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CORYMBIA PHLOEM PHENOLICS, TANNINS AND TERPENOIDS: INTERACTIONS WITH A CERAMBYCID BORER

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28
29 Running head: Chemistry of *Corymbia* bark

Plant secondary chemistry mediates the ability of herbivores to locate, accept and survive on potential host plants. We examined the relationship between attack by the cerambycid beetle *Phoracantha solida* and the chemistry of the secondary phloem (inner bark) of two differentially attacked plantation forestry taxa, *Corymbia variegata* and its hybrid with *C. torelliana*. We hypothesised that this differential rate of attack may have to do with differences in secondary chemistry between the taxa. We found differences in the bark chemistry of the taxa, both with respect to phenolic compounds and terpenoids. We could detect no difference between bored and non-bored *C. variegata* trees (the less preferred, but co-evolved host). Hybrid trees were not different in levels of total polyphenols, flavanols or terpenes according to attack status, but acetone extracts were significantly different between bored and non-bored trees. We propose that variations in the bark chemistry explain the differential attack rate between *C. variegata* and the hybrid hosts.

Key words: Bark chemistry, *Phoracantha solida*, eucalypt, GC-MS, HPLC, LC-MS

Introduction

Constitutive defences are a plant's "first line of defence" and include physical barriers such as thick bark and lignin (Franceschi *et al.* 2005) and chemical defences such as terpenes and phenolics (Hallgren *et al.* 2003). Inducible defences, on the other hand, are triggered by insect or pathogen invasion and include formation of necrotic tissue that confines the invader (Paine *et al.* 1988) and increased levels of chemicals, to deter establishment and further attack (e.g. Eyles *et al.* 2003). It is widely believed that the best explanation for intraspecific differences in host plant susceptibility to herbivory or pathogenic attack is variations in the chemistry of the host plant (Zangerl and Berenbaum 1993).

Eucalypts are some of the world's most important and widely planted forest species (Turnball 2000), with more than 19 609 670 ha planted globally (Iglesias-Trabado and Wilstermann 2008). Species of the three closely related eucalypt genera (*Eucalyptus, Angophora* and *Corymbia*) produce large amounts and a wide variety of plant secondary metabolites (PSMs), especially terpeneoids and phenolic esters (see Henery *et al.* 2008 and references within). Terpenoid compounds accumulate in glands distributed throughout the foliage and the bark (secondary phloem) of the plant (Carr and Carr 1969) and may function as repellents, attractants, feeding stimulants, etc., to herbivores (Paine *et al.* 2011). There is also a link between phenolic compounds (e.g. tannins) and herbivory (e.g. Bernays 1981). In general for cerambycids, attractants tend to be monoterpenoids and phenolic esters, while oviposition stimulants are monoterpenoids and flavanoids (Allison *et al.* 2004).

Corymbia (*Eucalyptus*) *torelliana* (F. Muell.) K.D. Hill & L.A.S. Johnson (section *Torellianae*) (Parra-O *et al.* 2009), is a rainforest tree whose endemic range occupies latitudes around 15.75 to 19°S (Boland *et al.* 2006), and is known to hybridise with the spotted gums (section *Maculatae*) including *C. variegata* (F.Muell.) K.D.Hill & L.A.S. Johnson (Parra-O *et al.* 2009). A hybrid between *C. torelliana* and *C. variegata* has been developed for commercial purposes (Lee 2007), and has become among the preferred plantation species in subtropical Queensland and New South Wales due to their significant advantages in growth, and tolerance to disease, insects, and frost (Lee 2007; Lee *et al.* 2009; Lee *et al.* 2010).

75 The eucalypt woodborers (*Phoracantha* Fabricius: Cerambycidae) attack eucalypts (Myrtaceae)
76 including *Eucalyptus* and *Corymbia* (Duffy 1963). Although the beetle genus is native to Australia,
77 most work has been conducted overseas where two species (*P. semipunctata* and *P. recurva*) have
78 become commercially important pests of eucalypt plantations (Paine *et al.* 2011). As with other
79 cerambycid beetles, olfaction is an important part of the biology of this genus used for host location
80 (Allison *et al.* 2004). In particular, *P. semipunctata* uses olfactory cues in host location in field trials,
81 laboratory trials and these volatiles are detected by the beetle electrophysiologically (Barata *et al.* 2002;
82 Barata and Araújo 2001; Barata *et al.* 1992; Barata *et al.* 2000). Three species of *Phorocantha* are pests
83 within eucalypt plantations in Australia, *P. solida, P. mastersi* and *P. acanthocera* (Elliott *et al.* 1998);
84 of these species *P. solida* has the broadest geographic range and is the most significant pest of the
85 three in the sub-tropics (Wang 1995). The life cycle of *P. solida* is approximately two years; females
86 oviposit single eggs or small batches on branch stubs or injuries of otherwise apparently healthy trees
87 ≥10 cm diameter; larvae create large amounts of damage beneath the outer bark, boring into the
88 sapwood several times, and then returning there to pupate (Wang 1995). *Corymbia variegata* is a
89 known host for the species (Griffiths *et al.* 2004), and *C. torelliana* × *C. variegata* hybrids developed
90 for plantation forestry have been shown to be more susceptible to longicorn beetles than pure *C.*
91 *variegata* (Nahrung *et al.* 2014).
92
93 We examined the impact of attack by *P. solida* on the chemistry of the secondary phloem (inner bark)
94 of *C. variegata* and its hybrid with *C. torelliana*. We hypothesised that differences in attack rate by the
95 borer on the two tree taxa would be as a result of differences in plant secondary chemistry. Samples
96 from bored and non-bored trees were examined for terpenoids and phenolic com pounds (e.g. tannins),
97 and differences between classes were determined. We propose that differences in the chemistry of the
98 secondary phloem between taxa will affect the beetle's ability to find, accept, and/or survive on hosts.
99 The implications of differences between taxa with respect to cues for host location and host defence are
100 discussed.
101
102 **Materials and methods**
103 *Phloem Sample Collection*

104 Phloem samples were collected on 24 October 2011 from a 3.83 ha site at Binjour Plateau, Queensland
105 (25.522 ºS 157.478 ºE) planted in May 2005 with *Corymbia variegata* (spotted gum) and its hybrid
106 with *C. torelliana* on a snuffy red ferrosol soil, which had boron treatments applied at the time of
107 planting. Samples were collected from representative bored and non-bored trees for each taxon from
108 throughout the site, with eleven replicate samples from each taxon-borer status combination. A tree was
109 defined as bored if any visible evidence existed of damage from one or more beetle borers, attacks on
110 these trees occur predominantly in the lower 50 cm of the trunk (Nahrung *et al.* 2014). Samples
111 comprised a cylindrical bark core (25.4 mm diameter) removed from the southwestern side of the
112 trunk, about 30 cm from the base, using a hammer and wad punch, extracting all phloem to the
113 cambium. Each core was stored separately in a labeled paper bag, stored cold during transit and frozen
114 until analysis.
115
116 *Analysis of total polyphenols and flavanols*
117 Samples were ground individually with a coffee grinder (Braun). The soluble phenolic compounds
118 were extracted twice from 50 mg of dry wood powder in 2 mL of a methanol: water solution (8:2, v/v).
119 The mixture was sonicated for 30 min at 4 °C. One mL of pooled supernatant was removed and dried
120 under vacuum, and re-dissolved in methanol (250 µL).
121
122 Total polyphenols were estimated by an adapted Folin-Ciocalteu method (Pizzo *et al.* 2011; Singleton
123 and Rossi 1965). Phenolic extract (20 µL) was diluted in 80 µL of ultra-pure water, 500 µL of Folin-
124 Ciocalteu's phenol reagent 2N (Sigma, diluted 10 times in ultra-pure water) and 400 µL of a 75 g/L
125 Na_2CO_3 solution were mixed and incubated for 5 min at 40 °C. The absorbance at 735 nm was
126 measured spectrophotometrically (Centra 202 double beam spectrophotometer, GBC) and the results
127 were expressed in mg of gallic acid equivalent (GAE) per gram dry weight (mg GAE/g d.m.).
128 Calibration was achieved with gallic acid (Sigma) aqueous solutions (0–20 µg/mL).
129
130 The quantification of flavanols was carried out by a colorimetric method using 4-
131 dimethylaminocinnamaldehyde (DMACA – Sigma) (Pizzo *et al.* 2011; Treutter 1989). Fifty µL of
132 phenolic extract, 930 µL of methanol and 20 µL of DMACA solution (100 mg DMACA in 10 mL of
133 1.5 M methanolic sulfuric acid) were mixed and incubated for 2 h at room temperature. The flavanol

134 content was determined spectrophotometrically (Centra 202 double beam spectrophotometer, GBC) at
135 630 nm using a standard curve based on catechin (Sigma) (0–15 µg/mL) and expressed in mg of
136 catechin equivalent (CE) per gram dry mass (mg CE/g d.m.).
137
138 *Phenolic compound analysis (LC-MS)*
139 Samples were ground individually with a coffee grinder (Braun), sub-samples of which (230 – 250 mg)
140 were then extracted in 1.5 mL of 70% aqueous acetone for 48 h in darkness at 4 ºC (after (Eyles *et al.*
141 2003). Solvent was removed by air-drying at ambient temperature, followed by drying under a stream
142 of N_2 at 40 ºC then under high vacuum. The extracts were re-dissolved in methanol to a concentration
143 of 10 mg/mL and filtered through a 0.45 µm PTFE filter.
144
145 High performance liquid chromatography–electrospray ionization mass spectrometry (HPLC-ESIMS)
146 was performed using an Agilent 1100-series chromatography system coupled to an Agilent 1100-series
147 single quadrupole mass spectrometer. HPLC gradient conditions were as follows: 1 mL/min gradient
148 elution from 90% H_2O/MeCN (0.05% HCOOH) to MeCN (0.05% HCOOH) over 15 min, followed by
149 a 5 min flush with MeCN using an Agilent Zorbax SB-C_8 column (150 mm × 4.6 mm; 5 µm). Peaks
150 were quantified by calculating area under the chromatogram at 254 nm.
151
152 High resolution HPLC-ESIMS was performed using a Dionex Ultimate 3000 chromatography system
153 coupled to a Bruker micrOTOF mass spectrometer. Mass calibration was performed before each
154 analysis using sodium formate clusters as an internal standard. HPLC gradient conditions were as
155 follows: 0.25 mL/min gradient elution from 90% H_2O/MeCN (0.025% HCOOH) to MeCN (0.025%
156 HCOOH) over 10 min, followed by a 5 min flush with MeCN using a Phenomenex Gemini-NX C_{18}
157 column (150 × 2.0 mm; 3 µm).
158
159 *Terpenoid analysis (GC-MS)*
160 Further sub-samples of the ground bark samples (230 – 250 mg) were extracted in methanol (1 mL) for
161 48 h in the dark at 4 ºC (after Cadahia *et al.* 1997; Eyles *et al.* 2003). Samples were then stored in the
162 freezer until analysis.
163

164 Samples (1 μL) were analysed using a gas chromatograph (GC) (Agilent 6890 Series) coupled to a
165 mass spectrometer (MS) (Agilent 5975) and fitted with a silica capillary column (Agilent, model HP5-
166 MS, 30 m × 250 μm ID × 0.25 μm film thickness). Data were acquired under the following
167 GC conditions - inlet temperature: 250 °C, carrier gas: helium at 51 cm/s, split ratio 13:1, transfer-line
168 temperature: 280 °C, initial temperature: 40 °C, initial time: 2 min, rate: 10 °C/min, final temperature:
169 260 °C, final time: 6 min. The MS was held at 280 °C in the ion source with a scan rate of 4.45 scans/s.
170
171 Peaks present in blank methanol (control) samples were discarded from analysis in test samples.
172 Tentative identities were assigned to peaks with respect to the National Institute of Standards and
173 Technology (NIST) mass spectral library. Mass spectra of peaks from different samples with the same
174 retention time were compared to ensure that the compounds were indeed the same.
175
176 *Statistical analysis*
177 Differences in mean total polyphenols (mg GAE/g dm) and flavanols (mg CE/g dm) were analysed by
178 a Mann-Whitney U test for differences between taxa, and between bored status across all trees. In
179 addition, differences in the bored status was analysed within each taxon separately.
180
181 The presence of peaks in the chromatograms, and their relative areas were analysed by nonparametric
182 multivariate statistical methods (Bray-Curtis cluster analysis and multidimensional scaling (MDS)
183 ordination) (Clarke 1993) to ascertain whether any differences could be detected between the samples.
184 Since they represent relative differences between samples, the axes of an MDS plot are dimensionless.
185
186 To determine whether clusters of individual plants relating to the taxa investigated were significantly
187 different from each other, an analysis of similarity (ANOSIM) was used. The ANOSIM tests are a
188 range of Mantel-type permutations of randomization procedures, which make no distributional
189 assumptions. These tests depend only upon rank similarities, and thus are appropriate for this type of
190 data. We used a similarity percentages (SIMPER) analysis to ascertain the relative contribution of each
191 of the components to assign the bark to the *a priori* determined groups and to assess similarity between
192 individuals within each group. Further analysis by ANOSIM and SIMPER were performed to detect
193 any differences between bark from trees that had or had not been attacked by the cerambycid beetle

194 borer. Differences in mean abundance were analysed by a Mann-Whitney U test for compounds which
195 were found to be important to distinguish these categories. The software used for the univariate
196 analysis was GenStat (V 14.2.0.6286) while that used for the multivariate analysis was Primer 5 for
197 Windows (V 5.2.9, Clarke and Gorley 2001). These analytical procedures have been used successfully
198 in previous studies to analyse chromatographic data (e.g. Hayes *et al.* 2006; Nahrung *et al.* 2009).
199
200 **Results**
201 *Analysis of total polyphenols and flavanols*
202 We detected no difference between taxa with respect to total polyphenol levels (mg GAE/g d.m.)
203 (Mann Whitney: $U_{41} = 170$, $P = 0.143$), and no difference between levels in bored and non-bored trees.
204 This lack of difference by bored status was true whether we considered all trees, or each of the taxa
205 separately (All data: Mann Whitney: $U_{41} = 212$, $P = 0.656$; *C. variegata*: Mann Whitney: $U_{21} = 54$, $P =$
206 0.699; Hybrid: Mann Whitney: $U_{20} = 49$, $P = 0.705$) (Table 1). Total flavanol (mg CE/g d.m.) levels
207 were significantly lower in hybrid trees when compared to the spotted gum (Mann Whitney: $U = 66$, P
208 < 0.001), but again there were no differences between bored and non-bored trees, whether we looked at
209 all trees or taxa separately (All data: Mann Whitney: $U_{41} = 189$, $P = 0.317$; *C. variegata*: Mann
210 Whitney: $U_{21} = 49$, $P = 0.478$; Hybrid: Mann Whitney: $U_{20} = 46$, $P = 0.557$) (Table 1).
211 [INSERT TABLE 1 NEAR HERE]
212
213 *Phenolic compound analysis (LC-MS)*
214 Molecular weight and tentative molecular formulae were determined for some of the components
215 detected in the aqueous acetone extracts of *Corymbia* bark (those shown to be important in
216 distinguishing taxa, see below). All the listed molecular formulae correspond to known flavanol and
217 flavanol glycosides (Table 2). For all samples, aqueous acetone extracts from the spotted gum and the
218 hybrid were significantly different (ANOSIM: Global $R = 0.79$, $P = 0.001$) (Figure 1a). The two taxa
219 were also distinct within trees that were not bored (Global $R = 0.723$, $P = 0.001$) and those that were
220 bored (ANOSIM: Global $R = 0.846$, $P = 0.001$). SIMPER analysis shows the most important
221 components contributing to these differences between the two taxa, while differences in mean area
222 under peaks in the chromatogram is shown also (Table 3)
223 [INSERT FIGURE 1 NEAR HERE]

224 [INSERT TABLE 2 NEAR HERE]

225 [INSERT TABLE 3 NEAR HERE]

226 To look for differences in chemicals extracted between bored and non-bored trees, we examined taxa
227 separately (as taxa differed significantly). In neither taxon did we detect a difference between bored
228 and non-bored trees (*C. variegata*: ANOSIM: Global R = 0.029, P = 0.272; Hybrid: ANOSIM: Global
229 R = 0.017, P = 0.33).

230

231 *Terpenoid analysis (GC-MS)*

232 Retention times and tentative identities of components detected in methanol extracts of *Corymbia* bark
233 are shown (Table 4). As above, the methanol extracts from the two taxa were also significantly
234 different, for all samples combined, (ANOSIM: Global R = 0.405, P = 0.001), within samples with
235 borers (ANOSIM: Global R = 0.585, P = 0.001), and without borers (ANOSIM: Global R = 0.429,
236 P = 0.001 (Figure 1b). The most important components (as determined by the SIMPER analysis) for
237 distinguishing the two taxa and differences between the mean area are shown (Table 5a).

238 [INSERT TABLE 4 NEAR HERE]

239 [INSERT TABLE 5 NEAR HERE]

240 Because taxa differed we tested for effect of boring on them separately, and found no detectable
241 differences between *C. variegata* that had or had not been bored (ANOSIM: Global R = 0.053,
242 P = 0.089). However *C. torelliana* × *C. variegata* hybrids, differed significantly according to bored
243 status (ANOSIM: Global R = 0.422, P = 0.001) (Figure 2), with the most important components to
244 distinguish the groups as determined by the SIMPER analysis, and differences between the mean area
245 listed (Table 5b).

246 [INSERT FIGURE 2 NEAR HERE]

247

248

249 **Discussion**

250 We examined the relationship between attack by *P. solida* and the chemistry of the secondary phloem
251 (bark) of two important plantation forestry taxa in southeast Queensland, *C. variegata* and its hybrid
252 with *C. torelliana*. Samples from bored and non-bored trees were examined for terpenoids and phenolic
253 compounds (e.g. flavanols, tannins), and differences between classes determined.

254

255 We found differences in the secondary phloem chemistry of the taxa, both with respect to phenolic
256 compounds and terpenoids. These differences concur with those reported previously for differences in
257 foliar chemistry between parent and hybrid taxa (Hayes *et al.* 2013). There are also differences in their
258 susceptibility to attack by *P. solida* with nine times more attacks on the hybrid than *C. variegata*
259 (Nahrung *et al.* 2014). Some aspect of this chemical variation may affect this differential attack rate.

260

261 It may be that the lower susceptibility is a result of greater chemical defences in the co-evolved host (*C.*
262 *variegata*) than in the "naïve" host (*C. torelliana* × *C. variegata*). Alternatively, the observed
263 differences in terpenoids between the taxa (Figure 1a) may explain this variation. Terpenoid differences
264 are associated with variation in host location by cerambycid beetles (Allison *et al.* 2004), and it may be
265 that the hybrid trees are easier for the beetles to recognise as a suitable oviposition site (both parental
266 taxa are sympatric to the beetle (Wang 1995)). Hybrid trees at this study site appeared more stressed
267 than *C. variegata* (Nahrung *et al.* 2014), and stress is well known to lead to differences in chemical
268 profiles of trees (e.g. Copolovici and Niinemets 2010; Niinemets 2010; Steindel *et al.* 2005).

269

270 There was no chemical difference detected between bored and non-bored *C. variegata* trees. This was
271 true whether we compared total polyphenols, flavanols, acetone or methanolic extracts. Interestingly, in
272 the case of the hybrid, although there was no chemical difference observed with the total polyphenols,
273 flavanols and acetone extracts, bored and non-bored hybrids were significantly chemically distinct in
274 the methanol extracts. Do the variations in these predominantly volatile components explain the ability
275 of the beetle to find the hybrid hosts? The presence of olfactory sensilla on the ovipositor of the
276 congeneric *P. recurva* suggests that olfactory cues from bark are important in oviposition in this
277 species (Faucheux 2012).

278

279 The bored and non-bored status of the trees used in our study arises through a combination of female
280 oviposition choice, and larval survival and it is difficult to differentiate these effects. However, the
281 differences in attack rate and secondary chemistry between taxa, and the general similarity in profiles
282 between bored and non-bored trees within taxa suggest that, if they are contributing to the distribution
283 of beetles, it is more likely due to differences in host location and acceptance than to larval

284 performance. Only one compound (4-methyl-3-oxovaleric acid, ethyl ester) was present at a higher
285 level in bored compared with non-bored hybrid hosts, and while this may represent an induced
286 response (see Eyles *et al.* 2010), we are unable to distinguish between an induced response and an
287 underlying constitutive difference between the trees.
288
289 The other three compounds that differed between bored and non-bored hybrids were higher in the
290 latter, and likely represent constitutive differences between the taxa. Indeed the compound that
291 contributes most to dissimilarity between the bored and non-bored groups is 1-methyl-2(1H)-
292 pyridinone. This compound is a known entomotoxin, and has been shown to have very high levels of
293 mortality in screwworm larvae feeding on it at even low concentrations (Oliver and Crystal 1972). It is
294 possible that even if there is no difference in oviposition preference between hybrid trees, levels of this
295 compound affect larval survival, and thus detectable damage in non-bored trees.
296
297 Overall there was no difference between bored and non-bored *C. variegata*, although upon examining
298 each compound individually we found significantly higher levels of β-pinene in bored rather than non-
299 bored (Mann-Whitney: $U_{20} = 24.0$, $P = 0.016$), while levels of this monoterpene were not significantly
300 different in the other combinations of taxon and status. This compound is electrophysiologically active
301 for the congeneric beetle *P. semipunctata* (Barata *et al.* 2002; Barata *et al.* 2000).
302
303 Factors other than the secondary metabolites may also influence the patterns of attack we report here.
304 For example bark moisture content has previously been found to be important in resistance of eucalypts
305 to borer attack (Hanks et al. 1991). In addition, reduction in larval performance of *P. semipunctata* in
306 two *Corymbia* species (*C. maculata* and *C. citriodora*) was recently attributed to sapwood reaction and
307 kino (resin) production (Haddan *et al.* 2010). Farr et al. (2000) reported a significant relationship
308 between kino and *Phorocantha* attack in Western Australia, and Carnegie et al. (2008) described kino
309 bleeding as characteristic in early stages of *P. solida* attack, and its role therefore warrants further
310 investigation. Nevertheless, we propose that variations in the chemistry of the secondary phloem (inner
311 bark) of these trees explain, at least in part, the differential attack rate or larval survival between *C.*
312 *variegata* and the hybrid hosts by the important boring pest *P. solida*.
313

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466 **Figure and Table headings**

467

468 **Figure 1**

469 a: Phenolic compounds: two-dimensional MDS ordination of the 44 *Corymbia* bark extracts in aqueous
470 acetone. The plot is based on fourth-root transformed abundances and a Bray-Curtis similarity matrix.
471 Extracts from each taxon cluster separately. Symbols: *Corymbia variegata* (Δ), *Corymbia torelliana* ×
472 *Corymbia variegata* (▲).

473 b: Terpenoids: two-dimensional MDS ordination of the 44 *Corymbia* bark extracts in methanol. The
474 plot is based on fourth-root transformed abundances and a Bray-Curtis similarity matrix. Extracts from
475 each taxon cluster separately. Symbols: *Corymbia variegata* (Δ), hybrid - *Corymbia torelliana* ×
476 *Corymbia variegata* (▲).

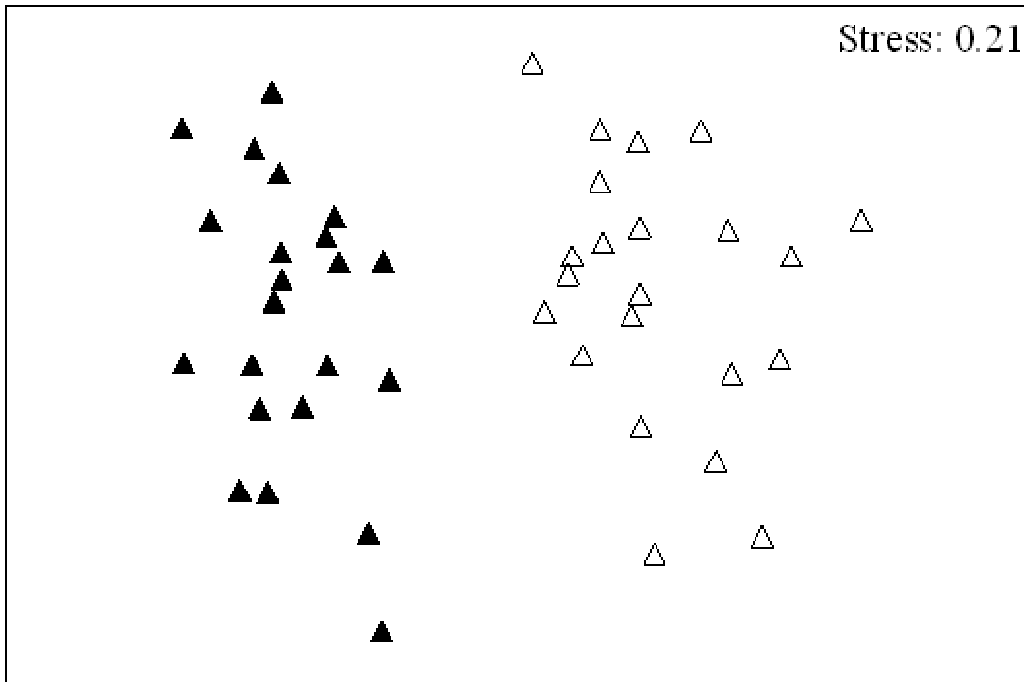
477

478 **Figure 2**

479 Terpenoid: two-dimensional MDS ordination of the 22 methanol bark extracts of *C. torelliana* × *C.*
480 *variegata*. The plot is based on fourth-root transformed abundances and a Bray-Curtis similarity
481 matrix. Extracts from bored (□) and non-bored (■) trees cluster separately.

482

483 a



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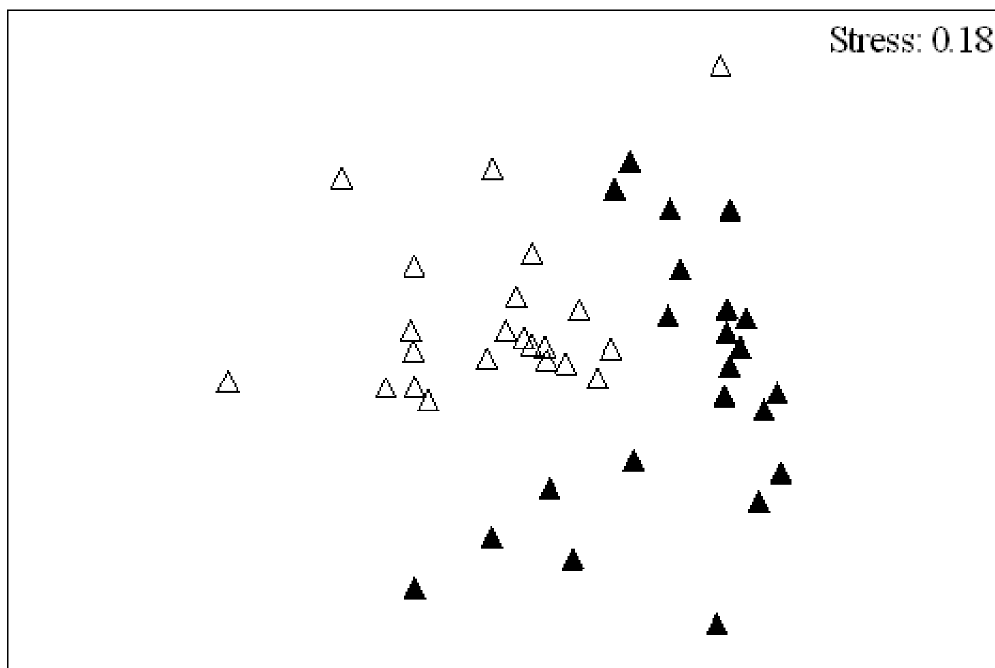
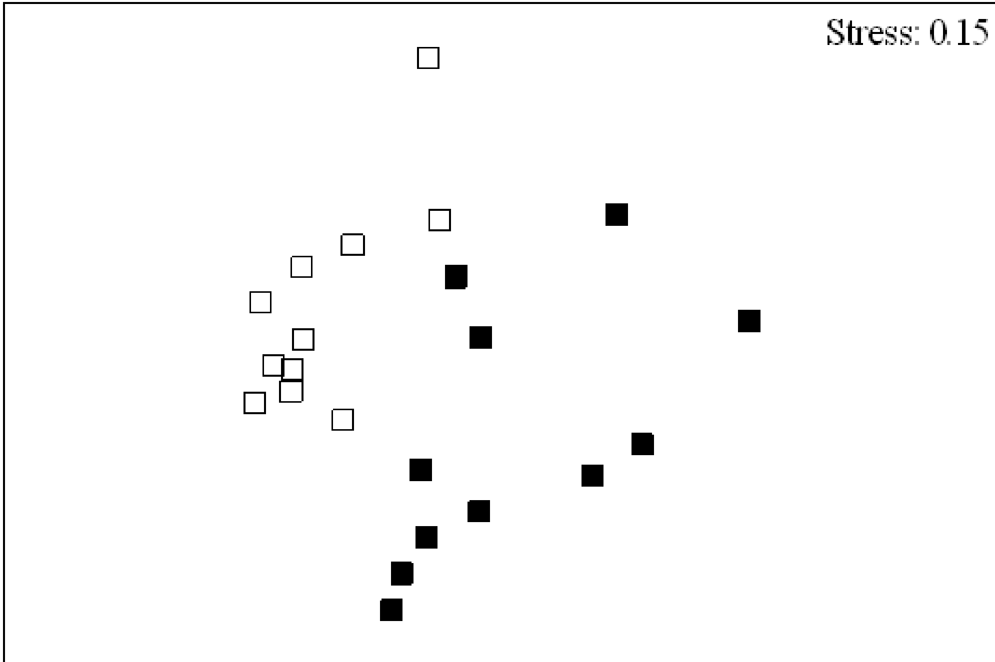
485 b



486

487 **Figure 1**

488



489

490

491 **Figure 2**

492

493 **Table 1**

494 Mean (± SEM) total polyphenol (gallic acid equivalents) and flavanol (catechin equivalents) content
495 detected in *Corymbia* bark from bored and non-bored trees of *Corymbia variegata* and *Corymbia*
496 *torelliana* × *Corymbia variegata*.

497

498 **Table 2**

499 Retention times, molecular weight and tentative molecular formulae of those components detected in
500 aqueous acetone extracts of *Corymbia* bark that were important in distinguishing between the taxa. The
501 number out of eleven replicates of each taxon in which the component was identified is also shown.

502

503 **Table 3**

504 Mean ± SE. percentage area under the peak for phenolic compounds (identified by retention time and
505 molecular formula) used to distinguish between the taxa, sorted by increasing contribution to group
506 dissimilarity.

507

508 **Table 4**

509 Retention times and tentative identities of components detected in methanol extracts of *Corymbia* bark
510 and the number out of eleven replicates of each taxon in which the component was identified.

511

512 **Table 5**

513 Mean ± SE. percentage area under the peak for compounds (identified by retention time) used to
514 distinguish between (a) the taxa and (b) the *C. torelliana* × *C. variegata* hybrids that had or had not
515 been bored.

516

517 **Table 1**

518

Taxon	Status	mg GAE/g d.m.	mg CE/g d.m.
C. variegata	Bored	1.94 ± 0.25	0.316 ± 0.077
	Non-bored	1.97 ± 0.27	0.368 ± 0.065
C. torelliana × *C. variegata*	Bored	1.62 ± 0.17	0.0918 ± 0.026
	Non-bored	1.52 ± 0.25	0.120 ± 0.035

519

520

521 **Table 2**

Ret Time (min)	MW	Molecular formula	*C. variegata*		*C. torelliana* × *C. variegata*	
			Bored	Non-bored	Bored	Non-bored
3.65	934	$C_{42}H_{46}O_{24}$	1/11	0/11	11/11	8/11
3.73	362	$C_{21}H_{14}O_{6}$	10/11	10/11	0/11	0/11
4.82	450	$C_{20}H_{18}O_{12}$	4/11	6/11	9/11	10/11
5.02	[a]	[a]	9/11	7/11	4/11	6/11
5.30	434	$C_{21}H_{22}O_{10}$	11/11	9/11	5/11	7/11
	478	[a]				
5.43	492	[a]	7/11	10/11	7/11	7/11
	498	$C_{22}H_{26}O_{13}$				
6.43	288	$C_{15}H_{12}O_{6}$	10/11	11/11	1/11	1/11
6.63	434	$C_{21}H_{22}O_{10}$	7/11	9/11	4/11	1/11
6.72	434	$C_{21}H_{22}O_{10}$	0/11	4/11	11/11	11/11
6.80	344	$C_{17}H_{12}O_{8}$	8/11	4/11	10/11	10/11
7.94	344 490	$C_{17}H_{12}O_{8}$ $C_{23}H_{22}O_{12}$	10/11	9/11	11/11	11/11

522

523 [a] Compounds for which we were unable to determine a molecular weight/formula.

524 Table 3

Ret Time (min)	Molecular formula	Mean % area *C. variegata*	Mean % area *C. torelliana* × *C. variegata*	% contribution to group dissimilarity	Mann-Whitney U
6.43	$C_{15}H_{12}O_6$	4.69 ± 0.55	0.10 ± 0.09	4.35	$U_{42} = 24, P < 0.001$
3.65	$C_{42}H_{46}O_{24}$	0.04 ± 0.04	7.3 ± 1.3	4.35	$U_{42} = 36.5, P < 0.001$
5.30	$C_{21}H_{22}O_{10}$	10.6 ± 1.0	1.76 ± 0.44	3.36	$U_{42} = 24, P < 0.001$
6.72	$C_{21}H_{22}O_{10}$	1.50 ± 0.37	1.50 ± 0.35	2.98	n.s.
7.94	$C_{17}H_{12}O_8$ $C_{23}H_{22}O_{12}$	0.39 ± 0.07	6.80 ± 0.83	2.89	$U_{42} = 0, P < 0.001$
3.73	$C_{21}H_{14}O_6$	0.85 ± 0.19	0	2.77	$U_{42} = 22, P < 0.001$
5.02	[a]	2.71 ± 0.49	2.00 ± 0.82	2.62	$U_{42} = 157, P = 0.039$
6.63	$C_{21}H_{22}O_{10}$	0.92 ± 0.33	0.08 ± 0.04	2.57	n.s.
4.82	$C_{20}H_{18}O_{12}$	1.87 ± 0.46	1.81 ± 0.24	2.41	n.s.
6.80	$C_{17}H_{12}O_8$	1.72 ± 0.83	3.40 ± 0.44	2.37	$U_{42} = 126, P = 0.006$
5.43	? $C_{22}H_{26}O_{13}$	2.93 ± 0.43	1.92 ± 0.52	2.19	$U_{42} = 134, P = 0.01$

525

526 [a] Compounds for which we were unable to determine a molecular weight/formula.

527 **Table 4**

Ret Time (min)	Compound id	*C. variegata*		*C. torelliana* × *C. variegata*	
		Bored	Non-bored	Bored	Non-bored
3.12	[a]	0/11	1/11	0/11	0/11
3.17	[a]	0/11	1/11	0/11	1/11
3.81	furfural	11/11	11/11	11/11	11/11
4.18	[a]	0/11	0/11	0/11	1/11
4.61	4-methoxy-2-butanone	10/11	11/11	11/11	3/11
4.72	[a]	0/11	0/11	0/11	3/11
5.50	[a]	0/11	0/11	0/11	3/11
5.54	β-methoxy-2-furanethanol	1/11	1/11	4/11	3/11
5.88	β-pinene	11/11	11/11	11/11	11/11
5.92	[a]	1/11	0/11	0/11	0/11
6.42	[a]	0/11	2/11	0/11	2/11
6.52	1-isopropyl-2-propylhydrazine	1/11	1/11	2/11	4/11
7.25	ethylacethydroxamate	8/11	11/11	3/11	5/11
7.28	[a]	0/11	0/11	0/11	1/11
7.59	furyl, hydroxymethylketone	1/11	0/11	10/11	5/11
8.60	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	11/11	11/11	11/11	11/11
8.71	[a]	0/11	0/11	2/11	2/11
9.03	1-methyl-2(1H)-pyridinone	10/11	11/11	2/11	11/11
9.13	4-methyl-3-oxovaleric acid, ethyl ester	0/11	0/11	11/11	4/11
9.63	1,1,2-trimethoxyethane	2/11	7/11	8/11	8/11
9.95	5-hydroxymethylfurfural (HMF)	11/11	11/11	11/11	11/11
10.10	[a]	2/11	1/11	3/11	1/11
10.20	[a]	1/11	0/11	1/11	0/11
11.15	[a]	1/11	0/11	0/11	0/11
17.84	14-methylpentadecanoic acid, methyl ester	7/11	7/11	0/11	1/11
17.84	[a]	0/11	0/11	1/11	0/11
18.24	n-hexadecanoic acid	9/11	10/11	11/11	11/11
18.62	[a]	0/11	1/11	3/11	2/11
19.00	[a]	1/11	1/11	0/11	0/11
19.35	[a]	1/11	0/11	0/11	0/11
19.45	8,11-octadecadienoic acid, methyl ester	8/11	6/11	3/11	2/11
19.52	linolenic acid, methyl ester	5/11	3/11	0/11	2/11
19.86	linoleic acid	1/11	0/11	2/11	11/11
20.12	[a]	0/11	0/11	1/11	0/11
25.00	β-sitosterol	10/11	11/11	10/11	11/11

528

529 [a] Compounds for which we were unable to determine an identity.

530

531 Table 5

a)

Ret Time (min)	Compound i.d.	Mean % area *C. variegata*	Mean % area *C. torelliana* × *C. variegata*	% contribution to group dissimilarity	Mann-Whitney U
7.25	ethylacethydroxamate	3.19 ± 0.71	1.03 ± 0.39	8.60	$U_{42} = 115$, $P = 0.002$
9.13	4-methyl-3-oxovaleric acid, ethyl ester	0	1.16 ± 0.19	7.37	$U_{42} = 77$, $P < 0.001$
7.59	furyl, hydroxymethylketone	0.04 ± 0.04	0.81 ± 0.15	6.55	$U_{42} = 82.5$, $P < 0.001$
19.45	8, 11-octadecadienoic acid, methyl ester	1.40 ± 0.47	0.42 ± 0.19	6.46	$U_{42} = 149$, $P = 0.015$
17.84	14-methylpentadecanoic acid, methyl ester	0.93 ± 0.28	0.07 ± 0.07	6.07	$U_{42} = 101$, $P < 0.001$
9.03	1-methyl-2(1H)-pyridinone	2.07 ± 0.27	1.10 ± 0.24	5.79	$U_{42} = 134.5$, $P = 0.01$
9.63	1, 1, 2-trimethoxy ethane	0.64 ± 0.19	0.80 ± 0.15	5.79	n. s.
4.61	4-methoxy-2-butanone	3.31 ± 0.68	1.25 ± 0.48	5.61	$U_{42} = 98$, $P < 0.001$
19.86	linoleic acid	0.03 ± 0.03	0.61 ± 0.12	5.49	$U_{42} = 103.5$ $P < 0.001$
25.00	β-sitosterol	26.8 ± 2.5	26.3 ± 2.5	3.99	n. s.
19.52	linolenic acid, methyl ester	0.62 ± 0.27	0.15 ± 0.10	3.88	n. s.

b)

Ret Time (min)	Compound i.d.	Mean % area bored	Mean % area non-bored	% contribution to group dissimilarity	Mann-Whitney U
9.03	1-methyl-2(1H)-pyridinone	0.41 ± 0.28	1.80 ± 0.25	9.73	$U_{20} = 15$, $P = 0.002$
19.86	linoleic acid	0.15 ± 0.10	1.08 ± 0.10	8.67	$U_{20} = 5$, $P < 0.001$
9.13	4-methyl-3-oxovaleric acid, ethyl ester	1.65 ± 0.12	0.68 ± 0.29	8.06	$U_{20} = 28$, $P = 0.034$
4.61	4-methoxy-2-butanone	1.25 ± 0.16	2.81 ± 0.97	7.55	$U_{20} = 23$, $P = 0.013$
7.59	furyl, hydroxymethylketone	1.11 ± 0.19	0.50 ± 0.19	6.24	n. s.
7.25	ethylacethydroxamate	0.30 ± 0.20	1.77 ± 0.70	6.22	n. s.
9.63	1, 1, 2-trimethoxy ethane	0.76 ± 0.20	0.84 ± 0.24	5.00	n. s.
5.54	β-methoxy-(S)-2-furanethanol	0.41 ± 0.19	0.55 ± 0.29	4.90	n. s.
5.88	β-pinene	1.69 ± 0.36	3.9 ± 1.3	4.08	n. s.
25.00	β-sitosterol	25.5 ± 3.8	27.1 ± 3.2	3.96	n. s.
6.52	1-isopropyl-2-propylhydrazine	0.09 ± 0.06	0.41 ± 0.19	3.95	n. s.

532

533