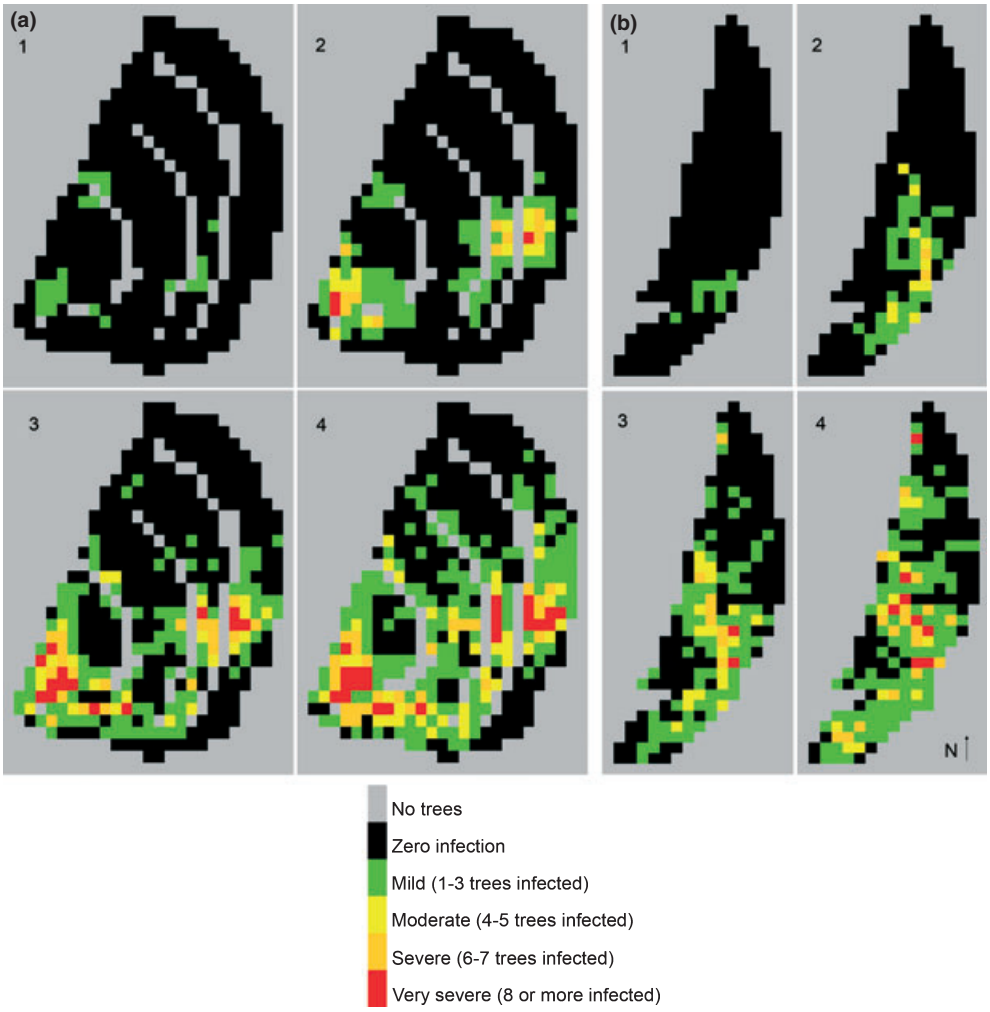

Figure 2 Development and spread of *Quambalaria pitereka* within spotted gum plantations at site 1, south-east Queensland, (a) block 1 and (b) block 2; on (1) 8 November, (2) 24 November, (3) 9 December and (4) 20 December 2005. Each square represents a 10- × 10-m grid in which the number of infected trees was counted.

also strongly correlated with each other ($\rho = 0{\cdot}96$; $P < 0{\cdot}0001$).

At site 2, incidence of disease within the plantation increased over time, with 60% of trees infected 87 days after disease was first detected (Fig. 7). An increase in the incidence of disease within the plantation was strongly correlated with an increase in the average disease incidence on individual trees ($\rho = 0{\cdot}99$; $P < 0{\cdot}0001$). Disease incidence increased significantly over time ($F_{6,6264} = 228{\cdot}2$; $P < 0{\cdot}0001$). Average disease incidence levels were not significantly different from day 1 to day 28, but increased significantly on day 40 ($P = 0{\cdot}001$) and continued to increase with time ($P < 0{\cdot}0001$; Fig. 7).

Inoculum source

Infected foliage

Spore survival, based on germination rates, decreased over time with significant differences between assessment times ($F_{3,24} = 84{\cdot}7$; $P < 0{\cdot}0001$). Spore germination rates were significantly higher in the first week after assessments commenced ($P < 0{\cdot}0001$). Spore germination decreased significantly in the second week but increased again in week 3 before declining again in week 4. The reason for the decreased level of spore germination in the second week is unclear. Spore production on lesions decreased significantly over time ($F_{3,24} = 52{\cdot}8$; $P < 0{\cdot}0001$). Spore production levels were significantly higher at the first assessment ($P < 0{\cdot}0001$), with a mean spore concentration of 190 000 spores mL^{-1} recorded. By week 4 spore production had reduced to below 20 000 spores mL^{-1}.

Senesced leaves

Significant differences in spore germination levels were identified between the different leaf types when assessing the viability of *Q. pitereka* spores on senesced foliage ($F_{4,20} = 13{\cdot}4$; $P < 0{\cdot}0001$). However, there was no signif-

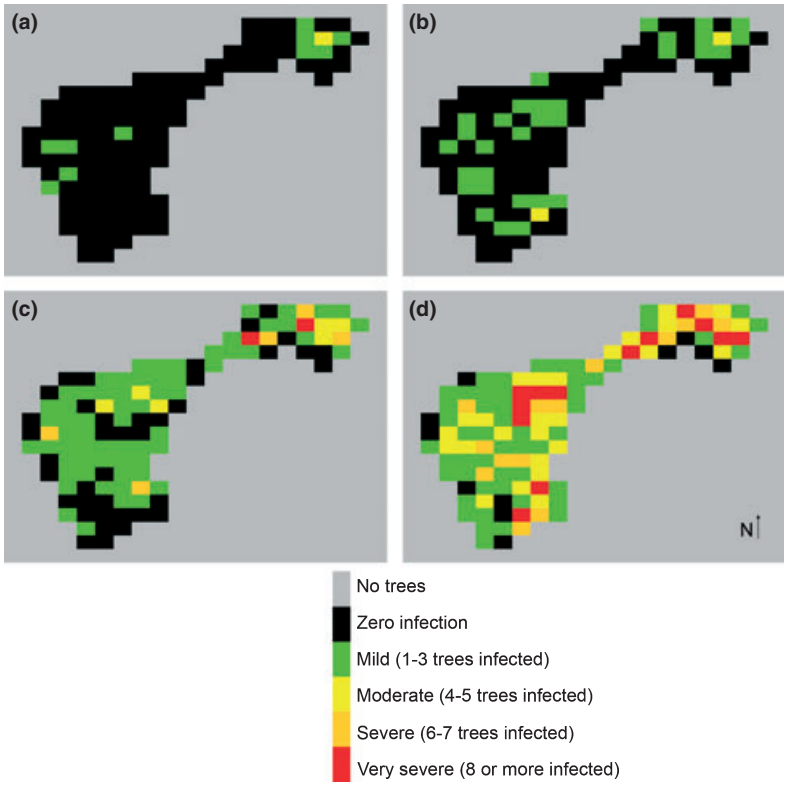

Figure 3 Development and spread of *Quambalaria pitereka* within spotted gum plantations at site 2, south-east Queensland on (a) 25 September, (b) 23 October, (c) 25 November and (d) 22 December, 2009. The planting was subdivided into 10- × 10-m plots and the number of infected trees within each counted.

icant difference between dried senesced leaves collected under the tree canopy and green senesced leaves and leaves still attached to the tree, but there was a significant ($P < 0{\cdot}0001$) reduction in spore germination on infected leaves air-dried for 10 days.

When examining *Q. pitereka*-infected leaf and shoot material 2 and 4 weeks after removing them and placing them under the tree canopy, significant differences in spore germination levels were found ($F_{1,30} = 203{\cdot}3$; $P < 0{\cdot}0001$). Spore germination levels 2 weeks after foliage removal was 40%. Four weeks after foliage removal it was difficult to detect spores and no spores germinated. There were no significant differences between foliage type or stems ($F_{3,28} = 0{\cdot}2$; $P = 0{\cdot}9$).

Spore concentration and disease development

Disease incidence levels were not significantly different at spore concentration levels between 1×10^5 and 1×10^6 spores mL^{-1} at 7 days ($F_{3,44} = 1{\cdot}7$; $P = 0{\cdot}19$) or 14 days ($F_{3,44} = 0{\cdot}4$; $P = 0{\cdot}8$) after inoculation. However, significant differences in disease severity were found 7 days ($F_{3,44} = 3{\cdot}4$; $P = 0{\cdot}02$) and 14 days ($F_{3,44} = 1{\cdot}5$; $P = 0{\cdot}2$) after inoculation. Seven days after infection, disease severity levels were significantly greater on seedlings infected with a spore concentration of 1×10^6 spores mL^{-1} than seedlings infected with 1×10^5 ($P = 0{\cdot}0006$) and $2{\cdot}5 \times 10^5$ ($P = 0{\cdot}03$) spores mL^{-1}. Fourteen days after inoculation, disease severity levels were only significantly greater on seedlings infected at 1×10^6 than seedlings infected at 1×10^5 spores mL^{-1} ($P = 0{\cdot}05$).

When there was a logarithmic reduction in spore load (1×10^6, 1×10^4 and 1×10^2 spores mL^{-1}), disease incidence ($F_{2,15} = 8{\cdot}3$; $P = 0{\cdot}004$) and severity levels ($F_{2,15} = 6{\cdot}2$; $P = 0{\cdot}01$) were significantly different on seedlings 14 days after inoculation. Disease incidence levels were significantly greater on seedlings inoculated with 1×10^6 than seedlings inoculated with 1×10^4 ($P = 0{\cdot}01$) and 1×10^2 ($P = 0{\cdot}001$) spores mL^{-1}. Disease severity levels were significantly greater on seedlings inoculated with a spore concentration of 1×10^6 than those inoculated with a spore concentration of 1×10^2 ($P = 0{\cdot}003$).

Discussion

This study represents the first consideration of the epidemiology of *Q. pitereka* in spotted gum plantations. Factors influencing the rate and patterns of disease spread, as well as the effect of disease incidence within the plantation and incidence and severity of disease on individual trees, were identified. Climatic factors, particularly temperature and relative humidity (Pegg *et al.*, 2009), have been shown to play an integral role in initiating disease.

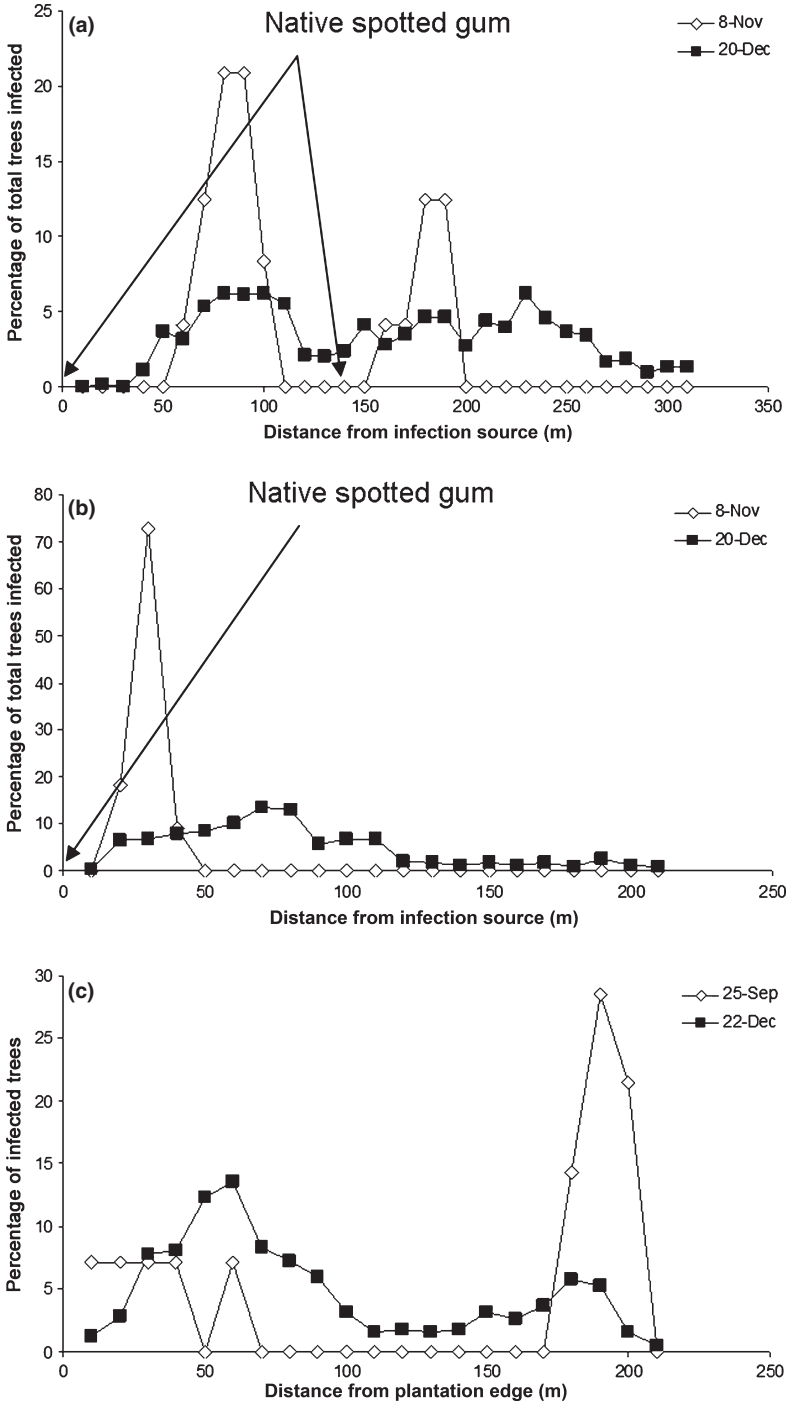

Figure 4 Disease gradients showing changes in distribution of *Quambalaria pitereka*-infected trees within the plantation over time in relation to native spotted gum trees in (a) site 1, block 1 (b) site 1, block 2, and (c) site 2, where no distinct inoculum source was identified.

Native spotted gum acted as a significant source of primary inoculum, followed rapidly by the production of secondary inoculum from infected trees within the plantation.

The distribution of infected plants within spotted gum plantations was aggregated throughout the period of assessment, despite the rapid spread of infection. Disease at site 1 was initially aggregated close to native spotted gum trees, both within and on the edge of the plantation, representing the primary sources of inoculum. From the distribution pattern over time it became clear that infected plantation trees acted as a source of secondary

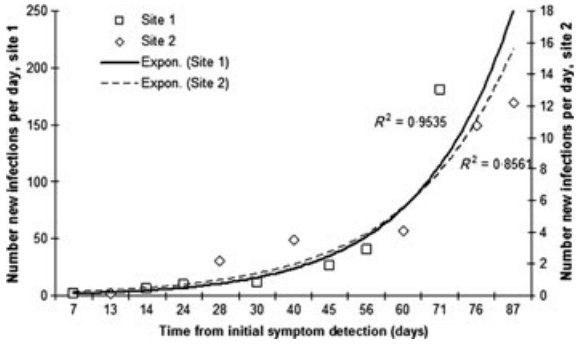

Figure 5 Rate of spread based on the number of new *Quambalaria pitereka*-infected trees per day after symptoms were first detected within a spotted gum plantation at site 1 and site 2.

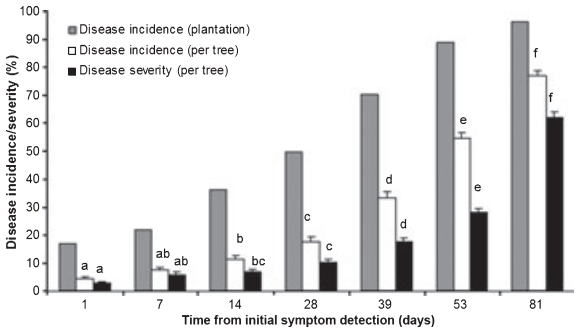

Figure 6 Disease incidence levels within the plantation and average incidence and severity (+1 SE) of *Quambalaria pitereka* infection on 304 trees assessed within a spotted gum plantation over a period of 81 days at site 1. The same letters above the bars designate means that do not differ significantly (Fisher's PLSD test $P < 0{\cdot}0001$).

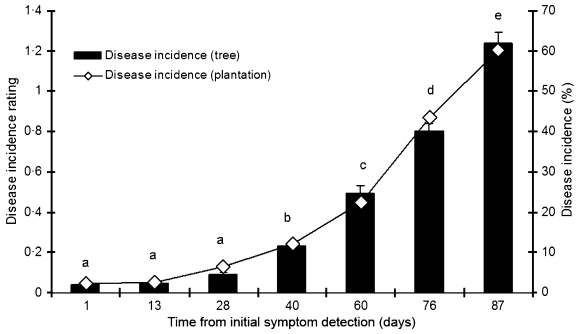

Figure 7 Changes in incidence levels within a spotted gum plantation and average disease incidence (+1 SE) levels on 808 trees assessed over an 87-day period based on a 0–4 rating scale at site 2. The same letters above the bars designate means that do not differ significantly (Fisher's PLSD test $P < 0{\cdot}0001$).

inoculum. *Quambalaria pitereka* is typical of a polycyclic fungus (Van der Plank, 1963) with the potential to produce multiple disease cycles within a short period of time when environmental conditions are favourable for disease development and spore production. Aggregation of infection is a common distribution pattern for plant diseases and usually indicates an initial random distribution of primary inoculum followed by secondary spread from newly diseased plants (Brown, 1997). The lack of an initial random distribution in this study reflects the fact that mature trees within the plantation represent point sources of inoculum. The maintenance of the spatially aggregated infection pattern over time, and disease gradients observed at the study sites, support the notion that subsequent disease spread occurred from newly infected plants within the plantations.

Disease gradients within both plantations were initially steep. However, at site 2, the primary inoculum source was less obvious than at site 1. The gradients at site 1 changed over time as the influence of the primary source of inoculum from native spotted gum was diluted by an increasing level of secondary inoculum from infected plantation trees. Despite spotted gum being common in surrounding vegetation, disease initiation was clearly from spotted gum trees at the edge of the plantation or within the plantation. Observation of a gradient in disease development implies the existence of a local source of inoculum, since background inoculum from a large number of distant sources is more likely to result in a uniform distribution of disease (Gregory, 1968).

While difficult to determine without spore trapping, this pattern of spread is most likely to be influenced by the height of the canopy of the native spotted gum at the edge of and within the plantation. The lowest branch with foliage was more than 10 m above the ground. It must also be considered that those plantation trees closest to the mature trees could be slower growing and flushes of new and susceptible foliage less common as a result of increased competition for nutrients (Bi *et al.*, 2002). Nutrient status, tree size, growth rate and canopy shape all have indirect effects on pathogen infection (Burdon & Chilvers, 1982).

Spread of primary inoculum is likely to be through splash-dispersal and wind-driven rain. Weather changes or storm fronts in the study areas develop in the south-west, generally moving in a north to north-easterly direction, similar to the direction of disease spread observed at site 1. Splash-dispersal and wind-driven rain are, therefore, likely mechanisms of spread for *Q. pitereka*, and for many pathogens deposition rates decrease with distance from the inoculum source (McCartney *et al.*, 2006). The high concentration of infected trees close to inoculum sources suggests that the distance of spread from primary infection sources is relatively short, although it is likely to be influenced by the height of the mature spotted gum trees, all of which were >20 m tall.

The disease gradient observed at site 2 could be the result of two factors: an internal source of inoculum through infected nursery stock, or spatial variation in the site where some sections have more favourable conditions for disease development (Madden *et al.*, 2008). The area in the north-eastern part of the block where the disease gradient was steepest is situated in a gully where

water was observed pooling at the base of trees during assessments. This would have an impact on leaf wetness periods and provided conditions more favourable for spore germination and infection. It would also influence host conditions with increased stomatal opening favoured by high relative humidity and moderate temperatures (Larcher, 1980). Conversely, the south-western part of the block is on a more exposed ridge where the leaf wetness period is likely to be shorter as a result of greater air flow and the absence of surface water. The peak in disease levels later in disease development in the south-west of the plantation may reflect the influence of an increased number of infected plantation trees south-west of the block assessed, or the increased influence of distant or other inoculum sources.

Species of *Corymbia* other than spotted gum may also be sources of *Q. pitereka* inoculum initiating disease development within plantations. *Corymbia tessellaris* is common in the surrounding vegetation at site 2 and has previously been reported as a host (Pegg *et al.*, 2011). However, Pegg *et al.* (2011) also found that isolates of *Q. pitereka* from a range of spotted gum species had relatively restricted host ranges and did not cause infection on all species of *Corymbia*. The interaction of different *Corymbia* spp. and *Quambalaria* isolates and variability in susceptibility associated with these species requires further investigation to determine their importance as sources of primary inoculum.

Rate of spread and development of *Q. pitereka* within both plantations was initially slow but increased exponentially over time. This acceleration in disease incidence is typical of many diseases of crop plants and reflects the initial phase of disease spread of polycyclic pathogens from a single or a couple of inoculum sources (Van der Plank, 1963; Madden, 1980; Scott *et al.*, 2003). In trees, this is typically followed by the influence of each subsequently infected tree providing a new source of inoculum (Brown, 1997). Conidiophore and conidial production by *Q. pitereka* occurs within 10–14 days after infection under favourable environmental conditions (Pegg *et al.*, 2009), so the rapid completion of a number of infection cycles is likely to occur.

As the number of infected trees increased across the plantation, the impact on individual infected trees, disease incidence and severity, became greater. Based on the results of the controlled inoculation studies, spore concentration was one of the major factors influencing disease incidence and severity levels. As the number of infected trees increases, not only will the spread of the disease across a plantation be affected, but the amount of inoculum produced will increase along with a subsequent increase in disease incidence and severity on individual trees. Each lesion represents a potential source of inoculum for spread within and between trees. The speed of a polycyclic epidemic is largely influenced by incubation and latent periods (Hau & De Vallavieille-Pope, 2006) and the disease cycle for *Q. pitereka* is rapid. Growing plants in dense stands is also likely to contribute to the development of severe epidemics. Increase in density increases the number of target plants and the probability that a unit of airborne inoculum will be intercepted over a given horizontal distance of flight (Burdon & Chilvers, 1982). Thus, current practice by plantation owners to plant at higher density in areas where *Q. pitereka* is prevalent (G. S. Pegg, unpublished data) to compensate for losses to disease, may inadvertently result in higher levels of infection and more severe damage to seedlings.

Infected foliage and green stems of plantation-grown spotted gum were clearly an important source of secondary *Q. pitereka* inoculum. Spore production on infected foliage declined dramatically over a matter of weeks, indicating persistence over extended periods of unfavourable climatic conditions is unlikely. In general, fungal spore production is influenced by a number of factors including low relative humidity, high or low temperatures, or even heavy rainfall or strong winds, resulting in removal and exhausting the source of spores (Hau & De Vallavieille-Pope, 2006). For *Q. pitereka*, spore production and survival on senesced spotted gum leaves appeared to be even shorter-lived than those on infected foliage and stem material still attached to the tree, making senesced leaves unlikely to have been a source of inoculum for further disease spread. Studies on rusts and mildews have shown that spore production increases rapidly to a maximum and then decrease gradually to zero, producing a typical asymmetrical bell shaped curve (Hau & De Vallavieille-Pope, 2006). While this was not observed in the present studies, spore production was highest when monitoring commenced less than a week after lesions were detected, suggesting a similarly rapid increase in spore production levels.

Although the role of mature, spotted gum trees as the primary source of inoculum of *Q. pitereka* is clear, there is little understanding of the process of spore production and survival of the pathogen during the cooler drier months. Simpson (2000) reported isolating *Q. pitereka* from symptomless foliage and stems, suggesting that the pathogen has an endophytic stage to its life cycle. However, this would then suggest that the pathogen would have no effect on tree growth until stress results in disease development, which has not been observed in the case of *Q. pitereka*. Pegg *et al.* (2008) identified *Q. pitereka* sporulating from cankers on woody stems on mature native spotted gum and in association with hail wounds on stems of 2-year-old trees. It is not, however, known how long these infections remain viable and what might trigger sporulation. It may be possible that *Q. pitereka* has an epiphytic stage similar to *Q. cyanescens*, which survives on bark of spotted gum (Pegg *et al.*, 2008). Weather patterns no doubt play a significant role in initiating spread and are also likely to trigger primary sporulation.

While understanding of *Q. pitereka* epidemiology is still limited, this study has clearly identified some of the key factors associated with initiation of disease and continued spread of disease within plantations of spotted gum. Breeding programmes to select resistance or identify

trends in susceptibility to *Q. pitereka* rely on field-based trials. The present study indicates that trials need to be strategically located to optimize exposure to repeated infection by *Q. pitereka* and that conditions conducive to repeated infection are needed to produce meaningful results. The results of this study should be considered in order to improve current breeding strategies and to provide industries with viable options and assessment strategies for future plantation development in subtropical and tropical regions of eastern Australia.

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