

Chemical signatures affecting host choice in the *Eucalyptus* herbivore, *Gonipterus* sp. (Curculionidae: Coleoptera)

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Abstract It is well known that herbivorous insects respond to host plant volatiles. Yet details of how these insects perceive the complex profile of volatiles from different potential host plants have not been studied for most insects. *Gonipterus* spp. are important pests of *Eucalyptus* worldwide, but differ in their preference for different species of this host. In this study, we consider whether host volatiles affect the host choice for a *Gonipterus* sp. and we characterize the response of the female insect to the volatile profiles from these hosts in an electro-antennographic experiment. We sampled volatiles from freshly damaged leaves of three *Eucalyptus* species and analysed the profiles by gas chromatography coupled to electro-antennography

(GC-EAD) and gas chromatography coupled to mass spectrometry. Female weevils gave a mixed range of electro-physiological responses to volatile puffs from leaves of different tree species. This suggests that differences in volatile profiles of different trees play a role in how these beetles discriminate between potential hosts. GC-EAD analysis showed that responses were as complex as the volatile chemical compositions of the leaves. A number of these chemicals were identified, and responses were mostly due to general green leaf volatiles. This was also evident from the fact that the insects showed a markedly greater response to the total volatile profile from freshly damaged leaves for all species. The females of the *Gonipterus* sp. can therefore detect damaged leaves, which may indicate host quality. Host specificity information is further expected to lie in the relative differences in emission ratios and synergism between different host chemical compounds, rather than specific individual compounds.

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Introduction

The *Eucalyptus* snout beetle originates from South-east Australia and Tasmania, but has been introduced to numerous countries around the world (Tooke 1953). The insect feeds on leaves of *Eucalyptus* trees during both larval and adult stages and consequently can cause significant damage to susceptible trees (Tooke 1953; Richardson and Meakins 1984). In many of these countries, the beetles have led to significant losses in plantation forests (Mally 1924; Clark 1931; Williams et al. 1951; Hanks et al. 2000;

Rivera and Carbone 2000; Lanfranco and Dungey 2001; Loch and Floyd 2001), including South Africa (Tooke 1953; Richardson and Meakins 1984). The collective name *Gonipterus scutellatus* has often been used for the Eucalyptus snout beetle in the past, but it is known today that this name represents a species complex (Mapondera et al. 2012). In South Africa, for example, the beetle has long been thought to represent the single species *G. scutellatus*, but recent studies suggest that collections most likely include *G. platensis* and an undescribed *Gonipterus* sp. 2 (Mapondera et al. 2012).

The literature is unclear as to which *Eucalyptus* species is the preferred host for invasive *Gonipterus* spp. (Clarke et al. 1998). For example, *E. globulus* has been reported as one of the most heavily damaged hosts for *Gonipterus* spp. in countries such as South Africa (Mally 1924; Tooke 1953; Richardson and Meakins 1984), New Zealand (Clark 1931), USA (Hanks et al. 2000), Spain (Rivera and Carbone 2000), Chile (Lanfranco and Dungey 2001) and Australia (Loch and Floyd 2001). It has, however, recently been shown that the beetles in South Africa survive better when feeding on *E. smithii* rather than *E. globulus*, which is in contrast to earlier reports (Newete et al. 2011). Furthermore, in the native range of Tasmania, where a wider host range is available, *G. scutellatus* is reported to prefer *E. pulchella* above *E. globulus* trees (Clarke et al. 1998). Host availability might thus be one of the factors influencing differences in reports about host preference of *Gonipterus* spp.

A number of reasons other than host availability might also influence differences in host preference reports. For example, techniques to score damage by *Gonipterus* spp. are not standardized and are interpreted across long time scales and broad geographical ranges. The host preference of a range of cryptic, related species has also not yet been considered, and studies prior to that of Mapondera et al. (2012) mostly refer to *G. scutellatus* in the broad sense. Environmental factors can also influence both the hosts and the beetles themselves (Clarke et al. 1998). Temperature, for example, is known to influence the beetle's activity levels (Tooke 1953) and the volatile emission rates of *Eucalyptus* trees (Guenther 1991; Nunes and Pio 2001). Furthermore, many *Eucalyptus* spp. carry two distinct types of foliage, which have different physical (Brooker and Kleinig 1996) and chemical (Guenther 1991; Nunes and Pio 2001; Pio et al. 2001) characteristics. These differences may influence the host choice of *Gonipterus* spp. (Richardson and Meakins 1984; Rivera et al. 1999).

Newete et al. (2011) showed that larvae of a *Gonipterus* sp. in South Africa could survive on a number of *Eucalyptus* species including some species (e.g. *Corymbia citriodora* and others) that are not selected for oviposition. Larval survival and adult oviposition for *Gonipterus* in

South Africa are therefore not necessarily correlated. The data provided by these authors, however, show that adult females preferentially lay eggs on *E. smithii*, *E. grandis*, *E. scoparia* and *E. viminalis* in the field (Newete et al. 2011). The mechanism by which female *Gonipterus* spp. selects oviposition material is largely unknown. What is known is that herbivorous insects are able to detect volatile organic compounds from plants (Visser 1986; Metcalf and Metcalf 1992; Dicke 2000). Compounds that are commonly found around green plants include green leaf volatiles, monoterpenes, sesquiterpenes and polyterpenes. It is thought that phytophagous insects may be able to select certain host plants based on these volatile chemicals (Bruce et al. 2005). This could also be the case for *Gonipterus* spp. in South Africa.

Odours from damaged plant tissue have been found to play a role in the behaviour of a number of weevil species. For example, the vine weevil, *Otiorhynchus sulcatus*, is known to prefer plant material that has been damaged by other vine weevils. Furthermore, these beetles appear not to be able to distinguish between mechanically damaged and weevil damaged plant material (van Tol et al. 2002). Research on the pepper weevil, *Anthonomus eugeni*, has shown that these beetles are attracted to damaged plants and in particular to plants freshly damaged by their conspecifics (Addesso et al. 2011). The sugarcane root-stalk borer weevil, *Diaprepes abbreviatus*, is also attracted to mechanically damaged plant tissue (Harari and Landolt 1997).

Tooke (1953), who studied *Gonipterus* (referred to as *G. scutellatus* in his studies) on *Eucalyptus* in South Africa, argued strongly that host selection behaviour of this insect was linked to some olfactory mechanism. He attempted to link the host preference of the insect to the essential oil composition of different *Eucalyptus* species by correlating the host susceptibility in the field to the major components in the essential oils made from these trees. This experiment, however, met with little success and Tooke (1953) could conclude only that the majority of preferred hosts had eucalyptol (cineol) in their essential oils.

If there is a host preference, as reported in the literature for *G. scutellatus* (which includes at least two different species), then it is likely that chemical cues might be involved in female host choice. These chemical cues could either be distinct or similar for each of the reported hosts. The aim of this study was to investigate the electro-physiological responses of females identified as *Gonipterus* sp. 2 (following Mapondera et al. 2012) beetles to the total volatile bouquet originating from foliage of eleven different *Eucalyptus* spp. A further aim was to identify individual host volatiles that are electro-physiologically active for *Gonipterus* sp. 2 females. For this latter part of the study, volatiles were sampled from the damaged leaves of

three *Eucalyptus* spp., two of reportedly susceptible hosts (*E. globulus* and *E. viminalis*) and one of a non-host, *Corymbia (Eucalyptus) citriodora* (Tooke 1953, Richardson and Meakins 1984) by an adsorption process. GC-EAD-active peaks were tentatively identified from the *E. globulus* volatile profile by GC-MS and confirmed with standards.

Materials and methods

Insect samples

Gonipterus sp. 2 samples were obtained from a *Eucalyptus* plantation in Pretoria, South Africa, near Tom Jenkins drive (S25°44'07.97 E28°14'18.08). Only *Gonipterus* sp. 2 is known from this area, and its identity has been confirmed using COI sequence data (Dr. J. Garnas unpublished, University of Pretoria, personal communication). Insects were fed on *E. smithii* and *E. globulus* foliage while being kept in wooden cages in a temperature-controlled (20–25 °C) room. Female insects were used in EAG recordings because they make the choice to find suitable oviposition material on which larvae will eventually develop. Females were identified based on the differences in the penultimate sternites as reported by Carbone and Rivera (1998).

Eucalyptus samples

Eleven *Eucalyptus* spp. were sampled from two sites in Pretoria. All species other than *E. saligna* have been reported as susceptible to infestation by *Gonipterus* spp. in South Africa by Tooke (1953), Richardson and Meakins (1984) or Newete et al. (2011). *Eucalyptus grandis* is widely planted in South Africa and is also known to be a host (Rivera and Carbone 2000), and it was therefore included in the analyses. *C. citriodora* (previously also classified in *Eucalyptus*) was chosen to represent a non-host (Tooke 1953). Six of the sampled *Eucalyptus* spp. were found at the same site as the insects. The remaining five *Eucalyptus* species were obtained from the Forestry and Agricultural Biotechnology Institute (FABI, www.fabinet.up.ac.za) nursery at the University of Pretoria. Cross-contamination between individual samples was avoided by separating them, upon sampling, in separately sealed poly-acetate cooking bags. These bags were stored in a fridge at 5 °C before the analyses were undertaken.

Volatile collection

Volatiles from the crushed juvenile foliage of three different *Eucalyptus* species were sampled by adsorption onto

standardized Tenax TA (200 mg) traps (MKIUNITY, Markes, Chemetrix, Midrand, South Africa). The sampling material was obtained from three trees at the same two sites in Pretoria. The leaves of each *Eucalyptus* sp. were cut into pieces of approximately 5 cm². The leaves were sampled for 30 min at a flow rate of 512 ml/min in duplicate for each *Eucalyptus* sp., and a sample blank was taken. The dry weight of the sampled leaves was measured as 6.1 g for *E. globulus*, 9.5 g for *E. viminalis* and 4.8 g for *C. citriodora*.

Electro-antennography

All EAG recordings were made with an EAG detector system (Syntech, Hilversum, The Netherlands). Live female beetles were used in these recordings because a decline in antennal sensitivity was observed when antennae were removed (data not shown). Individual beetles were secured with cotton wool inside a micropipette tip with only the head and antenna protruding from the end of the pipette tip. The pipette was secured to a mounting device, and a dissection microscope and micro-manipulator were used to position and connect glass capillary microelectrodes to the insect antenna and head. The recording electrode was connected to the tip of the club-shaped antenna with the reference electrode connected to the eye on the opposite side of the insect's head. Ag/AgCl electrodes were made from silver wire that were immersed in a 0.1 M KCl electrolyte solution with 2 % PVP (polyvinyl pyrrolidone) added to prevent desiccation. The entire preparation was moved to within one centimetre from a glass stimulus delivery tube. Filtered and humidified air was blown onto the insect preparation through the stimulus delivery tube at a flow rate of 150 ml/min, and sample volatiles were introduced into this air flow 170 mm upstream from the antennal preparation as 0.4 s puffs, at 30 ml/min at puff maximum.

Clean surgical blades were used to cut a 1 cm² piece of leaf from each of the eleven different *Eucalyptus* spp. samples. Each leaf piece was inserted into a different Pasteur pipette, and an empty pipette was used as a sample blank. A blank recording was made before and after sets of five sample recordings for each of the *Eucalyptus* samples. Each of the samples was freshly damaged after the first five recordings by mechanically scraping the cuticle of the leaf with a clean piece of glass. Five additional recordings were subsequently made of the freshly damaged plant material. A recovery period of one minute was allowed between each individual recording. The entire experiment was repeated three times with three different female insects. The order in which these recordings were made was kept constant for all three insects.

The absolute response intensity (mV) of each recording was measured. Four outliers ($1.5\times$ inter quartile range) were identified and discarded from the analysis. All blanks and respective recordings for each *Eucalyptus* species were pooled, and a global ANOVA analysis was done based on deflection intensity. Dunnett's test was used for joint ranking (control group = blank) with an α level equal to 5 % in order to determine which *Eucalyptus* species had larger responses than the blank recordings. Tukey honestly significant difference test was used to assign letters of significance.

Gas chromatography coupled to electro-antennography

All GC-EAD recordings were made with the same EAG detector system as reported above (Syntech) coupled to an Agilent 6890N gas chromatography system (Chemetrix, Midrand, South Africa). EAD signals were recorded at a sampling rate of a 100 samples/s. A 10 times external amplification was used, and the low cut-off filter was set to 0.05 Hz on the software. High-frequency noise was digitally removed, after the recording was made, by adjusting the low pass filter after the run to allow only a window of 0.05–3 Hz to pass. These settings were used for all thermally desorbed samples. Samples were injected onto a 60 m DB 624 column (J & W scientific, ID: 0.25 μm , film: 1.4 μm) with a thermal desorption system (MKIUNITY, Markes, Chemetrix, Midrand, South Africa) at a 17:1 split ratio. The transfer line between the thermal desorption system and GC was kept at 190 °C. Nitrogen was used as carrier gas, and constant column head pressure of 20.1 psi was used during separation. The GC oven was kept at 40 °C for 7 min and increased at 5 °C per minute to a maximum of 260 °C.

Antennae were removed at their bases from live female *Gonipterus* sp. 2 beetles using a surgical blade. A dissection microscope and micromanipulator were used to position and connect glass capillary microelectrodes to the insect antennae. The recording electrode was connected to the tip of the club-shaped antenna with the reference electrode connected to the base of the removed antenna. Ag/AgCl electrodes were prepared as reported above. The GC effluent was introduced into the air stream 90 mm upstream from the preparation. The transfer line between the GC and EAD detector was kept at a maximum temperature of 260 °C. Six GC-EAD recordings were performed for each *Eucalyptus* sp. in order to identify repeatable responses in the EAD data.

GC-EAD responses to the standard compounds as tentatively identified with the GC–MS analysis (described below) were also confirmed on the GC-EAD system by liquid injection of a mixture (1,000 ppm) made in

dichloromethane ($n = 9$). The liquid injector was operated in split mode (20:1) at a temperature equal to 200 °C. In order to avoid the automatic baseline correction, direct current (DC) recordings were performed during these liquid injection runs. Baseline correction was performed on the resulting EAD data (Time constant $r = 0.85$) (Slone and Sullivan 2007), and retention indices were used to match peaks between the two sample injection methods.

Gas chromatography coupled to mass spectrometry

GC–MS analysis was done in order to tentatively identify some of the EAD-active peaks. A Thermo Quest trace GC 2000 series coupled to a Finnigan Polaris ITD and a Perkin Elmer thermal desorption system with an identical 60 m DB624 analytical column as used during GC-EAD. A split ratio equal to 43.7:1 was used during desorption of samples on the GC–MS system. Helium was used as carrier gas, and the average linear velocity was matched with the GC-EAD system isothermally at 130 °C and required a column head pressure of 16.0 psi. The oven of the GC–MS system was set at 40 °C for 7 min and increased at 5 °C/minute to a maximum of 260 °C. The transfer line between the thermal desorption system and GC was kept at 190 °C, and the transfer line between the GC and MS was kept at 260 °C. The Finnigan Polaris ITD was operated with an ion source temperature equal to 200 °C and 70 eV ionization energy. The mass scan range was 50–285 m/z. Tentative identities were assigned based on a mass spectral comparison with library spectra and known retention indexes (Nist 2.0c, 2004). Sixteen standard reference compounds were purchased from reputable suppliers for confirmation of compound identity. Peak area was calculated by integration of the total ion chromatogram if the peaks were pure. Mass fragments were used when compounds could not be resolved from their total ion chromatograms.

Statistical analysis was conducted in R version 3.0.2 on relative percentage peak areas of the peaks confirmed with reference standards only (metaMDS, Vegan package, Oksanen et al. 2013). This gives a relative representation of identified compound distribution for each sample based upon these standards. Bray–Curtis distances (Bray and Curtis 1957) were calculated and used to locate the relative positions of species within a multidimensional space. Non-metric multidimensional scaling (NMDS) was used to find a low-dimensional representation with a maximum distance between points represented on the first dimension. This type of comparison was used successfully in other similar studies (Proffitt and Johnson 2009; Kotze et al. 2010).

Table 1 Differences between EAG response magnitude to the different leaf treatments

Level	<i>n</i>	Mean (mV)	SD (mV)	Letters of significance*
Freshly damaged <i>E. globulus</i>	16	0.768	0.201	A
Freshly damaged <i>E. tereticornis</i>	15	0.760	0.186	A
Freshly damaged <i>E. viminalis</i>	13	0.745	0.232	AB
Freshly damaged <i>E. robusta</i>	15	0.715	0.110	AB
Freshly damaged <i>E. smithii</i>	15	0.715	0.165	AB
Freshly damaged <i>E. camaldulensis</i>	15	0.666	0.148	ABC
Freshly damaged <i>E. scoparia</i>	14	0.655	0.247	ABC
Freshly damaged <i>E. punctata</i>	15	0.634	0.208	ABCD
Freshly damaged <i>E. saligna</i>	15	0.593	0.134	ABCD
Freshly damaged <i>E. grandis</i>	15	0.558	0.137	BCD
<i>E. viminalis</i>	15	0.514	0.165	CDE
<i>E. tereticornis</i>	15	0.483	0.126	CDEF
<i>E. smithii</i>	15	0.457	0.133	DEF
Freshly damaged <i>C. citriodora</i>	15	0.453	0.143	DEF
<i>E. globulus</i>	14	0.360	0.088	EFG
<i>E. robusta</i>	15	0.331	0.079	EFG
<i>E. camaldulensis</i>	15	0.327	0.076	FG
blank	72	0.297	0.132	G
<i>E. grandis</i>	15	0.256	0.064	G
<i>E. saligna</i>	15	0.230	0.042	G
<i>E. scoparia</i>	15	0.199	0.088	G
<i>E. punctata</i>	15	0.178	0.035	G
<i>C. citriodora</i>	15	0.174	0.062	G

Freshly damaged refers to leaves that were mechanically scraped before recordings were made

* Levels with the same letter are not significantly different, Tukey HOD $p < 0.05$

Results

Electro-antennography

Results of the EAG experiment with leaves before mechanical scraping showed that female beetles had a significantly greater responses to *E. viminalis*, *E. smithii* and *E. tereticornis* when compared to blank recordings. *E. tereticornis*, *E. smithii*, *E. globulus*, *E. robusta* and *E. camaldulensis* did not give statistically significantly different responses from each other. *E. globulus*,

E. robusta, *E. camaldulensis*, *E. grandis*, *E. saligna*, *E. scoparia*, *E. punctata* and *C. citriodora* could not be statistically separated from blank recordings (Table 1).

The EAG response of the beetles to the total volatile profile of freshly damaged leaves showed that all the *Eucalyptus* spp. tested elicited a response that was significantly greater than blank recordings. Among these, *E. globulus*, *E. tereticornis*, *E. viminalis*, *E. robusta*, *E. smithii*, *E. camaldulensis* and *E. scoparia* elicited larger EAG responses compared with the non-host *C. citriodora*, which showed the smallest responses (Table 1).

Gas chromatography coupled to electro-antennography

Electro-antennogram responses observed for the chromatograms of the different *Eucalyptus* spp. revealed that there are many different peaks that elicited responses from the female *Gonipterus* sp. 2 antennae. Many of these peaks were common for the three different *Eucalyptus* species that were sampled, but they occurred in different ratios for each species (Figs. 1, 2, 3).

Responses to the standard compounds revealed that (E)-2-hexenal, (Z)-3-hexen-1-ol, (Z)-3-hexenyl acetate, eucalyptol, γ -terpinene, α -pinene, 2-phenylethanol, benzyl acetate and ethyl phenylacetate were correctly identified as being antenna-active compounds. These standard compounds were confirmed to give measurable electro-physiological responses from the female antenna. The largest responses, among these, were observed for the green leaf volatiles (E)-2-hexenal, (Z)-3-hexen-1-ol, (Z)-3-hexenyl acetate and phenolic compounds 2-phenylethanol, benzyl acetate and ethyl phenylacetate (Fig. 4). Some of the compounds in the standard mixture were confirmed as antenna active, but could not be detected in the chromatographic profiles of any of the *Eucalyptus* samples tested in this experiment. These included camphene, β -pinene, 3-carene and m-cymene. These compounds were tentatively identified as being present in these profiles. However, retention index differences between the sample peaks and standard compounds showed that the initial tentative identification, which was based on library mass spectra, was incorrect.

Gas chromatography coupled to mass spectrometry

Standard compounds were confirmed to be correctly identified through retention index matches on both the GC-EAD and GC-MS systems (Tables 2, 3, 4). Mass spectral comparisons between standards and unknowns were also used to confirm tentative identities (see Table 4 for relative ion distributions of major ions). Inconsistency in retention indices (large difference in KI between the two systems) was observed for the alcohols 2-phenylethanol and

Fig. 1 FID chromatographic peaks (*top trace*) for *E. globulus* leaf volatiles and corresponding EAD responses (*bottom trace*) of *Gonipterus* sp. 2 antenna. Vertical lines correspond to elution times of peaks that were investigated in order to identify electro-physiologically active compounds. Peak letters correspond to those listed in Tables 3 and 4

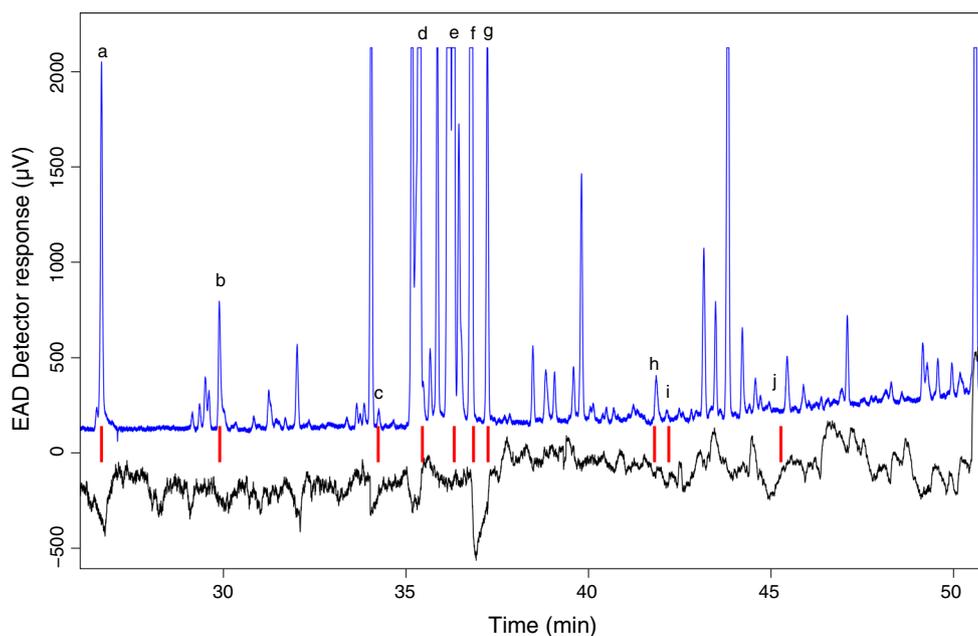
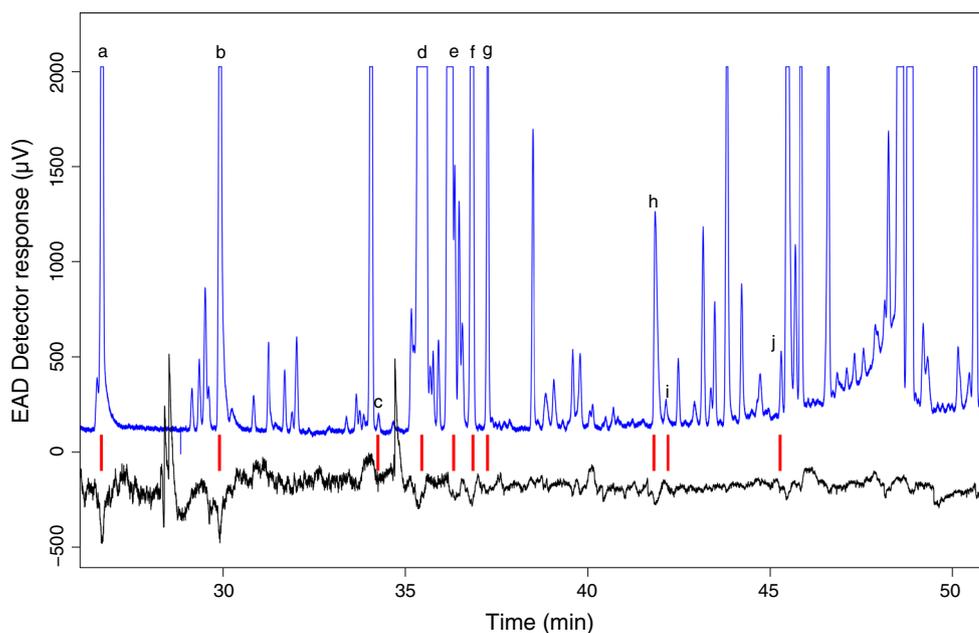


Fig. 2 FID chromatographic peaks (*top trace*) for *E. viminalis* leaf volatiles and corresponding EAD responses (*bottom trace*) of *Gonipterus* sp. 2 antenna. Vertical lines correspond to elution times

of peaks that were investigated in order to identify electro-physiologically active compounds. Peak letters correspond to those listed in Tables 3 and 4

(*Z*)-3-hexen-1-ol. These inconsistencies could be explained by column surface activity on the GC-MS instrument, which caused band broadening of alcohols through hydrogen bonding. Four of the identified compounds co-eluted under these chromatographic parameters. These include (*E*)-2-hexenal that co-eluted with (*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate that co-eluted with 3-carene.

Non-metric multidimensional scaling (NDMS) was used to plot samples in two dimensions in such a way that the distance between the points portrayed the relative differences between samples (Supplementary Figure 1). A larger distance is associated with a larger degree of dissimilarity. Caution was applied when interpreting these results since they are based on the presence and relative abundance of

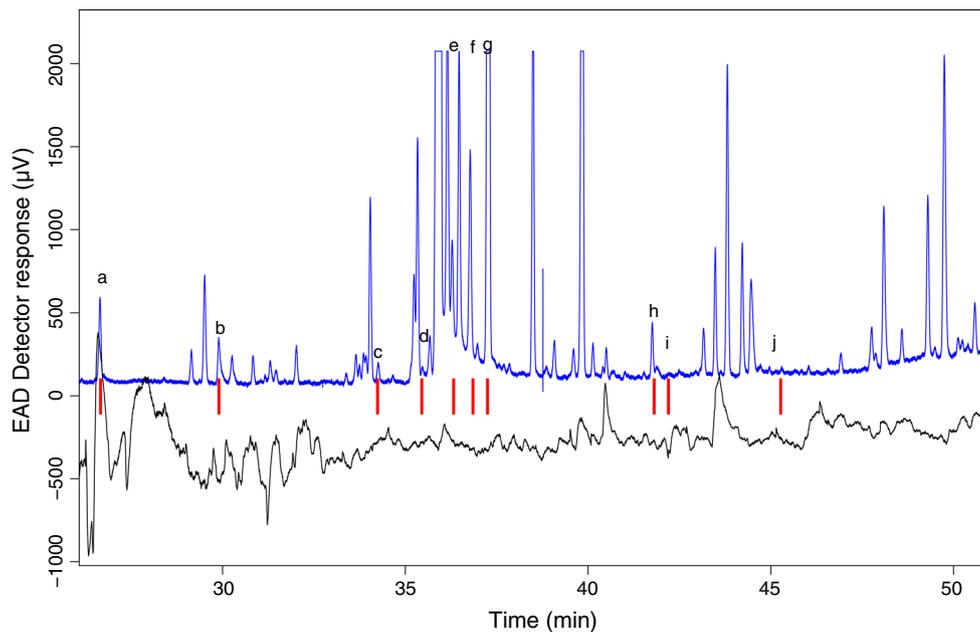


Fig. 3 FID chromatographic peaks (*top trace*) for *C. citriodora* leaf volatiles and corresponding EAD responses (*bottom trace*) of *Gonipterus* sp. 2 antenna. Vertical lines correspond to elution times

of peaks that were investigated in order to identify electro-physiologically active compounds. *Peak letters* correspond to those listed in Tables 3 and 4

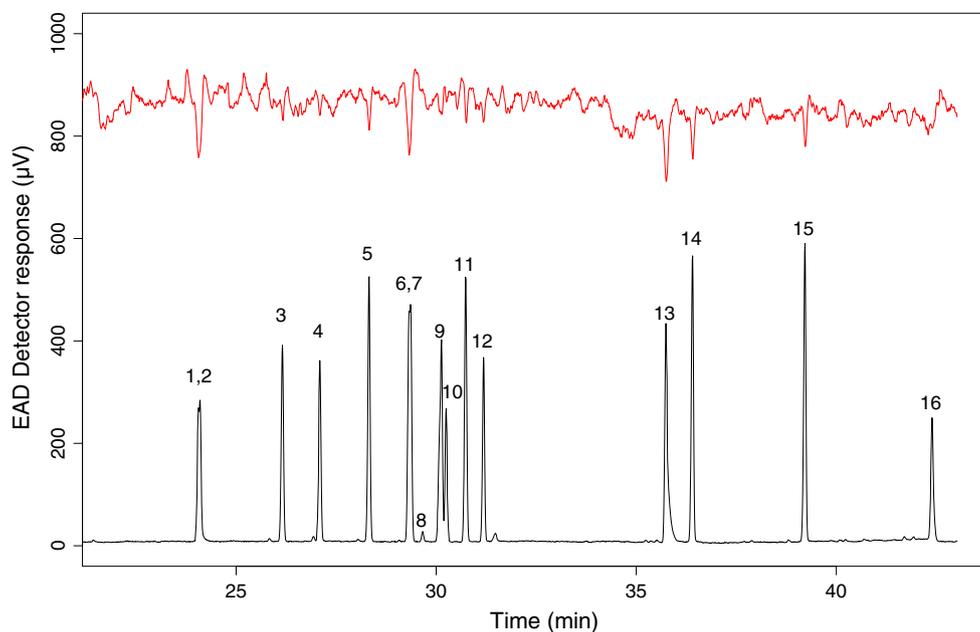


Fig. 4 GC-EAD responses towards identified standard compounds after liquid injection at 20.0 psi (25 ng at EAD). *Peak numbers* refer to standard numbers in Tables 2, 3 and 4. The *top trace* is the EAD response, and the *bottom trace* is the FID response

the 16 investigated compounds for only two samples of each species (stress ≈ 0). This plot separates the three sampled species by grouping them together based only on the presence and relative abundance of the identified compounds. The analysis separated *E. globulus* from *C. citriodora* and *E. viminalis*, largely based on the presence of 2-phenyl ethanol, benzyl acetate, ethyl phenylacetate

and terpenyl acetate. These compounds were not detected in *E. viminalis* and *C. citriodora*. Limonene, eucalyptol, (Z)-3-hexen-1-ol and (Z)-3-hexenyl acetate played a role in separating *E. viminalis* from the other two species. *C. citriodora* was mainly separated from *E. globulus* and *E. viminalis* due to the influence of γ -terpinene, which was present in relatively larger proportions.

Table 2 The standard compounds, purities and Kovats retention indexes as calculated for the different instruments and injection methods that were used

Standard no	Name	Cass no	% purity	GC-EAD ^a	GC-EAD ^a	GC-MS ^a
				KI liquid	KI thermal	KI thermal
1	(E)-2-hexenal	6728-26-3	98	910	910	912
2	(Z)-3-hexen-1-ol	928-96-1	99	910	910	912
3	α -Pinene	80-56-8	98	959	958	959
4	Camphene	79-92-5	99.3	982	980	981
5	β -Pinene	127-91-3	99.5	1,012	1,011	1,011
6	(Z)-3-hexenyl acetate	1708-82-3	99	1,038	1,038	1,038
7	3-Carene	13466-78-9	96.5	1,038	1,038	1,038
8	α -Terpinene	99-86-5	94	1,046	1,046	1,047
9	m-Cymene	535-77-3	99	1,058	1,058	1,058
10	Limonene	5989-27-5	99.3	1,058	1,058	1,058
11	Eucalyptol	470-82-6	–	1,074	1,073	1,074
12	γ -Terpinene	99-85-4	97	1,085	1,085	1,085
13	2-Phenylethanol	60-12-8	–	1,209	1,209	1,226
14	Benzyl acetate	140-11-4	99.5	1,229	1,228	1,231
15	Ethyl phenylacetate	101-97-3	99	1,311	1,311	1,314
16	Terpenyl acetate	80-26-2	–	1,411	1,411	1,413

^a Gas chromatography (GC), electro-antennography detector (EAD), mass spectrometry (MS)

Table 3 Identities of the compounds associated with EAD-active peaks and the retention indices for these peaks for comparison between the three tree species analysed on the GC-EAD instrument

Peak ^a	Standard no	Compounds	<i>E. globulus</i>		<i>E. viminalis</i>		<i>C. citriodora</i>	
			Rt (min)	KI	Rt (min)	KI	Rt (min)	KI
a		C6 alcohol	26.66	842.3	26.67	842.3	26.62	841.3
b	1	(E)-2-hexenal	30.01	912.7	29.87	909.4	29.87	909.3
	2	(Z)-3-hexen-1-ol	Present	–	–	–	–	–
	3	α -Pinene	32	958.2	31.99	957.8	31.99	957.9
	4	Camphene	–	–	–	–	–	–
c	5	β -Pinene	34.24	1,010	–	–	–	–
d	6	(Z)-3-hexenyl acetate	35.55	1,043	35.44	1,040	35.31	1,037
	7	3-Carene	–	–	–	–	–	–
	8	α -Terpinene	Present	–	35.7	1,047	35.65	1,045
e	10	Limonene	36.32	1,062	36.22	1,060	36.13	1,057
		Cymene	Present	–	36.35	1,063	36.25	1,061
f	11	Eucalyptol	36.85	1,076	36.81	1,075	36.75	1,073
g	12	γ -Terpinene	37.25	1,086	37.24	1,085	37.25	1,086
h	13	2-Phenylethanol	41.81	1,209	41.83	1,209	–	–
i	14	Benzyl acetate	42.45	1,227	–	–	–	–
j	15	Ethyl phenylacetate	45.27	1,310	–	–	–	–
	16	Terpenyl acetate	48.6	1,415	–	–	–	–

^a Peak letters displayed in table refer to the investigated peaks that were selected from the initial antennal responses as in Figs. 1, 2 and 3

Discussion

Results of this study showed that host volatiles could play a significant role in host choice for the *Eucalyptus* pest, *Gonipterus* sp. 2 in South Africa. This was evident from measurable electro-antennogram responses from the beetles to virtually all the *Eucalyptus* spp. tested, especially

when the leaves were freshly damaged. A number of specific volatiles to which the beetle responded were also identified.

Significantly larger antennal responses were recorded from freshly damaged leaves of *E. globulus*, *E. tereticornis*, *E. viminalis*, *E. smithii*, *E. camaldulensis* and *E. scoparia*, when compared to freshly damaged *C. citriodora*

Table 4 Identities and relative abundances of the compounds associated with EAD-active peaks and the Kovats retention indices for these peaks for comparison of the three tree species analysed on the GC–MS instrument

Peak ^a	Standard no	Compounds ^b	<i>E. globulus</i> (n = 2)			<i>E. viminalis</i> (n = 2)			<i>C. citriodora</i> (n = 2)		
			Rt (min)	KI	Area % ± std	Rt (min)	KI	Area % ± std	Rt (min)	KI	Area % ± std
a		C6 alcohol	27.63	844.3		27.63	844.2		27.62	844	
b	1	(E)-2-hexenal m/z 98*, 55 (100), 67 (19), 69 (57), 70 (20), 79 (31), 80 (21), 83 (65), 97 (16.3)	30.82	912.5	3.02 ± 3.37	30.83	912.7	1.13 ± 0.23	30.82	912.4	0.67 ± 0.37
	2	(Z)-3-hexen-1-ol m/z 100*, 55(100), 56 (25), 57 (20) 67 (21), 69 (57), 70 (25), 80 (25), 83 (67)	present	–	1.73 ± 0.74	–	–	1.26 ± 0.67	–	–	0 ± 0
	3	α-Pinene m/z 136*, 77 (38), 79 (28), 91 (100), 92 (50), 93 (64), 105 (13)	32.83	959.3	1.85 ± 1.37	32.83	959.1	3.50 ± 0.07	32.82	958.9	1.87 ± 0.24
	4	Camphene m/z 136*, 67 (28), 77 (31), 79 (52), 91 (70), 93 (100), 107 (23)	–	–	0 ± 0	–	–	0 ± 0	–	–	0 ± 0
c	5	β-Pinene m/z 136*, 77 (51), 79 (44), 80 (24), 91 (100), 93 (84), 107 (14), 107 (14), 121 (19)	–	–	0 ± 0	–	–	0 ± 0	–	–	0 ± 0
d	6	(Z)-3-hexenyl acetate m/z 142*, 65 (10), 67 (100), 82 (11)	36.15	1,040	21.78 ± 7.80	36.12	1,039	19.77 ± 6.05	36.11	1,039	4.55 ± 2.13
	7	3-Carene m/z 136*, 65 (20), 67 (96), 77 (48), 79(47), 91 (100), 92 (40), 93 (72), 105 (18), 121 (23)	–	–	0 ± 0	–	–	0 ± 0	–	–	0 ± 0
	8	α-Terpinene m/z 136* (70), 77 (43), 79 (39), 91 (100), 93 (92), 105 (32), 107 (12), 121 (71)	36.44	1,047	1.34 ± 0.06	36.43	1,047	2.67 ± 0.83	36.41	1,047	2.97 ± 0.18
e	10	Limonene m/z 136*, 67 (100), 79 (60), 91 (73), 92 (36), 93 (67), 94 (58), 107 (28), 119 (29), 121 (20)	36.93	1,060	7.15 ± 0.53	36.89	1,059	13.81 ± 0.25	36.87	1,058	4.18 ± 1.86
		Cymene m/z 134* (32), 67 (31), 79 (25), 91 (64), 115 (15), 117 (34), 119 (100)	–	–	0 ± 0	–	–	0 ± 0	–	–	0 ± 0
f	11	Eucalyptol m/z 154*, 67 (35), 69 (31), 79 (24), 81 (67), 93 (100), 107 (27), 108 (35), 111 (30), 139 (70)	37.53	1,075	14.2 ± 3.47	37.51	1,075	38.32 ± 8.09	37.5	1,074	11.89 ± 2.29

Table 4 continued

Peak ^a	Standard no	Compounds ^b	<i>E. globulus</i> (n = 2)			<i>E. viminalis</i> (n = 2)			<i>C. citriodora</i> (n = 2)		
			Rt (min)	KI	Area % ± std	Rt (min)	KI	Area % ± std	Rt (min)	KI	Area % ± std
g	12	γ -Terpinene m/z 136* (27), 77 (39), 79 (27), 80 (12), 91 (100), 92 (32), 93 (65), 105 (15), 121 (25)	37.95	1,086	6.98 ± 3.23	37.94	1,086	19.53 ± 0.62	37.94	1,086	73.86 ± 7.07
h	13	2-Phenylethanol m/z 122* (11), 65 (23), 91 (100), 92 (67)	43.05	1,228	0.11 ± 0.01	–	–	0 ± 0	–	–	0 ± 0
i	14	Benzyl acetate m/z 150* (14), 77 (12), 79 (40), 89 (21), 90 (14), 91 (23), 108 (100)	43.17	1,231	0.04 ± 0.02	–	–	0 ± 0	–	–	0 ± 0
j	15	Ethyl phenylacetate m/z 164* (28), 65 (16), 91 (100), 92 (15), 105 (10), 136 (14)	45.92	1,314	1.33 ± 0.08	–	–	0 ± 0	–	–	0 ± 0
	16	Terpenyl acetate m/z 196*, 67 (18), 79 (28), 91 (46), 92 (29), 93 (99), 105 (17), 107 (24), 108 (22), 121 (100), 136 (40)	49.04	1,415	40.48 ± 4.91	–	–	0 ± 0	–	–	0 ± 0

* The molecular ion is given first followed by fragments in ascending mass order. Ions in bold were used to extract peaks and for integration purposes if peaks were not resolved in the total ion chromatogram

^a Peak letters displayed in table refer to the investigated peaks that were selected from the initial antennal responses as in Figs. 1, 2 and 3

^b Mass fragments are indicated with relative intensities for unknown peaks and standards if not detected in the samples

leaves on which the beetle is known not to feed (Newete et al. 2011). Consistent with these results, the same *Eucalyptus* spp. have also been reported as being preferred hosts for *G. scutellatus* s.l. in South Africa (Mally 1924; Tooke 1953; Richardson and Meakins 1984; Newete et al. 2011). *E. dorrigoensis*, *E. nitens*, *E. scoparia*, *E. viminalis*, *E. grandis* and *E. smithii* were also found to bear more *G. scutellatus* eggs when compared to the other species surveyed from the field (Newete et al. 2011). Most of these species are known to occur near the suspected region of origin of the insect in eastern Australia (Newete et al. 2011). Female beetles therefore appear to be able to detect hosts that resemble some of the species found in their original habitat.

There are a number of factors that could result in the increased response magnitude observed for freshly damaged leaves. For example, different volatiles and mixtures of volatiles can be released after damaging the leaves (Kalberer et al. 2001). The differences in EAG responses observed between the different *Eucalyptus* species could arise due to different volatiles that are either unique to each species of tree or common between them. It is also possible

that *Gonipterus* females detect volatiles that originate specifically from the damaged foliage. Green leaf volatiles are known to originate from enzymatic reactions that occur when plant material is damaged (Gailliard and Matthew 1976; Matsui et al. 2000). These types of volatiles are known to stimulate the antennae of various phytophagous insects (Visser 1986; Metcalf and Metcalf 1992), and they are almost ubiquitous among all green plants. For example, certain phytophagous spider mites (*Tetranychus urticae*) are known to be attracted to foliage damaged by conspecific mites (Pallini et al. 1997). This is also known for weevils such as the vine weevil, *O. sulcatus*, which is strongly attracted to foliage that has been damaged by its conspecifics (van Tol et al. 2002). It is therefore possible that weevils such as *Gonipterus* spp. detect these compounds, because they convey information regarding the stress levels and general health of a potential host plant (D'Alessandro and Turlings 2006).

Gas chromatographic investigation of the damaged *Eucalyptus* leaves revealed that many of the volatiles that originate from leaves stimulate the antenna of the *Gonipterus* sp. 2 females, as could be seen in the complex

EAD traces matching the isolated volatiles. Compounds that were identified and confirmed as being antennally active for *Gonipterus* sp. 2 females included (E)-2-hexenal, (Z)-3-hexen-1-ol, α -pinene, camphene, β -pinene, (Z)-3-hexenyl acetate, 3-carene, limonene, eucalyptol, γ -terpinene, 2-phenylethanol, benzyl acetate and ethyl phenylacetate. These compounds are almost ubiquitous among all green plants (Metcalf and Metcalf 1992; Bruce et al. 2005).

The fact that *Gonipterus* sp. 2 detects a range of common compounds from *Eucalyptus* leaves may be explained by the high number of different *Eucalyptus* species that have been reported as hosts for this insect (see Clarke et al. 1998), which would require a general mechanism to identify the hosts more broadly. It is possible that *Gonipterus* sp. 2 distinguishes different *Eucalyptus* host species based on the relative emission rate and ratio differences of common compounds emitted from potential host trees. Unique combinations and ratios of some host volatiles could indicate more and less preferred hosts for *Gonipterus* sp. 2. If this is true, then the antenna would need a high degree of selectivity and sensitivity towards such volatiles. This phenomenon is known for other insect species. For example, females of the moth *Manduca sexta* are able to distinguish host species and quality based on host plant odour profiles (Späthe et al. 2012). The necessary selectivity appears to be present in the antenna or sensory periphery for that species (Späthe et al. 2012).

Results of this study showed that *Gonipterus* sp. 2 female antennae give relatively larger responses for the green leaf volatiles ((Z)-3-hexen-1-ol, (E)-2-hexenal and (Z)-3-hexenyl acetate) when compared to terpenes, including α -pinene, β -pinene, 1,8-cineol and γ -terpinene. This shows that the antennae are more sensitive to these compounds than towards the identified terpenes. This finding is consistent with an EAG study conducted on the vine weevil, *O. sulcatus*, which was shown to give larger EAG responses to (Z)-3-hexen-1-ol, (E)-2-hexenal, 2-phenylethanol and (Z)-3-hexenyl acetate, but showed weak responses towards terpenes (van Tol and Visser 2002). Measurement of single sensillum responses (SSR) in other weevil species have shown that these insects have specific neurons that are specialized for certain sets of volatiles. A SSR study on the white clover seed weevil (*Apion fulvipes*) has shown temporal differences in the response patterns of different receptor neuron classes towards different compounds (Andersson et al. 2012) including many compounds identified in the present study. These response differences were speculated to aid in discrimination of different odour filaments that the insect encounters as it flies. *Apion fulvipes* was also shown to possess a class of olfactory receptor neurons that specifically responds to damaged leaf odours (Andersson et al.

2012). In another weevil study, Blight et al. (1995) were able to show single sensillum responses to many of the same compounds as those identified in the present study, for the cabbage seed weevil (*Ceutorhynchus assimilis*). It is thus possible that *Gonipterus* sp. 2 uses a similar mechanism and similar receptor sets to discriminate different host odours.

A number of the identified compounds that elicited EAD responses in the *Gonipterus* sp. 2 females are also known to be antennally active for other insect species. These include 2-phenyl ethanol and (Z)-3-hexen-1-ol that were EAG active for the Colorado potato beetle, *Leptinotarsa decemlineata* (Weissbecker et al. 1999) and (Z)-3-hexen-1-ol, α -pinene, β -pinene, cymene, 1,8-cineole, and limonene being EAD active for the *Eucalyptus* woodborer, *Phoracantha semipunctata* (Barata et al. 2000). Five of the antenna-active compounds identified in the present study (2-phenylethanol, 1,8-cineol, (Z)-3-hexenyl acetate, (Z)-3-hexen-1-ol, (E)-2-hexenal) were found to be EAG active for the cabbage seed weevil (*Ceutorhynchus assimilis*) by Blight et al. (1995). 2-phenylethanol was also identified as being antenna active for the pollen beetle, *Astylus atro-maculatus*, and was shown to be behaviourally attractive to that species (Van den Berg et al. 2008). Two of the identified compounds, (Z)-3-hexenyl acetate and 2-phenyl ethanol, was found to be behaviourally attractive to adult tea weevil, *Myloccerinus aurolineatus*, females (Sun et al. 2012). These compounds could therefore be some of the volatiles that distinguish potential hosts for *Gonipterus* sp. 2 females. It is interesting that these two compounds were also in part responsible for separating the three species based on their presence and relative abundance within the damaged leaf profiles.

Although the behavioural function of the volatiles on *Gonipterus* sp. 2 remains unknown, our results have shown that there is a chemical interaction between *Gonipterus* sp. 2 female antennae and volatiles isolated from different *Eucalyptus* species. The olfactory interaction between *Gonipterus* sp. 2 females was further shown here to be very complex, but is mainly based on common green leaf volatiles that are released once the leaves are damaged. A number of electro-physiologically active volatile compounds were identified, and it is expected that some of these compounds may be involved in the insect behaviour, in particular female host choice. There is a possibility that some of the identified chiral terpenes (for example α -pinene, β -pinene, 3-carene, camphene and limonene) add an extra layer of complexity to the host selection behaviour for *Gonipterus* sp. 2. Enantiomeric ratios could also differ between crushed and non-crushed leaves of a single *Eucalyptus* species. Chiral separation to determine enantiomeric ratios could shed light on these complexities in future.

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References

- Adesso KM, McAuslane HJ, Alborn HT (2011) Attraction of pepper weevil to volatiles from damaged pepper plants. *Entomol Exp Appl* 138:1–11
- Andersson MN, Larsson MC, Svensson GP, Birgersson G, Rundlöf M, Lundin O, Lankinen Å, Anderbrant O (2012) Characterization of olfactory sensory neurons in the white clover seed weevil, *Apion fulvipes* (Coleoptera: Apionidae). *J Insect Physiol* 58:1325–1333
- Barata EN, Pickett JA, Wadhams LJ, Woodcock CM, Mustaparta H (2000) Identification of host and nonhost semiochemicals of the *Eucalyptus* woodborer *Phoracantha semipunctata* by gas chromatography-electroantennography. *J Chem Ecol* 26:1877–1895
- Blight MM, Pickett JA, Wadhams LJ, Woodcock CM (1995) Antennal perception of oilseed rape, *Brassica napus* (Brassicaceae), volatiles by the cabbage seed weevil *Ceutorhynchus assimilis* (Coleoptera, Curculionidae). *J Chem Ecol* 21:1649–1664
- Bray JR, Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. *Ecol Monogr* 27:325–349
- Brooker I, Kleinig D (1996) *Eucalyptus*: an illustrated guide to identification. Reed New Holland, Sydney. ISBN 1876334363
- Bruce TJA, Wadhams LJ, Woodcock CM (2005) Insect host location: a volatile situation. *Trends Plant Sci* 10:269–274
- Carbone SS, Rivera AC (1998) Sperm competition, cryptic female choice, and prolonged mating in the eucalyptus snout-beetle, *Gonipterus scutellatus* (Coleoptera, curculionidae). *Etologia* 6:33–40
- Clark AF (1931) The parasite control of *Gonipterus scutellatus* Gyll. *NZJ Sci Technol* 13:22–28
- Clarke AR, Paterson S, Pennington P (1998) *Gonipterus scutellatus* oviposition on seven naturally co-occurring *Eucalyptus* species. *For Ecol Manage* 110:89–99
- D'Alessandro M, Turlings TCJ (2006) Advances and challenges in the identification of volatiles that mediate interactions among plants and arthropods. *The Analyst* 131:24–32
- Dicke M (2000) Chemical ecology of host-plant selection by herbivorous arthropods, a multitrophic perspective. *Biochem Syst Ecol* 28:601–617
- Gailliard T, Matthew J (1976) Lipxygenase-mediated cleavage of fatty acids to carbonyl fragments in tomato fruits. *Phytochemistry* 16:339–343
- Guenther AB (1991) Isoprene and monoterpene emission rate variability: observations with *Eucalyptus* and emission rate algorithm development. *J Geophys Res* 96:10799–10808
- Hanks LM, Millar JG, Paine TD, Campbell CD (2000) Classical biological control of the Australian weevil *Gonipterus scutellatus* (Coleoptera: Curculionidae) in California. *Environ Entomol* 29:369–375
- Harari AR, Landolt PJ (1997) Orientation of sugarcane rootstalk borer weevil, *Diaprepes abbreviatus*, to weevil, frass, and food odors. *J Chem Ecol* 23:857–868
- Kalberer NM, Turlings TCJ, Rahier M (2001) Attraction of a leaf beetle (*Oreina cacaliae*) to damaged host plants. *J Chem Ecol* 27:647–661
- Kotze MJ, Jürgens A, Johnson SD, Hoffmann JH (2010) Volatiles associated with different flower stages and leaves of *Acacia cyclops* and their potential role as host attractants for *Dasineura dielsi* (Diptera: Cecidomyiidae). *S Afr J Bot* 76:771–777
- Lanfranco D, Dungey HS (2001) Insect damage in *Eucalyptus*: a review of plantations in Chile. *Austral Ecol* 26:477–481
- Loch AD, Floyd RB (2001) Insect pests of the Tasmanian blue gum, *Eucalyptus globulus*, in south-western Australia: history, current perspectives and future prospects. *Austral Ecol* 26:458–466
- Mally CW (1924) The *Eucalyptus* snout beetle *Gonipterus scutellatus* (Gyll.). *J Dep Agric S Afr* 9:3–31
- Mapondera TS, Burgess T, Matsuki M, Oberprieler RG (2012) Identification and molecular phylogenetics of the cryptic species of the *Gonipterus scutellatus* complex (Coleoptera: Curculionidae: Gonipterini). *Aust J Entomol* 51:175–188
- Matsui K, Ujita C, Fujimoto S, Wilkinson J, Hiatt B, Knauf V, Kajiwara T, Feussner I (2000) Fatty acid 9- and 13-hydroperoxide lyases from cucumber. *FEBS Lett* 481:183–188
- Metcalfe RL, Metcalfe ER (1992) Plant kairomones in insect ecology and control. Chapman and Hall, London
- Newete SW, Oberprieler RG, Byrne MJ (2011) The host range of the *Eucalyptus* Weevil, *Gonipterus "scutellatus"* Gyllenhal (Coleoptera: Curculionidae). *Ann For Sci* 68:1005–1013
- Nunes TV, Pio CA (2001) Emission of volatile organic compounds from Portuguese *Eucalyptus* forests. *Chemosph Glob Change Sci* 3:239–248
- Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara B, Simpson GL, Solymos P, Stevens MHH, Wagner H (2013) Community ecology package. R package version 2.0-10. <http://cran.r-project.org>, <http://vegan.r-forge.r-project.org/>
- Pallini A, Janssen A, Sabelis MW (1997) Odour-mediated responses of phytophagous mites to conspecific and heterospecific competitors. *Oecologia* 110:179–185
- Pio CA, Nunes TV, Castro LM, Lopes DA (2001) Volatile and particulate organic compounds in the ambient air of a *Eucalyptus* forest in Portugal during the FIELDVOC94 campaign. *Chemosph Glob Change Sci* 3:283–293
- Proffitt M, Johnson SD (2009) Specificity of the signal emitted by figs to attract their pollinating wasps: comparison of volatile organic compounds emitted by receptive syconia of *Ficus sur* and *F. sycomorus* in Southern Africa. *S Afr J Bot* 76:701–709
- Richardson KF, Meakins RH (1984) Inter- and intra-specific variation in the susceptibility of eucalypts to the snout beetle *Gonipterus scutellatus* Gyll. (Coleoptera: Curculionidae). *S Afr For J* 139:21–31
- Rivera AC, Carbone SS (2000) The effect of tree species of *Eucalyptus* on the growth and fecundity of the *Eucalyptus* snout beetle (*Gonipterus scutellatus*). *Forestry* 73:21–29
- Rivera AC, Carbone SS, Andrés JA (1999) Life cycle and biological control of the *Eucalyptus* snout beetle (Coleoptera, Curculionidae) by *Anaphes nitens* (Hymenoptera, Mymaridae) in north-west Spain. *Agric For Entomol* 1:103–109
- Slone DH, Sullivan BT (2007) An automated approach to detecting signals in electroantennogram data. *J Chem Ecol* 33:1748–1762
- Späthe A, Reinecke A, Olsson SB, Kesavan S, Knaden M, Hansson BS (2012) plant species- and status-specific odorant blends guide oviposition choice in the moth *Manduca sexta*. *Chem Senses* 38:147–159
- Sun XL, Wang GC, Gao Y, Chen ZM (2012) Screening and field evaluation of synthetic volatile blends attractive to adults of the tea weevil, *Myloccerinus aurolineatus*. *Chemoecology* 22:229–237

- Tooke FGC (1953) The Eucalyptus Snout-beetle, *Gonipterus scutellatus* Gyll. A study of its ecology and control by biological means. Entomology Memoir 3. Department of Agriculture, Pretoria, South Africa, pp 1–53
- Van den Berg J, Torto B, Pickett JA, Smart LE, Woodcock LJWCM (2008) Influence of visual and olfactory cues on field trapping of the pollen beetle, *Astylus atromaculatus* (Col.: Melyridae). *J Appl Entomol* 132:490–496
- van Tol RWHM, Visser JH (2002) Olfactory antennal responses of the vine weevil *Otiiorhynchus sulcatus* to plant volatiles. *Entomol Exp Appl* 102:49–64
- van Tol RWHM, Visser JH, Sabelis MW (2002) Olfactory responses of the vine weevil, *Otiiorhynchus sulcatus*, to tree odours. *Physiol Entomol* 27:213–222
- Visser J (1986) Host odor perception in phytophagous insects. *Annu Rev Entomol* 31:121–144
- Weissbecker B, Loon JAV, Dike M (1999) Electroantennogram responses of a predator, *Perillus bioculatus*, and its prey, *Leptinotarsa decemlineata*, to plant volatiles. *J Chem Ecol* 25:2313–2325
- Williams JR, Moutia LA, Hermelin PR (1951) The biological control of *Gonipterus scutellatus* Gyll. in Mauritius. *Bull Entomol Res* 42:23–28