Screening for host responses in Acacia to a canker and wilt pathogen, Ceratocystis manginecans

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<th>Journal:</th>
<th>Forest Pathology</th>
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<td>EFP-OA-2017-052.R1</td>
</tr>
<tr>
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</table>
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Mohammed, Caroline; University of Tasmania, School of Land and Food |
| Subject Area: | Canker < Disease type, Acacia < Host genus, chemical ecology |
Screening for host responses in *Acacia* to a canker and wilt pathogen, *Ceratocystis manginecans*

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Summary

In Vietnam, the productivity of *Acacia* hybrid (*Acacia mangium* x *A. auriculiformis*) plantations is being threatened by an aggressive canker pathogen, *Ceratocystis manginecans* and selection for tolerance is the main control strategy. A pot trial was established in Binh Duong province to screen for the host response of nine *Acacia* genotypes (six *Acacia* hybrid clones, two *A. auriculiformis* clones and mixed provenance seedlings of *A. mangium*) to artificial inoculation with three isolates of *C. manginecans*. Lesion lengths as measured on the inner bark suggested that the two *A. auriculiformis* clones were relatively more tolerant to *C. manginecans* than the *A. mangium* genotype. In contrast, the lesion lengths of all six *Acacia* hybrid clones fell between the *A. auriculiformis* and *A. mangium* genotypes. The results of this study indicate that among the *Acacia* hybrid clones, BV10 showed the most tolerance to *C. manginecans*. Chemical analysis of crude sapwood extracts sampled from the lesion provided some evidence that induced phenolic compounds, particularly
tetrahydroxyflavanone and condensed tannins may have a defensive role in the Acacia – C. mangoecans pathosystem. However, results were not consistent across individual Acacia hybrid clones and A. mangium genotypes.

Key words: Acacia genotypes, basidiomycetes, Ceratocystis mangoecans, phenolic compounds
1 INTRODUCTION

Over the last decade, a vascular wilt and stem canker disease caused by a species of Ceratocystis has become the most damaging disease of Acacia, especially A. mangium, causing large scale mortalities in Indonesia, Vietnam and Malaysia (Tarigan, Roux, Van Wyk, Tjahjono & Wingfield 2011; Thu, Quynh & Dell 2012; Brawner, Japarudin, Lapammu, Rauf, Boden & Wingfield 2015). First described in Indonesia as C. acaciivora Tarigan & M. van Wyk (Tarigan et al. 2011), recent molecular studies have identified this pathogen as C. manginecans M. van Wyk, AlHAdawi & M.J. Wingf. (Fourie, Wingfield, Wingfield & Barnes 2015). Other authors consider that several recently described new species including C. acaciivora and C. manginecans, are populations within a large species complex for which the most appropriate name is C. fimbriata Ellis & Halst. (Oliveira, Harrington, Ferreira, Damacena, Al-Sadi, Al-Mahmooli & Afenas 2015). By 2015, this Ceratocystis wilt and canker pathogen was affecting approximately 2000 ha of Acacia plantations across Vietnam (Plant Protection Department 2015). A recent study estimated that the incidence of this disease on A. auriculiformis, A. mangium and Acacia hybrid plantations ranged from 7.1 – 12.5%, 9.2 – 18.4% and 10.2 – 18.2%, respectively (Thu, Chi & Tam 2016).

Clones of Acacia hybrid, the natural hybrid between Acacia auriculiformis Benth. and A. mangium Willd, are the most widespread plantation species established in Vietnam (Beadle et al. 2013) with a total of approximately 400,000 ha planted (Nambiar & Harwood 2014). Although Acacia hybrid is largely grown to supply the domestic demand for pulpwood and wood chips for the export market (Bueren 2004; Nambiar, Harwood & Kien 2015), a significant proportion of the Acacia hybrid estate is increasingly being managed for solid wood, mainly for furniture (Kha, Harwood, Kien, Baltunis, Hai & Thinh 2012). Silvicultural practices required to produce solid wood from Acacia include singling, pruning
and thinning (Trang, Glen, Eyles, Ratkowsky, Beadle & Mohammed 2017). Wounds thus created have been shown to facilitate the entry of pathogens including *C. manginecans* (Tarigan, Wingfield, van Wyk, Tjahjono & Roux 2011).

In host-pathogen interactions, phenolic compounds such as stilbenes, flavonoids, lignans and tannins have been shown to play a major role in chemical defence following fungal invasion in some woody plants (Eyles, Davies & Mohammed 2003; Eyles, Davies, Yuan and Mohammed 2003; Woodward, Bianchi, Bodles, Beckett & Michelozzi 2007; Wallis, Eyles, Chorbadjian, Gardener, Hansen, Cipollini, Herms & Bonello 2008; Eyles, Bonello, Ganley & Mohammed 2010). In temperate plantations of pruned *E. nitens* (Deane & Maiden) Maiden, the reaction zone typically contained four- to six-fold more polyphenolic compounds than the sound sapwood (Barry, Pearce & Mohammed 2000; Barry, Pearce, Evans, Hall & Mohammed 2001), although the amount was influenced by the extent of wood decay caused by the different decay fungi present (Barry, Davies & Mohammed 2002). The phenolic chemistry of *A. auriculiformis* and *A. mangium* has been examined previously however, these studies focused on heartwood extractives (Barry, Mihara, Davies, Mitsunaga & Mohammed 2005; Mihara, Barry, Mohammed & Mitsunaga 2005; Barry, Irianto, Tjahjono, Tarigan, Agustini, Hardiyanto & Mohammed 2006). To our knowledge, this is the first paper to characterize the phenolic profile induced by fungal inoculation in the sapwood of *Acacia* species.

This paper investigated the host responses of nine *Acacia* plantation genotypes to three isolates of the canker and wilt pathogen, *C. manginecans*. This study aimed to link host tolerance, as indicated by lesion size with the localised accumulation of phenolic chemistry (i.e. condensed tannins, total phenolics as well eight selected individual phenolic compounds)
in the sapwood of all Acacia genotypes. Understanding potential chemical markers of
tolerance or susceptibility could be of value for determining Acacia hybrid clones showing
higher host tolerance to fungal attack.

2 MATERIALS AND METHODS

2.1 Plant material

A total of nine Acacia genotypes comprising two A. auriculiformis, six Acacia hybrids and
mixed provenance seedlings of A. mangium were used in this study. Full details of the genetic
history of each Acacia genotype are detailed in Table 1.

2.2 Fungal material

Three C. manginecans cultures isolated from Acacia hybrid trees in Vietnam were selected as
inoculum (Table 2). The identities of C. manginecans were determined from DNA sequence
data of the rDNA ITS and β-tubulin genes. DNA fragments were amplified using primers
ITS1-F/ITS4 (White, Bruns, Lee & Taylor 1990) and Bt1a/Bt1b primers (Glass & Donaldson
1995), respectively. All isolates are being stored at the Vietnamese Academy of Forest
Sciences. Cultures were prepared by subculturings from stock culture to PDA in 90-mm-
diameter Petri dishes and incubating at room temperature (25 °C) for 15 days.

2.3 Pot trial site and experiment design
The pot trials were located at Bau Bang station, Binh Duong province, southern Vietnam (Latitude: 11°27'74.3"N and Longitude: 106°63'35.5"E). The climate in southern Vietnam is characterised by distinct dry and wet seasons, the latter receiving > 90% of the total annual rainfall of 1500 – 2500 mm from May to November; the mean annual temperature is 27.6 – 28.6 °C with little monthly variation. In June 2013, 32 clonally replicated trees from each of eight Acacia clones provided by the South-eastern Forest Research and Experimental Centre and 32 seedling trees of A. mangium provided by the Institute of Forest Tree Improvement and Biotechnology were planted in 20 cm diameter pots. In September 2013, the trees were transferred to 50 cm diameter pots. Pots were spaced 1 x 1.5 m apart. Each pot was irrigated daily with 3 L of water using an automatic irrigation water system.

The experiment was set up as a randomised complete block design, with five fungal treatments for each of the nine Acacia genotypes and four blocks (replicates). Fungal treatments consisted of three isolates of C. mangenicans (C1, C2 and C3) and two types of controls (mock wounded and unwounded trees), giving a total of 20 trees per Acacia genotype.

The diameters (at 1.3 m tree height above pot surface) and heights of trees were measured once, just prior to inoculation. All trees of each of the genotypes were of similar diameter (3.76 ± 0.11 cm; mean ± standard error) and height (491 ± 8 cm).

2.4 Experimental fungal inoculation

In August 2014, 14-month-old trees were inoculated with a fungal isolate on the stem 50 cm above the soil. In brief, the bark was removed with a sterile borer (10 mm diameter) and a 10
mm diameter PDA plug colonized with 15-day-old mycelia (fungal inoculation) or no fungi (mock inoculation: to control for potential effects of wounding alone on induced responses (Eyles et al. 2007) was placed mycelium-side down onto the cambium. The wounds were wrapped with Parafilm to retain the inoculum and limit desiccation and contamination.

2.5 Lesion length assessment

Host resistance was based on lesion length, which is an appropriate estimate of relative host resistance in this and other canker and heart rot systems (Blodgett, Eyles & Bonello 2007; Guimaraes, Resende, Lau, Rosse, Alves & Alfenas 2010; Brawner et al. 2015). Trees were destructively harvested 23 days after inoculation with three C. manginecans isolates. The lesion length that developed over bark (OB) was measured first and then the bark was removed to measure the under bark (UB) lesion length.

2.6 Wood extraction and analysis of phenolic compounds

An 80-cm length of stem centered on the inoculation site was cut from the main seedling. This stem length was halved longitudinally through the inoculation wound with a blade (Fig. 1). A cordless drill was used to obtain shavings of sapwood from the following locations:

- in inoculated treatment — the infected region (Fig. 1),
- in wounded control treatment — adjacent to (above) the inoculation site,
- in unwounded control treatment — healthy sapwood adjacent to (above) at a similar height to the other treatments.

Drill bits were sterilised with ethanol (70%) and flamed for 30 seconds between each sampling. Fresh shavings (0.5 mg) were extracted twice with 1 mL of 100% grade methanol
over 24 hours in the dark at 4 °C. The pooled extracts were transferred to a 2 mL tube and stored in a freezer (-20 °C) until transported to the University of Tasmania under quarantine permit (IP14010539) and then stored at -80 °C in a freezer until analysed.

Samples were analysed by UPLC-UV-MS using a Waters Acquity H-series UPLC coupled to a Waters Acquity Photo Diode Array (PDA) detector connected in series with a Waters Xevo triple quadrupole mass spectrometer. A Waters Acquity UPLC BEH C18 column (2.1 x 100 mm x 1.7 µ particles) was used. The solvents were 1% acetic acid in water (Solvent A) and acetonitrile (Solvent B) at a flow rate of 0.35 mL/min, with initial conditions of 98%A: 2%B for 0.5 min then a linear ramp to 44%A:56%B at 15 minutes, followed by a linear ramp to 5 %A:95 %B at 20 min, with a 1 min hold at the final value before re-equilibration for 3 minutes to initial conditions. Injection volume was 2.5 µL. The PDA was monitored from 230 nm to 500 nm at a resolution of 1 nm and data for quantitative measurements were extracted at 280 nm. A small number of peaks observed on the 280 nm chromatogram were selected for individual quantitation. Condensed tannin response was estimated from the area of the ‘hump’ observed underneath all the individually eluting phenolic compounds by subtracting the area of all individual peaks from the area of the whole chromatogram.

The mass spectrometer was operated in negative ion electrospray ionisation mode with needle voltage of 2.8 KV, scanning from m/z 120 to 1200 every 0.25 s with a cone voltage of 45 V. Phenolic compounds potentially present based on previous studies on Acacia heartwood were also initially monitored by Selected Ion Monitoring (SIM) at m/z 271, 287, 289, 303, 305 and 479, with 35 ms dwell time on each ion. The ion source temperature was 130 °C, the desolvation gas was nitrogen at 950 L/hr, the cone gas flow was 50 L/hr and the desolvation temperature was 450 °C. Data were analysed using MassLynx and TargetLynx software.
Reference standards of teracacidin and 2,3-trans-3,4',7,8-tetrahydroxyflavanone were available. Eight individual phenolic compounds were measured with reference to a catechin (Merck) standard curve (1-20 µg mL\(^{-1}\) dissolved in acetone) and results were expressed as catechin equivalent per mg fresh weight of wood.

Individual phenolic compounds 1 to 8 were denoted as Cp1 to Cp8, respectively. They were observed at 4.20, 5.02, 5.68, 5.92, 6.52, 6.80, 7.40 and 8.33 minutes, respectively (Fig. 2).

**2.7 Statistical analysis**

Two Acacia genotypes, BV10 and BV33, were characterised by very thick bark and exploratory analysis of the OB lesion lengths for these genotypes showed that they were very short compared to the UB lesion lengths (i.e. mean OB lesion lengths were 3.3 and 2.8 cm whereas averaged UB lesion lengths were 16.0 and 20.8 cm, respectively for BV10 and BV33). As such, UB rather OB lesion lengths were used to examine treatment effects – previous screening trials of Ceratocystis sp. have similarly measured lesions formed under the bark (Roux, Van Wyk, Hatting & Wingfield 2004; Brawner et al. 2015).

Two-way analysis of variance (ANOVA) was used to test the effects of block, Acacia genotype, fungal isolates and the interactions of genotype and isolate on diameter, height, lesion length and phenolic chemistry (total phenolic concentration, condensed tannins and eight selected phenolic compounds).

The concentrations of phenolic compounds in both the mock wounded and unwounded control treatments were very low compared with the inoculated treatment, therefore the
treatment effects were examined for inoculated trees only. Full details of effect of treatment
on constitutive chemistry are presented in supplementary tables (Supplementary Tables 1 –
3). The assumptions of ANOVA such as homogeneity of variance and the Gaussian
distribution were evaluated by the use of quantile – quantile plots and residual plots for all
variables. Only the phenolic data required log transformation to produce normalised
distributions of residuals. Fisher’s protected least significant difference post hoc tests were
used to determine significant differences among treatment means. All analyses was

3 RESULTS

3.1 Relative host response of nine Acacia genotypes to inoculation with three
C. manginecans isolates

Lesion length was significantly influenced by Acacia genotype (Fig. 3 and Table 3). The
lesion length of AM were significantly higher than that of AA1 and AA9 by 3.1-fold and 3.6-
fold, respectively. The lesion length of the six Acacia hybrid clones (AH1, AH7, BV10,
BV33, TB12 and TB6) fell between that of AA1, AA9 and AM. Out of the six Acacia hybrid
clones, the lesion length of BV10 was most similar to that of AA1.

Lesion length was significantly affected by fungal isolate (Table 3). Lesion length of isolate
C3 was significantly higher by 93.1% and 36.6% than that of isolate C2 and C1, respectively.
Lesion length of isolate C2 was significantly longer by 41.4% than that of isolate C1 (Fig. 4a).

3.2 Characterisation of phenolic compounds
Analysis of *Acacia* crude wood extracts by UPLC-UV-MS indicated the presence of a complex range of phenolic compounds (Fig. 2 and Table 4). The identity of Cp2 was unequivocally confirmed by direct comparison with a standard. Other related flavanones, Cp4 and Cp6, were identified on the basis of UV, MS, and tandem MS evidence only and not by comparison with authentic standards. The other five phenolic compounds were tentatively identified as unknown flavonoids.

### 3.2.1 Induced phenolic chemistry

With the exceptions of Cp6 and Cp7, phenolic chemistry was significantly influenced by *Acacia* genotype (Tables 3 and 5). The concentrations of total peaks, condensed tannins and seven compounds (except Cp2) were similar for AA1, AA9 and AM. Among all of the *Acacia* genotypes, BV10 had the highest concentrations of total peaks, condensed tannins, and Cp1, Cp3, Cp4, Cp5 and Cp8.

Fungal isolates did not affect the concentrations of total peaks, condensed tannins and seven phenolic compounds with the exception of Cp4 (Table 3). Concentration of Cp4 (a tetrahydroxyflavanone) induced by *C. manginecans* isolate C3 was significantly lower than isolates C1 and C2 by approximately 65 and 53%, respectively (Fig. 4b).

### 4 DISCUSSION
In this study, lesion lengths in response to inoculation with *C. manginecans* varied significantly among *Acacia* genotypes. These data indicated that *A. auriculiformis* was significantly more tolerant to *C. manginecans* than *A. mangium*, and this response was consistent for all three isolates of *C. manginecans*. Since the discovery of *C. manginecans*, there has been a series of resistance screening trials with *Acacia* in Indonesia, Malaysia and Vietnam (Tarigan et al. 2011; Thu et al. 2012; Chen, Wyk, Roux, Wingfied, Xie & Zhou 2013; Brawner et al. 2015; Tarigan, Yuliarto, Gafur, Yong & Sharma 2016). Levels of tolerance to *C. manginecans* in *A. mangium* are low and resistance is rarely observed but other species such as *A. auriculiformis* show greater tolerance. The lesion length of the five *Acacia* hybrid clones in this study fell between the two *A. auriculiformis* clones and *A. mangium* genotypes, confirming that a gradient of tolerance exists in hybrids.

Reports of host tolerance or resistance for the same *Acacia* genotype have not always been consistent. For example, in our study of young trees, *C. manginecans* elicited lesions in AH1 and AH7 but these same genotypes appeared resistant in a previous field trial (Nghia, Thu & Chi 2013). Such variation in response may indicate evidence of ontogenetic resistance or conversely, tolerance as indicated in artificial inoculation trials at a young age may not be indicative of field tolerance at a later age when trees are exposed to conditions that may promote disease such as regular wounding by animals, high loads of inoculum and strains with different virulence. In our study, lesion length indicated that isolate C3 was the most aggressive while isolate C1 was the least aggressive of the three isolates, regardless of *Acacia* genotype. A wide variation in the pathogenicity of *C. manginecans* has been shown in other studies such as Thu et al. (2012).
Given the observed higher degree of host tolerance of *A. auriculiformis* and its hybrids to *C. manginecans* and the variation in the response to three isolates, we hypothesised that these differences could be related to the induction of phenolic compounds, as has been previously reported in many woody tree species (Barry et al. 2005; Mihara et al. 2005; Woodward et al. 2007; Sherwood & Bonello 2013; Chen, Chen, Yeh & Chang 2014; Araujo, Bispo, Rios, Fernandes & Rodrigues 2016). The concentration of Cp4 induced by isolate C3 was significantly lower but the lesion length of isolate C3 was the longest, providing some evidence, although correlational, that the induction of this compound may have a defensive role in the *Acacia – C. manginecans* pathosystem. However, although significant differences in phenolic profiles were generally demonstrated among the *Acacia* genotypes regardless of *C. manginecans* isolate, the changes in the concentrations of the eight selected phenolic compounds and total phenolic compounds did not consistently relate well to the observed variation in host tolerance as indicated by lesion size. Although the lesion lengths of the *Acacia* hybrid clones ranged between the *A. auriculiformis* clones and *A. mangium* genotypes, the concentrations of phenolic compounds of the *Acacia* hybrid clones were, in general, the same or higher than that observed for *A. auriculiformis* clones and *A. mangium* genotypes. For example, the concentrations of total peaks, Cp1, Cp3, Cp4, Cp5 and Cp8 in BV10 were significantly higher than in either the *A. auriculiformis* clones or *A. mangium* genotypes.

Phenolic Cp2, identified as 2,3-trans 3,4',7,8 tetrahydroxyflavanone has been previously identified at significantly higher levels in the heartwood of *A. auriculiformis* compared to *A. mangium* (Barry et al. 2005; Mihara et al. 2005; Barry et al. 2006). This compound showed antifungal activity against *Phellinus noxius* and *P. badius* using *in vitro* bioassays (Mihara et al. 2005) and it was suggested that it accounted for the lower susceptibility of *A. auriculiformis* to heart rot. However, Cp2 did not appear to be associated with tolerance to
C. manginecans as concentrations induced in sapwood were higher in A. mangium compared to A. auriculiformis. Cp2 was even detected in sapwood of A. mangium (0.16 µg/mL) in unwounded control trees (Supplementary Table 3).

There was a trend for higher concentrations of condensed tannins associated with shorter lesions, e.g. the concentrations of condensed tannins in AA1, AA9 and BV10 clones were significantly higher than in TB6 and TB12 whereas the lesion length was significantly shorter in AA1, AA9 and BV10 than in TB6 and TB12. The accumulation of condensed tannins as an indicator of tolerance to Ceratocystis pathogens has been previously described (El Modafar, Clerivet & Macheix 1996; Brignolas, Lieutier, Sauvard, Christiansen & Berryman 1998; Hammerbacher, Paetz, Wright, Fischer, Bohlmann, Davis, Fenning, Gershenzon & Schmidt 2014) and for A. auriculiformis and Acacia hybrid BV10, condensed tannins may have a defensive role in the Acacia – C. manginecans pathosystem. The high concentrations of condensed tannins in A. mangium appears to contradict the involvement of condensed tannins in a defensive role but those accumulated in A. mangium may be of a different type to those in the more tolerant Acacia genotypes.

This pioneer study has revealed some promising phenolic markers for investigating host responses in Acacia to invasion by fungi although more research is required to understand the phenolic chemistry associated with host tolerance. We can confirm that a clear gradient of tolerance to C. manginecans, as indicated by lesion lengths, exists in Acacia species. This variation must be fully exploited, especially the transference of tolerance from A. auriculiformis to A. mangium through hybridisation. Acacia hybrid, the natural hybrid between A. mangium and A. auriculiformis, is a key multipurpose plantation species that is increasingly being planted across Vietnam for both sawn timber and pulpwood products.
Acknowledgments

This work was supported by the Australian Centre for International Agricultural Research (ACIAR) through a John Allwright Fellowship to the senior author and ACIAR Project FST/2006/087 and the Tasmanian Institute of Agriculture and School of Land and Food, University of Tasmania.

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caused by *Chrysoporthe cubensis*. *Genetics and Molecular Biology*, 33, 525-531.

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fungus *Ceratocystis polonica*. *Plant Physiology*, 164, 2107-2122.

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Forests*, 43, 13-29.

antifungal and antioxidant activities of *Acacia mangium* and *A. auriculiformis* heartwood


Plant Protection Department, (2015). Dispatch Number 2400/BVTV-QLSVGHR dated 01/12/2015 of Plant Protection Department on reporting on a number of emerging pests and prevention results, 9 pp.


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FIGURE 1 Representative photo showing inoculation wound and lesion caused by *Ceratocystis mangenicans* on *Acacia* hybrid (BV33) observed on sapwood. Black arrow indicates where the tissue was sampled.
FIGURE 2 HPLC-UV chromatogram (280 nm) of a 100% methanol extract of (A) *Acacia auriculiformis*, (B) *A. mangium* and (C) *Acacia* hybrid (TB12) 23 days after inoculation with *Ceratocystis manginecans* isolate C1. Identities of peaks are as follows: 1, unknown flavonoid; 2, 2,3-trans 3,4',7,8 tetrahydroxyflavanone; 3, unknown flavonoid; 4, a tetrahydroxyflavanone; 5, unknown flavonoid; 6, Putative 4',7,8 trihydroxyflavanone; 7, unknown flavonoid; 8, unknown flavonoid.
FIGURE 3 Effect of *Acacia* genotype on lesion lengths 23 days after inoculation with three *Ceratocystis manginecans* isolates. Different letters show significant differences at \( p \leq 0.001 \) (N = 12 trees). See Table 1 for details of *Acacia* genotypes.
FIGURE 4 Effects of Ceratocystis manginecans isolates (C1, C2 and C3) on lesion lengths 23 days after inoculation on nine Acacia genotype (A) and concentrations of phenolic compound Cp4 (a tetrahydroxyflavanone) extracted from the sapwood of Acacia trees (B). Different letters show significant differences at $p < .001$. (N = 36 trees)
TABLE 1 Genetic background of the selected *Acacia* planting material

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<th>Genotype</th>
<th>Origin of genetic material</th>
<th>Note</th>
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<tr>
<td></td>
<td>AA9</td>
<td>AA9 trial in Dong Nai (Nghia &amp; Chien 2007a).</td>
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<tr>
<td><em>auriculiformis</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>Acacia</em> hybrid</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>Father = <em>A. auriculiformis</em> Darwin (Northern Territory, Australia) provenance (Kha 2000).</td>
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<tr>
<td><em>Acacia</em> hybrid</td>
<td>AH1</td>
<td><em>Acacia</em> hybrid plantations in Dong Nai and Binh Duong,</td>
<td>AH1 and AH7: recognised as superior clones by</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Acacia hybrid plantations in Dong Nai and Binh Duong, Vietnam (Nghia &amp; Chien 2007b).</td>
<td></td>
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<tr>
<td>---------------------------------</td>
<td>-------------------------------------------------------------------</td>
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<tr>
<td>TB12 Mother = Mossman (Queensland, Australia) provenance.</td>
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<tr>
<td>TB6 Mother = Mossman (Queensland, Australia) provenance.</td>
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<tr>
<td>TB6 Father = possibly Oenpelli (Northern Territory, Australia) provenance (Chis Harwood pers.comm.).</td>
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<td>TB12 Father = possibly Oenpelli (Northern Territory) provenance.</td>
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<table>
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<th>Acacia mangium (seedlings)</th>
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<tr>
<td>AM</td>
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<td>Mixed provenance seedlings - Papua New Guinea (PNG).</td>
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**TABLE 2** GenBank accession numbers for ITS and β-tubulin sequences of *Ceratocystis manginecans* isolates.

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<th>ITS accession #</th>
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<td><em>Ceratocystis manginecans</em></td>
<td>C3</td>
<td>MF033457</td>
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TABLE 3 Summary of a two-way ANOVA that examined the effects of nine *Acacia* genotypes and three *Ceratocystis manginecans* isolates on lesion lengths and concentrations of induced phenolic compounds. The two controls were not included in this analysis. N = 4

<table>
<thead>
<tr>
<th>Response variables*</th>
<th>Acacia Genotype</th>
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<td>0.01</td>
<td>0.06</td>
<td>0.10</td>
</tr>
<tr>
<td>Condensed tannins</td>
<td>0.003</td>
<td>0.20</td>
<td>0.08</td>
</tr>
<tr>
<td>Cp1</td>
<td>&lt;0.001</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>Cp2</td>
<td>&lt;0.001</td>
<td>0.52</td>
<td>0.02</td>
</tr>
<tr>
<td>Cp3</td>
<td>&lt;0.001</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Cp4</td>
<td>&lt;0.001</td>
<td>0.01</td>
<td>0.17</td>
</tr>
<tr>
<td>Cp5</td>
<td>&lt;0.001</td>
<td>0.40</td>
<td>0.03</td>
</tr>
<tr>
<td>Cp6</td>
<td>0.05</td>
<td>0.20</td>
<td>0.12</td>
</tr>
<tr>
<td>Cp7</td>
<td>0.28</td>
<td>0.84</td>
<td>0.05</td>
</tr>
<tr>
<td>Cp8</td>
<td>0.008</td>
<td>0.50</td>
<td>0.20</td>
</tr>
</tbody>
</table>

*See Table 4 for details of phenolic compounds Cp1 -8.
### TABLE 4

Characterisation of eight selected phenolic compounds from the crude wood extracts of *Acacia* genotypes after infection with *Ceratocystis manginecans*

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>Molecular weight</th>
<th>UV maxima</th>
<th>Tentative identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cp1</td>
<td>286</td>
<td>289</td>
<td>Unknown flavonoid</td>
</tr>
<tr>
<td>Cp2</td>
<td>288</td>
<td>294</td>
<td>2,3-trans 3,4',7,8 tetrahydroxyflavanone*</td>
</tr>
<tr>
<td>Cp3</td>
<td>318</td>
<td>286</td>
<td>Unknown flavonoid</td>
</tr>
<tr>
<td>Cp4</td>
<td>288</td>
<td>289</td>
<td>a tetrahydroxyflavanone</td>
</tr>
<tr>
<td>Cp5</td>
<td>302</td>
<td>286</td>
<td>Unknown flavonoid</td>
</tr>
<tr>
<td>Cp6</td>
<td>272</td>
<td>293</td>
<td>Putative 4',7,8 trihydroxyflavanone</td>
</tr>
<tr>
<td>Cp7</td>
<td>286</td>
<td>284</td>
<td>Unknown flavonoid</td>
</tr>
<tr>
<td>Cp8</td>
<td>328</td>
<td>277</td>
<td>Unknown flavonoid</td>
</tr>
</tbody>
</table>

* Identification based on retention time, mass spectral and UV spectrum consistent with those of a standard.
**TABLE 3** Effects of nine *Acacia* genotypes on the induced phenolic chemistry 23 days after inoculation with *Ceratocystis manginecans*. Values shown are the means of concentrations (µg/mL) of 12 trees. Means with different letters in the same row are significantly different ($p < 0.05$)

<table>
<thead>
<tr>
<th>Phenolic compounds*</th>
<th><em>A. auriculiformis</em></th>
<th><em>Acacia hybrid</em></th>
<th><em>A. mangium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA1</td>
<td>AA9</td>
<td>AH1</td>
</tr>
<tr>
<td>Total peaks</td>
<td>251.3$^a$</td>
<td>237.8$^d$</td>
<td>265.1$^a$</td>
</tr>
<tr>
<td>Condensed tannins</td>
<td>502.5$^{de}$</td>
<td>472.1$^{cde}$</td>
<td>371.4$^a$</td>
</tr>
<tr>
<td>Cp1</td>
<td>7.4$^{ab}$</td>
<td>6.4$^{ab}$</td>
<td>7.9$^{ab}$</td>
</tr>
<tr>
<td>Cp2</td>
<td>13.3$^{ab}$</td>
<td>10.1$^a$</td>
<td>34.0$^d$</td>
</tr>
<tr>
<td>Cp3</td>
<td>6.3$^{bc}$</td>
<td>6.3$^{bc}$</td>
<td>3.1$^a$</td>
</tr>
<tr>
<td>Cp4</td>
<td>12.1$^{bcd}$</td>
<td>12.9$^{cd}$</td>
<td>6.2$^a$</td>
</tr>
<tr>
<td>Cp5</td>
<td>8.0$^c$</td>
<td>8.2$^e$</td>
<td>3.7$^a$</td>
</tr>
<tr>
<td>Cp6</td>
<td>18.4</td>
<td>18.8</td>
<td>21.3</td>
</tr>
<tr>
<td>Cp7</td>
<td>11.5</td>
<td>11.4</td>
<td>9.8</td>
</tr>
<tr>
<td>Cp8</td>
<td>24.8$^{ab}$</td>
<td>32.7$^{ab}$</td>
<td>39.7$^{bc}$</td>
</tr>
</tbody>
</table>

*See Tables 1 and 4 for details of *Acacia* genotypes and phenolic compounds Cp1 – 8, respectively