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Jeremy Brawner[a], Yani Japarudin[b], Mahadir Lapammu[b], Redzuan Rauf[b], David Boden[c] & Michael J Wingfield[d]

[a] Forest Industries Research Centre, University of the Sunshine Coast, Maroochydore, Queensland, Australia

[b] Sabah Softwood Berhad, Tawau, Sabah, Malaysia

[c] Boden and Associates Pty Ltd, Cooroy, Queensland, Australia

[d] Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa

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Evaluating the inheritance of *Ceratocystis acaciivora* symptom expression in a diverse *Acacia mangium* breeding population§

Jeremy Brawner[1]*, Yani Japarudin[2], Mahadir Lapammu[2], Redzuan Rauf[2], David Boden[3] and Michael J Wingfield[4]

[1] *Forest Industries Research Centre, University of the Sunshine Coast, Maroochydore, Queensland, Australia*
[2] *Sabah Softwood Berhad, Tawau, Sabah, Malaysia*
[3] *Boden and Associates Pty Ltd, Cooroy, Queensland, Australia*
[4] *Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa*
* *Corresponding author, e-mail: jbrawner@usc.edu.au*

A dramatic rise in the incidence of a serious canker and wilt disease of *Acacia mangium* has led to the replacement of thousands of hectares of plantation forests in eastern Sabah. A disease screening program was initiated to evaluate levels of disease resistance and tolerance to the causative fungus, *Ceratocystis acaciivora*, in an *A. mangium* breeding population. Resistance was evaluated as the presence or absence of external symptoms in two open-pollinated progeny trials. In addition, tolerance was evaluated in one of these trials by measuring the size of lesions produced following a controlled inoculation with *C. acaciivora*. Heritability estimates were low to moderate for growth traits but were close to zero for the range of traits used to evaluate *Ceratocystis* resistance and tolerance. Nevertheless, significant differences were found among the three sources (families from the local land race, Queensland and Papua New Guinea origins, all selected in progeny trials in Sabah) and among populations within these sources for many of the traits used to assess damage by the pathogen. Significant differences in lesion length among sources were evident, but no differences among populations within sources were found. Differences among sources and populations would have been reduced as seed was sourced from a progeny within provenance trial where hybridisation among origins and populations would have occurred. Results of this study suggest that modest genetic improvement may be realised from selecting among populations and sources for resistance and tolerance to this pathogen. However, the lack of additive genetic variation will make the development of resistant breeds challenging.

Keywords: *Acacia mangium*, *Ceratocystis*, genetic parameters, resistance, tolerance

Introduction

Acacia mangium plantations have expanded in the tropics over the past two decades following trials that identified this species as highly productive and suitable for the development of planted forests (Turnbull et al. 1983; Harwood and Williams 1991; Griffin et al. 2011). For some time, the species was relatively disease-free in plantations and extensive areas were established to supply fibre for pulp and paper making. First-rotation plantations of *A. mangium* have been highly productive in many countries but subsequent rotations have been increasingly damaged by various pathogens. In advanced rotations, the most significant disease in *A. m*angium has been root rot, primarily caused by *Ganoderma philippii* (Lee 2000; Rimbawanto 2006; Coetzee et al. 2011). This root rot is widespread in many regions of South-east Asia. Other diseases that have increased in severity in acacia plantations as the estate size has increased are pink disease (*Corticum salmonicolor*; Old et al. 2000) and gall rust (*Atelocauda digitata*; Lee 1999).

In areas where *A. mangium* has been planted for multiple rotations in Indonesia, Malaysia and Vietnam, a new disease caused by the canker and wilt pathogen *Ceratocystis acaciivora* has become established. This pathogen is very closely related to the mango wilt pathogen *C. magninecans* (Tarigan et al. 2011). Given that the taxonomy of this group of fungi is complex and is currently being resolved, the name *C. acaciivora* is used herein.

Ceratocystis fungi are virulent pathogens of a wide range of plants and have been associated with many commercially important forest trees (Wingfield et al. 1993). Symptoms of *Ceratocystis* canker and wilt disease in *A. mangium* include cracked or sunken bark above cankers and blackened streaks within the vascular tissue. Infections are associated with wounds on stems caused by pruning or animal damage and there appears to be a close relationship between infection and insect vectors. Ambrosia beetles (*Coleoptera*: *Scolytinae*) such as *Xylosandrus crassiusculus* commonly infest the stems of infected trees and are thought to be the primary vectors of the pathogen in Malaysia. Often, a fermentation exudate including yeasts and bacteria is associated with foam arising from beetle entrance holes near stem cankers. This foam appears to attract nitidulid beetles (*Coleoptera*: *Nitidulidae*), which are often found

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aggregating on the bark above cankers and are also thought to be involved in the dissemination of the pathogen.

Following widespread mortality in *A. mangium* plantations in south-east Sabah, two experiments were established to evaluate systems that could be used to clarify the genetic architecture of *Ceratocystis* resistance and tolerance. The concept was to develop a rapid and cost-effective screening system that could be used to screen large numbers of clones, families or seed sources in replicated field trials. Resistance to the disease was evaluated using the presence or absence of external disease symptoms and tolerance was evaluated following controlled inoculation of stems with cultured *C. acaciivora*. Evaluating resistance using natural infection would be the preferred option given its simplicity, but this approach suffers from problems such as uneven infection in field trials. Controlled inoculation with the causal pathogen provides a consistent disease challenge but is more complicated as this involves careful choice of isolates following DNA-based identification, production of sufficient quantities of inoculum and destructive assessment following inoculation. This study presents results from a pair of pedigreed progeny trials assessed to evaluate *Ceratocystis* symptoms in order to estimate the genetic control of resistance and tolerance in a diverse *A. mangium* population.

Methods

Two progeny trials of *A. mangium* were established to evaluate the genetic control of resistance and tolerance to *Ceratocystis* canker and wilt disease. A total of 93 open-pollinated families, comprised of seed collected from selected trees with above-average growth and stem form, were evaluated. The families were from three seed sources: selected mother trees of the local Sabah land race (source 1), and mother trees of Papua New Guinea (PNG) origin (source 2) and Queensland (QLD) origin (source 3) selected in a local Sabah provenance–progeny trial. A total of 15 populations were designated within the three sources (Table 1). The Malaysian land race was principally derived from Queensland populations (Sim 1984). The PNG- and QLD-origin selections were trees in seedling seed orchards incorporating families from a range of PNG and QLD provenances. It is highly likely that these family seedlots contain a proportion of inter-source hybrids arising from pollen movement within the seed orchards and from nearby Malaysian land race plantations. Therefore, families from the three sources are more closely related than families from natural provenances would be.

The families were established in two progeny trials using single-tree-plot designs with 20-tree incomplete blocks arranged within 20 replications. A total of 2 000 trees were established in each trial with some imbalance among families caused by limitations in the number of seedlings available for some families. Because the trials were planned to run for less than one year, a very compact spacing of 1.5 m × 1.5 m was used to increase the number of families that could be evaluated within a limited area. The two trials were established adjacent to *A. mangium* stands that were severely damaged by *Ceratocystis* in order to increase the chances that natural infection would occur.

Assessment of symptoms from natural infection

Both trials were assessed for symptoms caused by natural infection six months after establishment. As there were few differences in disease incidence between the trials, Trial 1 was selected for the controlled inoculation study. Assessments were undertaken when mean tree height was 2.5 and 3.0 m in trials 1 and 2, respectively. Symptoms assessed included a subjective score of crown health on a 1–4 ordinal scale (1 = least healthy, to 4 = most healthy) and the following absence/presence (0 or 1) variables: borer damage (evidence of beetle damage), presence of white foam exuding from cankers, nitidulid beetle presence, gummosis (presence of resin or black sap stain on bark), sunken bark indicative of cankers, presence of fungal fruiting bodies (excluding those of known root rot fungi), bark cracking often found above cankers and root rot. As well, a derived binomial (0 or 1) variable termed 'Cerato' was created with the value of 1 assigned to a tree where any of the following

Table 1: Genetic material included in two *A. mangium* screening trials to evaluate resistance and tolerance to *Ceratocystis acaciivora*

Source	Population	Trial 1		Trial 2	
		Families	Seedlings	Families	Seedlings
Malaysian land race	Gum Gum	12	241	12	233
	SFI	16	368	15	347
	SSB	1	19	1	19
Papua New Guinea	Bandanber	2	35	2	39
	Bimadebun	2	30	2	31
	Deri-Deri	1	18	1	14
	Dimisisi	7	130	8	148
	Gubam Boite	14	255	10	180
	Pongaki	5	88	5	89
	Unknown PNG	2	36	2	39
Queensland	Bloomfield	5	90	5	93
	Ingham	7	128	6	114
	Unknown QLD	3	52	3	48
	Shelbourne Bay	14	294	15	297
	Cairns	1	20	2	37
	Total	92	1 804	89	1 728

symptoms were present: white foam, gummosis, sunken bark, fruiting bodies or bark cracking.

Inoculation study

Prior to the inoculation study in progeny Trial 1, *C. acaciivora* was collected from symptomatic trees and isolated on malt extract agar (MEA; 20 g malt extract, 20 g Biolab agar per 1 litre water). The identity of pure cultures was verified by sequencing the ITS region of the ribosomal DNA and comparing the sequences to those of known cultures of this fungus (Tarigan et al. 2010). The isolate of the fungus chosen for inoculation is maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Inoculum was bulked on MEA in petri dishes to obtain sufficient material for the inoculation study.

Inoculation was carried out by removing a piece of bark 1 cm in diameter and replacing this with a similar-sized plug of agar taken from the edges of a freshly grown culture. The extracted bark disc was replaced above the inoculum to ensure close contact of the culture with the cambium and the entire inoculation site was sealed tightly with plastic film to reduce chances of contamination or desiccation. While the inoculation was typically undertaken on the main stem at 1 m above ground, the height varied in order to avoid inoculations near stem defects, abnormally large branches or multiple leaders. Lesions resulting from the inoculation were measured after stripping the bark from above and below the point of inoculation. Tolerance to fungal development was evaluated as the ability of the tree to restrain fungal growth, with the length and width of the lesions resulting from fungal infection used to quantify tolerance. The area of the lesion was also evaluated, assuming each lesion presented as an oval ($\pi/4 \times$ length $\times$ width).

At seven months after planting, a preliminary inoculation of 200 trees (20 trees from each of 10 randomly selected families) was undertaken to determine the time required between inoculation and assessment. One of these 10 families was used as a control with sterile media used for inoculation. Rather than the expected six weeks between inoculation and assessment, a period of three weeks was determined to be sufficient for lesion growth. The main inoculation study was conducted between eight and nine months after planting when trees averaged 3.3 m in height. Given the large number of trees involved, the inoculation study was undertaken in four stages, separated by two weeks, to ensure that the time between inoculation and assessment was consistent across all families in each stage. Families were selected at random for each stage, with all trees in each selected family inoculated in sequence. Two families used as controls in the main inoculation study were inoculated using sterile media. Three weeks after inoculation, the bark was removed from each stem for lesion assessment.

Statistical analysis

Data from the assessment of natural infection were used to estimate the heritability of traits using the Markov Chain Monte Carlo generalised linear mixed model (MCMCglmm) package (Hadfield 2010) within R (R Development Core Team 2013) as all *Ceratocystis* variables were either ordinal or binomial. For all generalised linear mixed models, the error variance was fixed at one and the variance of the link function (logit for binomial traits and probit for ordinal traits) was included in the estimate of phenotypic variance. Each trial was analysed separately using a reduced model that included fixed replication effects and a random effect for open-pollinated families. The incomplete block effect was not included in the final model as *a priori* analyses showed this random effect was not significant for any trait. The heritability estimates derived from these variance components are biased upwards as seed-source and population effects were not accounted for so that the variation from these factors was pooled with family effect. Further analyses with expanded models that included seed-source and population-within-source effects were undertaken to assess the significance of these effects using the glm procedure of the base R installation. The models used for these expanded analyses were the same as the complete models used for the inoculation study described below.

Data from the assessment of the inoculation study were analysed with ASReml (Gilmour et al. 2009) to produce genetic parameter estimates of heritability and the proportion of population variance (Brawner et al. 2011). A complete linear model and various reduced models were used to evaluate the importance of the three genetic strata represented in the trial (Pegg et al. 2013; Lee et al. 2015). The complete linear model included fixed effects for replication, source and stage of inoculation with random effects included for incomplete blocks, population within source and family within population. A covariate indicating the height of inoculation was included in the model. Reduced models that did not account for source and population effects were used to examine the degree of upward bias in heritability estimates that resulted from the inflation of additive variance caused by excluding these higher level genetic strata in the linear model. Taylor series approximations were used to estimate standard errors of genetic parameters and Wald *F*-tests were used to determine the significance of including fixed effects in the mixed model.

Heritability estimates ($\hat{h}^2$) from the generalised linear model of binomial or ordinal data were calculated as where the error variance was fixed at one and the variance of the link function is $\pi^2/3$ for the binomial traits and 1 for the ordinal crown health trait. For the normally distributed height, diameter and lesion length assessments of the inoculation study, the error variance was estimated from the model and there was no link function variance. The proportion of population variance ($\hat{p}^2$) was also estimated as a ratio of the population variance to within-population phenotypic variance. The numerator of the heritability and the proportion of population variance estimates differed while the denominator was the same so that comparisons between these estimates are direct (Hodge and Dvorak 2012).

Results

Assessment of resistance using external symptoms

The absence of external symptoms was used to infer resistance to natural infection six months after the establishment of both trials. A list of symptoms and their incidence

is provided in Table 2 with box plots describing mean family disease incidence in Figure 1. In both trials, the incidence of symptoms was low (<5%) with the exception of the presence of gummosis, sunken bark, fruiting bodies and bark cracking. These four prevalent symptoms were combined into a derived trait named 'Cerato', which designated the disease to be present if one or more of these symptoms was evident. Figure 1 presents box plots for each source depicting the median, quartiles and range of family mean disease incidence in these trials as well as tree height six months after planting. Table 2 presents the overall trial average for each trait and degree of differentiation among the various genetic strata. The tests of significance among source and population effects presented in Table 2 are derived using a conservative likelihood ratio based chi-square test.

For height assessed six months after planting, analysis of variance showed no significant difference among the three sources in either trial ($p > 0.15$ and $p > 0.13$), whereas there were highly significant differences ($p < 0.01$) among populations within sources. Heritability estimates, which were biased upward with the use of a reduced model, indicated high levels of genetic control over height growth in both trials (Trial 1 $\hat{h}^2 = 0.75$, 95% CI = [0.54, 0.93], Trial 2 $\hat{h}^2 = 0.27$, 95% CI = [0.21, 0.46]). The lack of differences among the three sources for tree height was further examined using less conservative single degree of freedom contrasts between the local land race and the PNG or QLD sources, which both indicated that the local land race was smaller ($p < 0.05$).

Heritability estimates for the symptom presence/absence data used to infer resistance are presented in Table 2. Heritability estimates were practically zero for most of the traits, with the exception of the sunken bark symptom and the derived Cerato variable in Trial 2 ($\hat{h}^2 = 0.12$). Comparisons for the incidence of the derived Cerato variable were made among the three sources using a reduced generalised linear model that included replication, source and population effects. The Cerato estimate for the local land race was significantly ($p < 0.01$) lower than estimates for both the PNG and QLD sources in Trial 1 (35%, vs 40% and 41%) and Trial 2 (34%, vs 43% and 41%), respectively. Tests of significance for among-source and among-population differences are listed in Table 2 for all traits. Genetic parameter estimates indicated there was little additive genetic variation within these populations available for selection against any of the symptoms assessed in these two trials. On the other hand, significant differences were found among sources and populations within sources for some of the symptoms (Table 2) with the derived Cerato trait consistently demonstrating significant differences. Identifying populations with lower disease incidence may be achieved using the natural infection approach.

Assessment of lesion size in the inoculation study

The inoculation study was used to produce estimates of heritability and the proportion of population variance for height, diameter, lesion length, lesion width and lesion area approximately three weeks after inoculation. For all traits, heritability estimates were produced using a complete model as well as models that were reduced by removing the source, population or both higher-level genetic effects from the complete model. This model reduction procedure was used to evaluate the degree of inflation in heritability estimates caused by not accounting for differing degrees of population structure.

When considering the complete rather than the reduced linear model, heritability estimates were relatively low for both growth traits assessed in these trials, which may be expected at such a young age. For height, a slightly higher level of variation was noted among populations ($\hat{p}^2$) than within populations ($\hat{h}^2$). Estimates of genetic variation among populations for lesion traits were effectively zero. Heritability estimates indicate the derived 'lesion area' trait provides better discrimination among families relative to measurements of lesion width or length alone. Nevertheless, all estimates of genetic control for lesion assessments were low, with REML estimates of variation among families for lesion width typically constrained at the theoretical limit of zero. When both source and population were dropped from the model, a consistent upwards bias in estimates of additive genetic variation provided evidence that population structure is present. Examination of the standard errors for $\hat{h}^2$ and $\hat{p}^2$ in Table 3 indicated that these estimates did not differ from zero, and log-likelihood tests comparing complete and reduced models verified that among-family variation was not significant for lesion traits ($p > 0.1$). There appeared to be very little or zero additive genetic variation or variation among the populations tested

Table 2: Average crown health scores and incidence (%) of *Acacia mangium* trees with symptoms associated with *Ceratocystis acaciivora* infection at six months in two screening trials. Heritability estimates ($\hat{h}^2$) and 95% confidence intervals and the significance of differences among seed sources (S) and populations (P) are also provided

	Trial 1				Trial 2			
	Average (%)	$\hat{h}^2$ (±95%)	S	P	Average (%)	$\hat{h}^2$ (±95%)	S	P
Crown health (1–4)	3.6	0.43 (0.24, 0.61)	ns	***	3.7	0.25 (0.16, 0.37)	ns	ns
Borer	2.1	0.001 (0, 0.22)	ns	ns	0.6	0.003 (0, 1.20)	ns	ns
Nitidulid	0.2	0.01 (0, 0.08)	.	ns	0.1	0.001 (0, 0.02)	ns	ns
Gummosis	10.2	0.001 (0, 0.14)	**	.	8.6	0.001 (0, 0.25)	ns	ns
White foam	0.5	0.001 (0, 1.15)	ns	ns	0.1	0.001 (0, 0.07)	ns	ns
Sunken bark	23.6	0.001 (0, 0.17)	*	.	24.4	0.12 (0, 0.24)	***	ns
Fruiting body	7.8	0.0004 (0, 0.10)	ns	*	5.8	0.01 (0, 0.08)	ns	ns
Bark cracking	23.7	0.001 (0, 0.21)	.	*	25.1	0.001 (0, 0.21)	**	ns
Cerato	37.8	0.001 (0, 0.18)	**	*	38.7	0.12 (0, 0.23)	***	.

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, . $p < 0.1$, ns $p > 0.1$

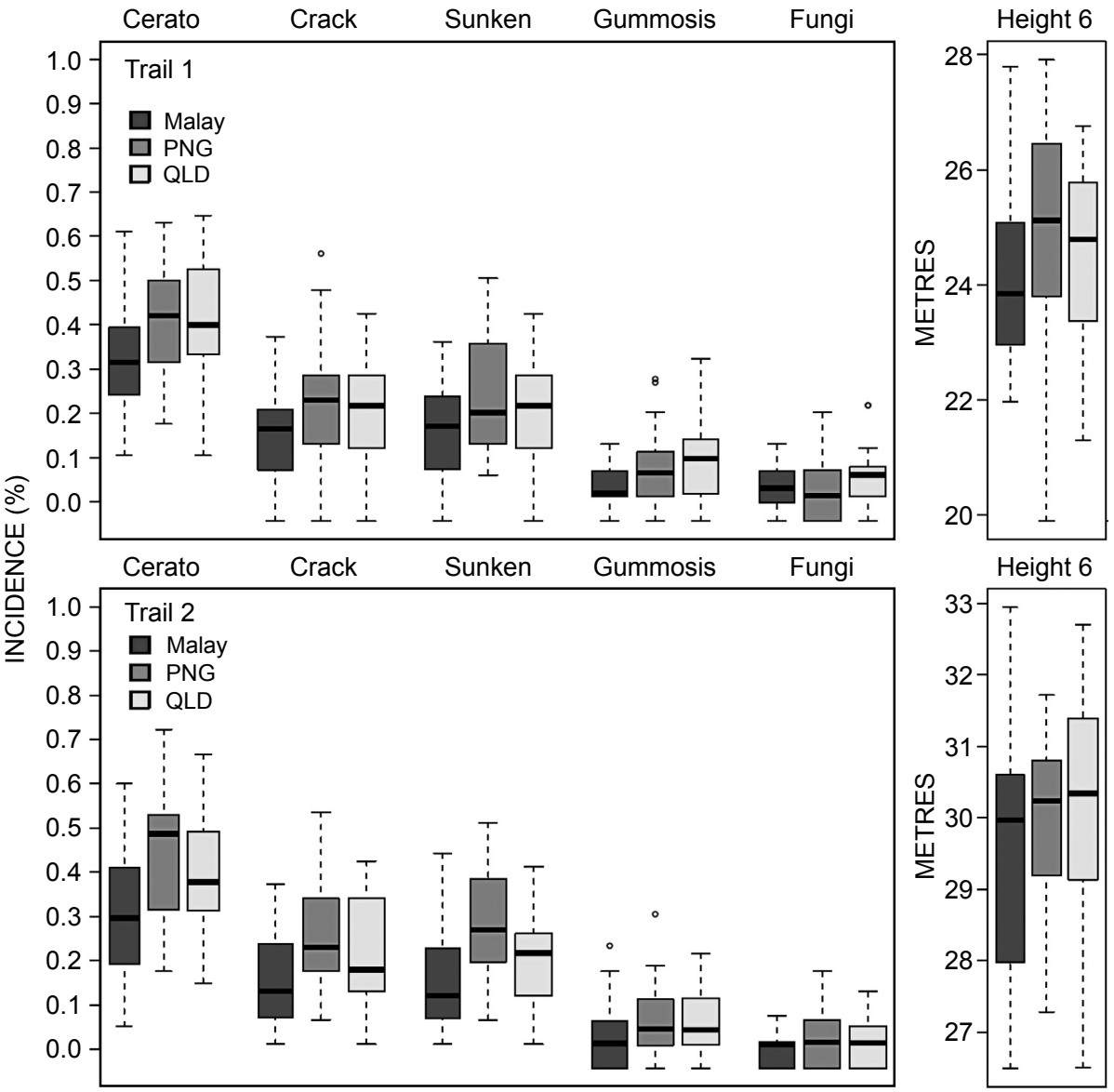

Figure 1: Box and whisker plots showing the median, quartiles and range of family means for external symptoms and height for each source assessed six months after planting in Trials 1 and 2. External symptoms include the derived Cerato variable (presence of one or more of the following symptoms: white foam, gummosis, sunken bark, fruiting bodies or cracks in the bark), cracks in the bark, sunken bark, gummosis or exudation, and the presence of fruiting bodies from fungi

Table 3: Genetic parameter estimates indicating low levels of tolerance attributable to additive genetic effects (heritability; $\hat{h}^2$) and proportion of variance attributable to populations ($\hat{p}^2$) within sources from Queensland, New Guinea and the Malaysian local land race for diameter at breast height (DBH), height, lesion length, lesion width and lesion area in a disease screening trial established in eastern Sabah. Parameters were estimated using models that include various levels of genetic control including source, population within source and family within population. The significance of differences among sources estimated using a complete model assuming fixed source effects is also provided

Model	DBH		Height		Lesion length		Lesion width		Lesion area	
	$\hat{h}^2$	$\hat{p}^2$	$\hat{h}^2$	$\hat{p}^2$	$\hat{h}^2$	$\hat{p}^2$	$\hat{h}^2$	$\hat{p}^2$	$\hat{h}^2$	$\hat{p}^2$
Source, Population, Family	0.09 (0.05)	0.03 (0.05)	0.03 (0.02)	0.05 (0.05)	0.02 (0.04)	0.00 (0.00)	0*	0*	0.05 (0.04)	0.00 (0.00)
Source, Family	0.20 (0.07)	NE**	0.13 (0.06)	NE	0.02 (0.04)	NE	0*	NE	0.05 (0.04)	NE
Population, Family	0.10 (0.05)	0.03 (0.05)	0.04 (0.03)	0.05 (0.05)	0.02 (0.04)	0.01 (0.01)	0*	0*	0.04 (0.05)	0.00 (0.01)
Family	0.28 (0.08)	NE	0.15 (0.06)	NE	0.04 (0.04)	NE	0.00 (0.00)	NE	0.06 (0.05)	NE
p Source	$p < 0.05$		$p < 0.05$		$p < 0.001$		$p > 0.05$		$p < 0.001$	

* Family and/or population variance constrained at zero boundary, ** $\hat{p}^2$, NE = not estimable

in their ability to constrain growth of the *Ceratocystis* fungus once it entered the stem. On the other hand, the significantly greater lesion length of the local land race indicated there are differences in tolerance among sources.

Significance tests for differences among fixed source effects ('*p* Source' row in Table 3) were undertaken using the complete model. The differences among sources in diameter at breast height (DBH) and height were slight but significant ($p < 0.05$). Significant differences ($p < 0.001$) among the three sources for lesion length and area were evident, whereas there were no differences among sources for the lesion width assessment. For both DBH and height, the Malaysian land race was significantly smaller than either of the PNG or QLD sources, whereas there were slight differences between the PNG and QLD sources. For the lesion length, width and area assessments, the material sourced from the local land race produced significantly larger lesions than the other two sources. The proportion of population variance ($\hat{p}^2$) estimates indicated there were no differences among populations within sources for lesion size. For all three lesion traits, this was verified with standard Wald tests ($p > 0.3$) using alternative mixed models that included population as a fixed effect.

The two families used as controls in the inoculation study were excluded from the genetic analysis. Contrasts between the control and inoculated trees showed highly significant differences in lesion size An average lesion length of 37.9 cm (±0.5 cm) was found for inoculated trees and an average lesion length of 11.6 cm (±4.0 cm) for control trees. A few of the control trees were infected, either in the inoculation process or naturally, as evidenced by the large standard error for the controls.

The height of inoculation covariate recorded for the lesion traits was useful for investigating the impact of changing the height at which inoculations were made. Changing the location of the point of inoculation was necessary at times because of branches or stem deformities. The covariate of inoculation height was significant for both lesion length and area but was not significant for lesion width. The covariate estimate indicated that a 1.47 cm increase in lesion length is expected for each 10 cm increase in the height at which the inoculation is carried out. Given the average lesion length of 40 cm, increasing the height of inoculation by 10 cm would produce a lesion that is 3.7% longer.

Discussion

The study provides several clear results useful when considering the development of *Ceratocystis*-resistant breeds of *A. mangium*. Importantly, the evaluation of natural infection in provenance–progeny trials of *A. mangium* did not show any heritable variation (Table 2). Insufficient time may have been provided for the development of some symptoms and additional assessments over time could be used to identify the age at which to assess symptoms that are useful for predicting susceptibility. At the time of assessment, overall crown health scores remained high, with means of 3.61 and 3.74 in trials 1 and 2, respectively. However, inferred rates of infection by *C. acaciivora* were already high, with 37.8% and 38.1% of trees in trials 1 and 2 positive for the derived variate 'Cerato' (presence of white foam, gummosis, sunken bark, fungal fruiting bodies and/or cracks in the bark).

It must be noted that there was some uncertainty as to whether external disease symptoms scored in the trials resulted in every case from attack by *C. acaciivora*. For example, fungal fruiting bodies were not cultured or typed, and sunken bark and bark cracking might have been associated with other fungal diseases. While external symptoms will be useful for studies of disease development over time, there is clearly more work to be done before external symptoms may be reliably used to identify resistant material. It is likely that future assessments of external symptoms would focus on the presence of sunken bark as this is the only trait that appears to present a genetic signal and therefore may be amenable to improvement via parental selection.

Interestingly, the finding of significant differences among populations for external symptoms in the natural infection assessment contrasts with the near-zero estimates of the proportion of population variance in the inoculation study. The intra-population hybridisation that would have occurred in the trials where these families were sourced from would have tended to reduce among-population differences. Nevertheless, based upon the inoculation study and the most highly heritable trait of lesion area, it may be possible to identify families within the best source that better tolerate *Ceratocystis* infections. However, with a biased heritability estimate of 0.06 for lesion area, there is a low probability of identifying families that effectively reduce the size of *Ceratocystis* lesions. Similar findings have been noted in South African experiments using *A. mearnsii*, where resistant individuals were identified but no differences among families were found (Roux et al. 2000). This quantitative genetic study supports the idea that resistance genes are relatively rare in this population and either source-specific genes, or dominant or interacting alleles induce non-additive variation that regulates the traits assessed in these trials. These sources of genetic variation would only be useful if control-pollinated seed or clones were used for reforestation. Given the difficulty in producing control-pollinated seed and maintaining the juvenility required for vegetatively propagating selections of *A. mangium*, deploying genetic improvements based on non-additive effects will be extremely difficult. The constraint of physiological ageing has been overcome in the *A. auriculiformis* × *A. mangium* hybrid, providing other options for this breed.

Combining a rapid screening system with a suitable propagation strategy will be required to deploy resistance at scale. Alternative screening systems should be evaluated to reduce the time required for screening (van Wyk et al. 2010; Newhouse et al. 2013). Although this time might be reduced considerably, verification of stability between seedling and sapling assessments of lesion length requires longitudinal studies complicated by a response variable that involves death. Understanding the variability within the pathogen is also required. The extent to which these results may be generalised is unclear as only one *Ceratocystis* strain was used and many races of the pathogen have been described (Witthuhn et al. 1999; Ferreira et al. 2010). For example, one particularly virulent strain may have been selected for this inoculation. Further screening with an increased

number of strains is planned and a reduced number of clonally replicated host genotypes is planned so that the importance of differences in strains and their interaction with hosts may be understood.

Within the controlled inoculation experiment, a clear contrast in the genetic architecture of growth and disease traits with and without population structure is evident. For growth, there is clear population structure, with the exclusion of the population term leading to large upward biases in the heritability estimates. This supports evidence provided by Arnold and Cuevas (2003) that demonstrated large differences among provenances and smaller family within provenance differences in an *A. mangium* progeny trial established in the Philippines. On the other hand, there is little additive variance accounted for by differences among sources for lesion size, as evidenced by the small change in heritability estimates between a complete model and one that excludes a source effect. Genetic variation for a wide range of disease-related traits is low; however families derived from the local land race produce larger lesions when inoculated with *Ceratocystis* than families from QLD and PNG. Whether or not this variability in tolerance will lead to differences in survival and productivity in operational plantation requires further investigation (Brawner et al. 1999). For now, families with differences in their level of tolerance have been identified so that further studies may be undertaken to confirm the repeatability of results and determine the practical significance of these differences in a plantation environment. Further screening involving clonally replicated parents as well as their offspring is underway to examine the genetic architecture of *Ceratocystis* tolerance in more detail.

While operational decisions to drop *Acacia mangium* from the plantation program in certain parts of eastern Sabah may be disappointing, one may remain optimistic that resistance could be developed over time. Until that time, reliance on alternative species will be required and further work on developing appropriate silvicultural systems for these species will be a priority for research and development teams. If a positive outcome may be found from this outbreak of *Ceratocystis*, it would be that this has served as a reminder that alternative species domestication programs must be maintained in commercial forestry research and development programs. Specifically, this has resulted in a large number of species-provenance trials being established in eastern Malaysia and more generally there is a genuine interest in hedging bets across a diverse set of species rather than planting only one highly productive species.

Conclusions

Screening *A. mangium* for resistance and tolerance to *C. acaciivora* may be completed in a relatively short time frame; however, developing a *Ceratocystis*-resistant breed of *A. mangium* will not be a simple process. Results from the evaluation of external symptoms in two diverse provenance–progeny trials revealed that there is little heritable variation in the expression of *Ceratocystis* symptoms and that there are some significant differences in disease incidence among populations and sources. While intra-population hybridisation in the breeding population would be expected to reduce among-family, among-population and among-source differences, statistically significant differences were found among sources and populations for many of the traits used to assess resistance or tolerance. Significant differences between the local land race and the QLD or PNG origins indicated that the local land race exhibited a lower incidence of external symptoms than the two other sources. However, when tolerance following inoculation was evaluated the local land race developed significantly larger lesions and there were no significant differences in lesion length found among populations within sources. Although little heritable variation was evident in this screening, individuals with no external symptoms and high tolerance following infection were present in this study. It is recommended that further work to improve the disease assay be undertaken and that these improved methods are used to evaluate clonally replicated *A. mangium* populations for tolerance to *Ceratocystis* infection.

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