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

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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

26 tetrahydroxyflavanone and condensed tannins may have a defensive role in the *Acacia* – *C.*
27 *manginecans* pathosystem. However, results were not consistent across individual *Acacia*
28 hybrid clones and *A. mangium* genotypes.
29
30 Key words: *Acacia* genotypes, basidiomycetes, *Ceratocystis manginecans*, phenolic
31 compounds
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1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

33 # 1 INTRODUCTION

34 Over the last decade, a vascular wilt and stem canker disease caused by a species of
35 *Ceratocystis* has become the most damaging disease of *Acacia*, especially *A. mangium*,
36 causing large scale mortalities in Indonesia, Vietnam and Malaysia (Tarigan, Roux, Van Wyk,
37 Tjahjono & Wingfield 2011; Thu, Quynh & Dell 2012; Brawner, Japarudin, Lapammu, Rauf,
38 Boden & Wingfield 2015). First described in Indonesia as *C. acaciivora* Tarigan & M. van
39 Wyk (Tarigan et al. 2011), recent molecular studies have identified this pathogen as
40 *C. manginecans* M. van Wyk, Al-Adawi & M.J. Wingf. (Fourie, Wingfield, Wingfield &
41 Barnes 2015). Other authors consider that several recently described new species including
42 *C. acaciivora* and *C. manginecans*, are populations within a large species complex for which
43 the most appropriate name is *C. fimbriata* Ellis & Halst. (Oliveira, Harrington, Ferreira,
44 Damacena, Al-Sadi, Al-Mahmooli & Alfenas 2015). By 2015, this *Ceratocystis* wilt and
45 canker pathogen was affecting approximately 2000 ha of *Acacia* plantations across Vietnam
46 (Plant Protection Department 2015). A recent study estimated that the incidence of this
47 disease on *A. auriculiformis*, *A. mangium* and *Acacia* hybrid plantations ranged from 7.1 –
48 12.5%, 9.2 – 18.4% and 10.2 – 18.2%, respectively (Thu, Chi & Tam 2016).
49

50 Clones of *Acacia* hybrid, the natural hybrid between *Acacia auriculiformis* Benth. and
51 *A. mangium* Willd, are the most widespread plantation species established in Vietnam
52 (Beadle et al. 2013) with a total of approximately 400,000 ha planted (Nambiar & Harwood
53 2014). Although *Acacia* hybrid is largely grown to supply the domestic demand for
54 pulpwood and wood chips for the export market (Bueren 2004; Nambiar, Harwood & Kien
55 2015), a significant proportion of the *Acacia* hybrid estate is increasingly being managed for
56 solid wood, mainly for furniture (Kha, Harwood, Kien, Baltunis, Hai & Thinh 2012).
57 Silvicultural practices required to produce solid wood from *Acacia* include singling, pruning

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58 and thinning (Trang, Glen, Eyles, Ratkowsky, Beadle & Mohammed 2017). Wounds thus
59 created have been shown to facilitate the entry of pathogens including *C. manginecans*
60 (Tarigan, Wingfield, van Wyk, Tjahjono & Roux 2011).

61

62 In host-pathogen interactions, phenolic compounds such as stilbenes, flavonoids, lignans
63 and tannins have been shown to play a major role in chemical defence following fungal
64 invasion in some woody plants (Eyles, Davies & Mohammed 2003; Eyles, Davies, Yuan and
65 Mohammed 2003;Woodward, Bianchi, Bodles, Beckett & Michelozzi 2007; Wallis, Eyles,
66 Chorbadjian, Gardener, Hansen, Cipollini, Herms & Bonello 2008; Eyles, Bonello, Ganley &
67 Mohammed 2010). In temperate plantations of pruned *E. nitens* (Deane & Maiden) Maiden,
68 the reaction zone typically contained four- to six-fold more polyphenolic compounds than the
69 sound sapwood (Barry, Pearce & Mohammed 2000; Barry, Pearce, Evans, Hall &
70 Mohammed 2001), although the amount was influenced by the extent of wood decay caused
71 by the different decay fungi present (Barry, Davies & Mohammed 2002). The phenolic
72 chemistry of *A. auriculiformis* and *A. mangium* has been examined previously however, these
73 studies focused on heartwood extractives (Barry, Mihara, Davies, Mitsunaga & Mohammed
74 2005; Mihara, Barry, Mohammed & Mitsunaga 2005; Barry, Irianto, Tjahjono, Tarigan,
75 Agustini, Hardiyanto & Mohammed 2006). To our knowledge, this is the first paper to
76 characterize the phenolic profile induced by fungal inoculation in the sapwood of *Acacia*
77 species.

78

79 This paper investigated the host responses of nine *Acacia* plantation genotypes to three
80 isolates of the canker and wilt pathogen, *C. manginecans.* This study aimed to link host
81 tolerance, as indicated by lesion size with the localised accumulation of phenolic chemistry
82 (i.e. condensed tannins, total phenolics as well eight selected individual phenolic compounds)

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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 subculturing 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

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107 The pot trials were located at Bau Bang station, Binh Duong province, southern Vietnam
108 (Latitude: 11°27′74.3″N and Longitude: 106°63′35.5″E). The climate in southern Vietnam is
109 characterised by distinct dry and wet seasons, the latter receiving > 90% of the total annual
110 rainfall of 1500 – 2500 mm from May to November; the mean annual temperature is 27.6 –
111 28.6 °C with little monthly variation. In June 2013, 32 clonally replicated trees from each of
112 eight *Acacia* clones provided by the South-eastern Forest Research and Experimental Centre
113 and 32 seedling trees of *A. mangium* provided by the Institute of Forest Tree Improvement
114 and Biotechnology were planted in 20 cm diameter pots. In September 2013, the trees were
115 transferred to 50 cm diameter pots. Pots were spaced 1 x 1.5 m apart. Each pot was irrigated
116 daily with 3 L of water using an automatic irrigation water system.
117
118 The experiment was set up as a randomised complete block design, with five fungal
119 treatments for each of the nine *Acacia* genotypes and four blocks (replicates). Fungal
120 treatments consisted of three isolates of *C. mangenicans* (C1, C2 and C3) and two types of
121 controls (mock wounded and unwounded trees), giving a total of 20 trees per *Acacia*
122 genotype.
123
124 The diameters (at 1.3 m tree height above pot surface) and heights of trees were measured
125 once, just prior to inoculation. All trees of each of the genotypes were of similar diameter
126 (3.76 ± 0.11 cm; mean ± standard error) and height (491 ± 8 cm).
127

128 **2.4 Experimental fungal inoculation**

129
130 In August 2014, 14-month-old trees were inoculated with a fungal isolate on the stem 50 cm
131 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

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157 over 24 hours in the dark at 4 °C. The pooled extracts were transferred to a 2 mL tube and
158 stored in a freezer (-20 °C) until transported to the University of Tasmania under quarantine
159 permit (IP14010539) and then stored at - 80 °C in a freezer until analysed.
160

161 Samples were analysed by UPLC-UV-MS using a Waters Acquity H-series UPLC coupled to
162 a Waters Acquity Photo Diode Array (PDA) detector connected in series with a Waters Xevo
163 triple quadrupole mass spectrometer. A Waters Acquity UPLC BEH C18 column (2.1 x 100
164 mm x 1.7 μ particles) was used. The solvents were 1% acetic acid in water (Solvent A) and
165 acetonitrile (Solvent B) at a flow rate of 0.35 mL/min, with initial conditions of 98%A: 2%B
166 for 0.5 min then a linear ramp to 44%A:56%B at 15 minutes, followed by a linear ramp to
167 5 %A:95 %B at 20 min, with a 1 min hold at the final value before re-equilibration for 3
168 minutes to initial conditions. Injection volume was 2.5 μL. The PDA was monitored from
169 230 nm to 500 nm at a resolution of 1 nm and data for quantitative measurements were
170 extracted at 280 nm. A small number of peaks observed on the 280 nm chromatogram were
171 selected for individual quantitation. Condensed tannin response was estimated from the area
172 of the 'hump' observed underneath all the individually eluting phenolic compounds by
173 subtracting the area of all individual peaks from the area of the whole chromatogram.
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175 The mass spectrometer was operated in negative ion electrospray ionisation mode with needle
176 voltage of 2.8 KV, scanning from *m/z* 120 to 1200 every 0.25 s with a cone voltage of 45 V.
177 Phenolic compounds potentially present based on previous studies on *Acacia* heartwood were
178 also initially monitored by Selected Ion Monitoring (SIM) at *m/z* 271, 287, 289, 303, 305 and
179 479, with 35 ms dwell time on each ion. The ion source temperature was 130 °C, the
180 desolvation gas was nitrogen at 950 L/hr, the cone gas flow was 50 L/hr and the desolvation
181 temperature was 450 °C. Data were analysed using MassLynx and TargetLynx software.

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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

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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 performed using SAS Enterprise Guide 6.1 (SAS Institute Inc., Cary, NC, USA).

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

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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

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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).

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3 282 Given the observed higher degree of host tolerance of *A. auriculiformis* and its hybrids to
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5 283 *C. manginecans* and the variation in the response to three isolates, we hypothesised that these
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7 284 differences could be related to the induction of phenolic compounds, as has been previously
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9 285 reported in many woody tree species (Barry et al. 2005; Mihara et al. 2005; Woodward et al.
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11 286 2007; Sherwood & Bonello 2013; Chen, Chen, Yeh & Chang 2014; Araujo, Bispo, Rios,
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13 287 Fernandes & Rodrigues 2016). The concentration of Cp4 induced by isolate C3 was
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15 288 significantly lower but the lesion length of isolate C3 was the longest, providing some
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17 289 evidence, although correlational, that the induction of this compound may have a defensive
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19 290 role in the *Acacia* – *C. manginecans* pathosystem. However, although significant differences
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21 291 in phenolic profiles were generally demonstrated among the *Acacia* genotypes regardless of
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23 292 *C. manginecans* isolate, the changes in the concentrations of the eight selected phenolic
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25 293 compounds and total phenolic compounds did not consistently relate well to the observed
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27 294 variation in host tolerance as indicated by lesion size. Although the lesion lengths of the
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29 295 *Acacia* hybrid clones ranged between the *A. auriculiformis* clones and *A. mangium* genotypes,
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31 296 the concentrations of phenolic compounds of the *Acacia* hybrid clones were, in general, the
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33 297 same or higher than that observed for *A. auriculiformis* clones and *A. mangium* genotypes.
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35 298 For example, the concentrations of total peaks, Cp1, Cp3, Cp4, Cp5 and Cp8 in BV10 were
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37 299 significantly higher than in either the *A. auriculiformis* clones or *A. mangium* genotypes.
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41 301 Phenolic Cp2, identified as 2,3-trans 3,4',7,8 tetrahydroxyflavanone has been previously
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43 302 identified at significantly higher levels in the heartwood of *A. auriculiformis* compared to *A.*
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45 303 *mangium* (Barry et al. 2005; Mihara et al. 2005; Barry et al. 2006). This compound showed
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47 304 antifungal activity against *Phellinus noxius* and *P. badius* using *in vitro* bioassays (Mihara et
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49 305 al. 2005) and it was suggested that it accounted for the lower susceptibility of
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51 306 *A. auriculiformis* to heart rot. However, Cp2 did not appear to be associated with tolerance to
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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.

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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

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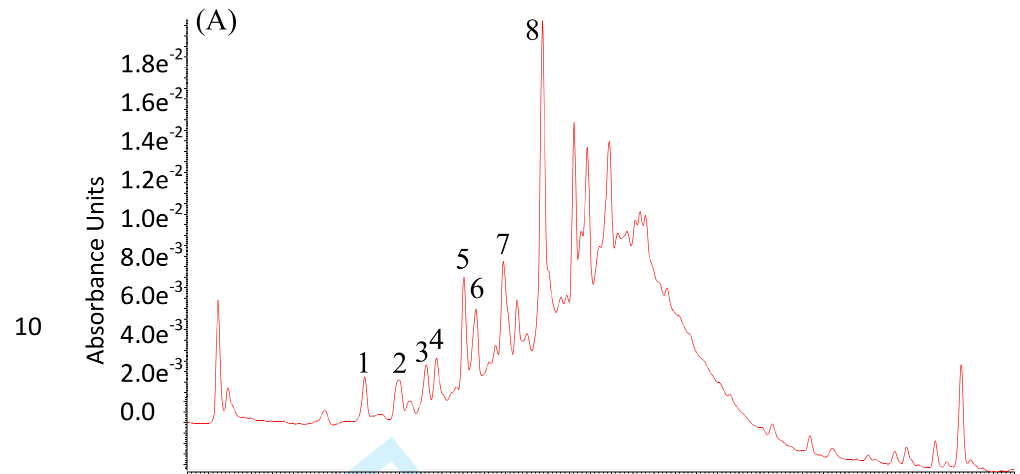

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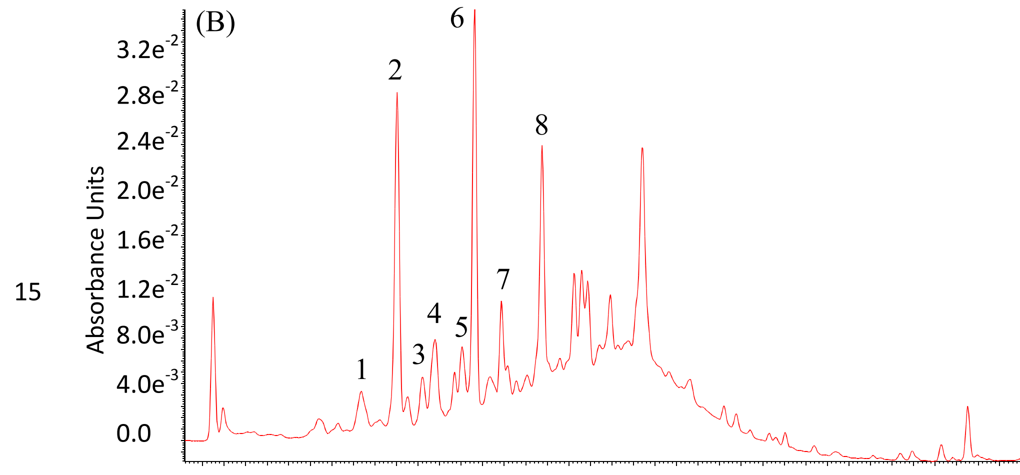

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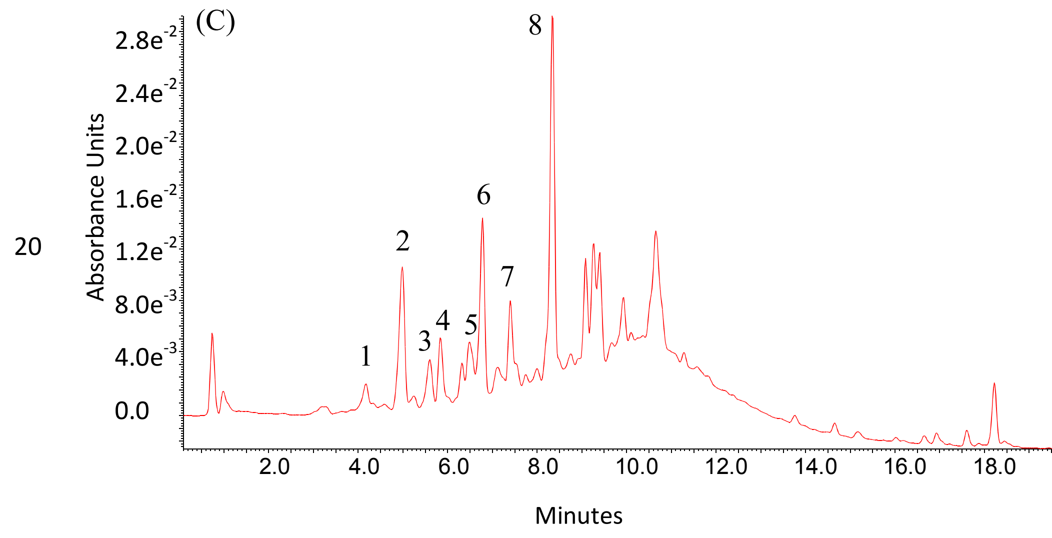

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25 **FIGURE 2** HPLC-UV chromatogram (280 nm) of a 100% methanol extract of (A) *Acacia auriculiformis*, (B) *A. mangium* and (C) *Acacia* hybrid (TB12) 23 days after inoculation with *Ceratocystis manginecans* isolate C1. Identities of peaks are as 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,
30 unknown flavonoid; 8, unknown flavonoid.

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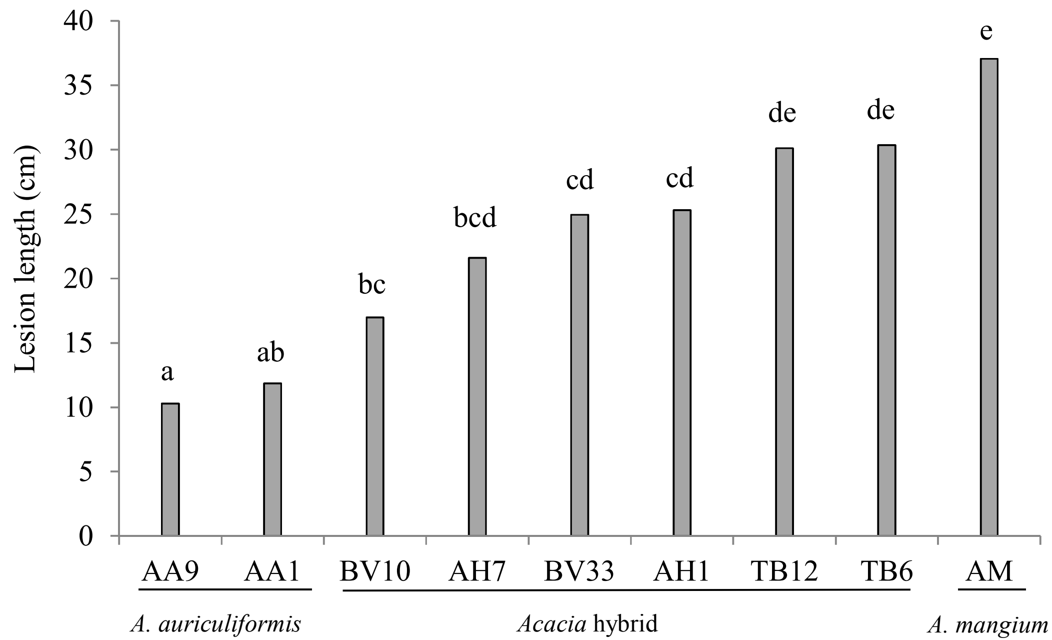

35 **FIGURE 3** Effect of *Acacia* genotype on lesion lengths 23 days after inoculation with three *Ceratocystis manginecans* isolates. Different letters show significant differences at p 0 < .001 (N = 12 trees). See Table 1 for details of *Acacia* genotypes.

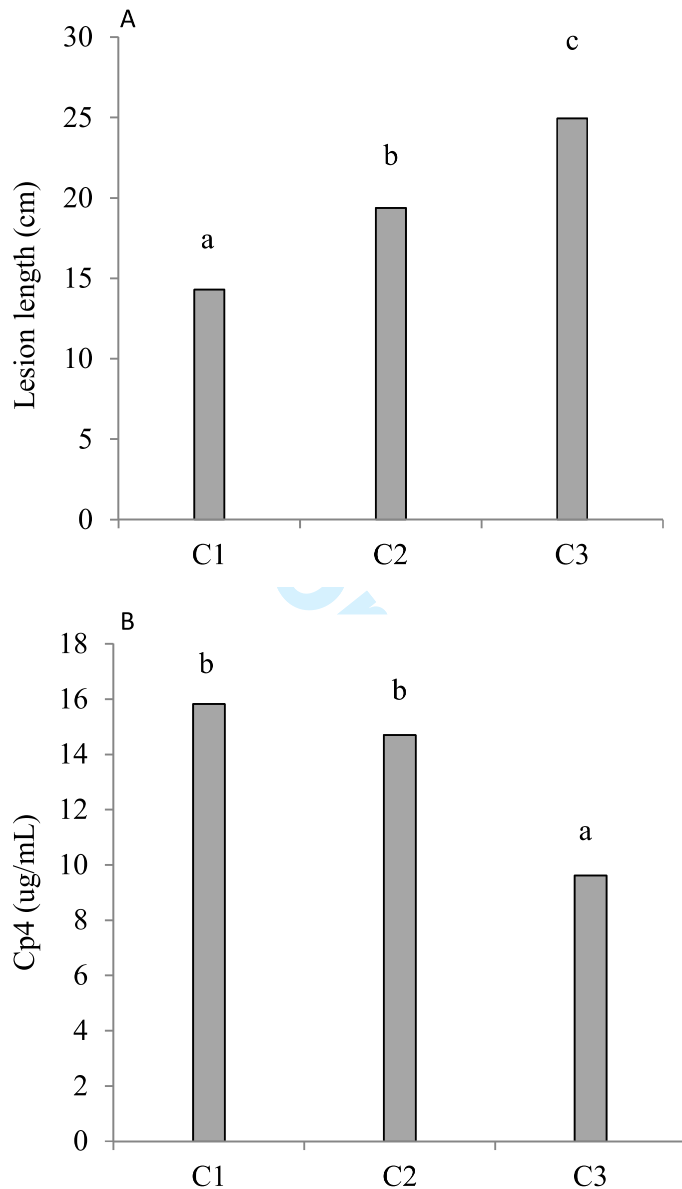

FIGURE 4 Effects of *Ceratocystis manginecans* isolates (C1, C2 and C3) on lesion lengths 23 days after inoculation on nine *Acacia* genotype (A) and concentrations of phenolic compound Cp4 (a tetrahydroxyflavanone) extracted from the sapwood of *Acacia* trees (B). Different letters show significant differences at p 0 < .001. (N = 36 trees)

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TABLE 1 Genetic background of the selected *Acacia* planting material

Taxon	Genotype number	Origin of genetic material	Note
Acacia auriculiformis	AA1	FORTIP trial in Binh Duong (Nghia & Chien 2007a).	AA1 and AA9: recognised as superior clones by the Ministry of Agricultural and Rural Development (MARD) of Vietnam in Decision No: 3377/QĐ-BNN-TCLN dated 16/12/2010.
	AA9	AA9 trial in Dong Nai (Nghia & Chien 2007a).	
Acacia hybrid (*A. mangium* x *A. auriculiformis*)	BV10	Mother = *A. mangium* Daintree (Queensland, Australia) provenance. Father = *A. auriculiformis* Darwin (Northern Territory, Australia) provenance (Kha 2000).	BV10: recognised as a superior clone by MARD in Decision No: 132/QĐ/BNN-KHCN dated 17/1/2000.
	BV33	Mother = *A. mangium* Daintree (Queensland, Australia) provenance. Father = *A. auriculiformis* Darwin (Northern Territory, Australia) provenance (Kha 2000).	BV33: recognised as a superior clone by MARD in Decision No: 1998/QĐ/BNN-KHCN dated 11/7/2006.
	AH1	*Acacia* hybrid plantations in Dong Nai and Binh Duong,	AH1 and AH7: recognised as superior clones by

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		Vietnam (Nghia & Chien 2007b).	MARD in Decision No: 3905/QĐ-BNN-TCLN dated 11/12/2007.
	AH7	*Acacia* hybrid plantations in Dong Nai and Binh Duong, Vietnam (Nghia & Chien 2007b).	
	TB12	Mother = Mossman (Queensland, Australia) provenance Father = possibly Oenpelli (Northern Territory, Australia) provenance (Chis Harwood pers.comm.).	TB6 and TB12: recognised as superior clones by MARD in Decision No: 3118/QĐ/BNN-KHCN dated 9/8/2000.
	TB6	Mother = Mossman (Queensland, Australia) provenance Father = possibly Oenpelli (Northern Territory) provenance (Chris Harwood pers.comm.).	
Acacia mangium (seedlings)	AM	Mixed provenance seedlings - Papua New Guinea (PNG).	Seeds were imported from PNG by Institute of Forest Tree Improvement and Biotechnology.

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TABLE 2 GenBank accession numbers for ITS and β-tubulin sequences of *Ceratocystis manginecans* isolates.

Species	Isolate	ITS accession #	β-tubulin accession #
Ceratocystis manginecans	C1	MF033455	MF040712
Ceratocystis manginecans	C2	MF033456	MF040713
Ceratocystis manginecans	C3	MF033457	MF040714

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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

Response variables*	*Acacia* Genotype *p*-value	*C. manginecans* Isolate *p*-value	*Acacia* genotype x fungal isolate *p*-value
Lesion length	<0.001	<0.001	0.20
Total peaks	0.01	0.06	0.10
Condensed tannins	0.003	0.20	0.08
Cp1	<0.001	0.07	0.08
Cp2	<0.001	0.52	0.02
Cp3	<0.001	0.06	0.06
Cp4	<0.001	0.01	0.17
Cp5	<0.001	0.40	0.03
Cp6	0.05	0.20	0.12
Cp7	0.28	0.84	0.05
Cp8	0.008	0.50	0.20

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

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TABLE 4 Characterisation of eight selected phenolic compounds from the crude wood extracts of *Acacia* genotypes after infection with *Ceratocystis manginecans*

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

* Identification based on retention time, mass spectral and UV spectrum consistent with those of a standard

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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 ($p\ 0 < 0.05$)

Phenolic compounds*	*A. auriculiformis*		*Acacia* hybrid						*A. mangium*
	AA1	AA9	AH1	AH7	BV10	BV33	TB12	TB6	AM
Total peaks	251.3^{a}	237.8^{a}	265.1^{a}	294.8^{ab}	435.5^{c}	407.3^{bc}	249.0^{a}	318.1^{abc}	277.6^{a}
Condensed tannins	502.5^{de}	472.1^{cde}	371.4^{a}	451.9^{abcde}	543.5^{e}	429.5^{abcd}	380.1^{ab}	381.8^{abc}	462.4^{bcde}
Cp1	7.4^{ab}	6.4^{ab}	7.9^{ab}	8.1^{ab}	37.3^{d}	22.4^{cd}	6.8^{ab}	12.1^{bc}	6.1^{a}
Cp2	13.3^{ab}	10.1^{a}	34.0^{d}	25.8^{bcd}	15.1^{abc}	73.8^{e}	30.4^{cd}	32.6^{d}	33.7^{d}
Cp3	6.3^{bc}	6.3^{bc}	3.1^{a}	6.4^{bc}	24.6^{e}	13.4^{d}	4.8^{ab}	9.1^{cd}	4.0^{ab}
Cp4	12.1^{bcd}	12.9^{cd}	6.2^{a}	12.8^{bcd}	62.8^{e}	20.7^{d}	6.7^{ab}	13.3^{cd}	7.0^{abc}
Cp5	8.0^{c}	8.2^{c}	3.7^{a}	6.7^{bc}	19.1^{d}	9.0^{c}	4.4^{ab}	6.8^{bc}	5.5^{abc}
Cp6	18.4	18.8	21.3	27.4	29.8	43.6	21.5	31.2	33.9
Cp7	11.5	11.4	9.8	12.0	9.1	15.0	10.1	11.1	15.6
Cp8	24.8^{ab}	32.7^{ab}	39.7^{bc}	43.5^{bc}	53.7^{c}	28.7^{a}	34.7^{ab}	35.6^{ab}	33.7^{ab}

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

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