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

Authors:

1. Marc. Clement. Bouwer (Corresponding)

   Affiliations: Department of Chemistry, Forestry and Agricultural Biotechnology Institute, University of Pretoria

   Address: Cnr Lynnwood and University Roads, Hatfield 0083, Pretoria, South Africa

   Email address: marc.bouwer@fabi.up.ac.za

   Tel: +27124285821

   Fax: +27124203960

2. Bernard Slippers

   Affiliations: Department of Genetics, Forestry and Agricultural Biotechnology Institute, University of Pretoria

   Address: Cnr Lynnwood and University Roads, Hatfield 0083, Pretoria, South Africa

3. Michael John Wingfield

   Affiliations: Forestry and Agricultural Biotechnology Institute, University of Pretoria

   Address: Cnr Lynnwood and University Roads, Hatfield 0083, Pretoria, South Africa

4. Egmont Richard Rohwer

   Affiliations: Department of Chemistry, University of Pretoria

   Address: Cnr Lynnwood and University Roads, Hatfield 0083, Pretoria, South Africa

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 has 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 (EAG) experiment. We sampled volatiles from freshly damaged leaves of three *Eucalyptus* species, and analyzed the profiles by gas chromatography coupled to electroantennography (GC-EAD) and gas chromatography coupled to mass spectrometry (GC-MS). 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.

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 *G. 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 *Gonipterus scuttelatus*, 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 geographic 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 is, 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. select 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, sequiterpenes 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 eugenii* has shown that these beetles are attracted to damaged plants and in particular to plants freshly damaged by their conspecifics (Addesso et al. 2001). The sugarcane root-stalk borer weevil, *Diaprepes abbreviatus*, is also attracted to mechanically damaged plant tissue (Harari et al. 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 electrophysiological 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. *Corymbia 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 cm^2. The leaves were sampled for 30 minutes 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 *Corymbia 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 pyrolidone) added to prevent desiccation. The entire preparation was moved to within one centimeter 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 second puffs, at 30 ml/min at puff maximum.

Clean surgical blades were used to cut a 1 cm^2 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 X 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 Hz to 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 minutes 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 steam 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 (1000 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 minutes 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 to 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 (Proffit and Johnson, 2009; Kotze et al 2010).

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 to the non-host *Corymbia 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 (Figure 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, amongst 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 (Figure 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 (Table 2, 3 and 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 (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 the 16 investigated compounds for only 2 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. *Corymbia citriodora* was mainly separated from *E. globulus* and *E. viminalis* due to the influence of γ-terpinene, which was present in relatively larger proportions.

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* 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, *Otiorhynchus 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 toward 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, *Otiorhynchus 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) was 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 atromaculatus* 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, *Myllocerinus 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 insects 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.

Acknowledgements

We are grateful to the members of the Tree Protection Co-operative Programme (TPCP), the THRIP initiative of the Department of Science and Technology (DST), South Africa and the National Research Foundation (NRF) for providing financial support for this study. We also thank Dr. Jeff Garnas for assistance with some of the statistical tests and for providing unpublished data pertaining to the identification of the *Gonipterus* sp. used in this study.

References

Addesso KM, McAuslane HJ and 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 and 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 and Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. *Ecological monographs* 27: 325-349.

Brooker I and Kleinig D (1996) *Eucalyptus*: An illustrated guide to identification. *Sydney: Reed New Holland* 230p. -. ISBN 1876334363.

Bruce TJA, Wadhams LJ, Woodcock CM (2005) Insect host location: a volatile situation. *Trends in Plant Science* 10:269-274.

Carbone SS and 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. Tech* 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) Lipoxygenase-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 and 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 and 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 Ecology* 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 Ecology* 26:458-466.

Mally CW (1924) The *Eucalyptus* snout beetle *Gonipterus scutellatus* (Gyll.). J Dep Agric South Africa: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 Ent* 51:175-188.

Matsui K, Ujita C, Fujimoto S, Wilkinson J, Hiatt B, Knauf V, Kajiwara T and Feussner I (2000) Fatty acid 9- and 13-hydroperoxide lyases from cucumber. *FEBS Letters* 481:183-188.

Metcalf RL, Metcalf ER (1992) Plant kairomones in insect ecology and control. Chapman and Hall.

Newete SW, Oberprieler RG, Byrne MJ (2011) The host range of the *Eucalyptus* Weevil, *Gonipterus* "*scutellatus*" Gyllenhal (Coleoptera: Curculionidae), *Ann For Sci* Vol. 68:1005-1013.

Nunes TV, Pio CA (2001) Emision of volatile organic compounds from Portuguese *Eucalyptus* forests. *Chemosphere-Global 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. *Chemosphere-Global Change Sci* 3:283-293.

Proffit M and 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). South African Forestry Journal 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 and Chen ZM (2012) Screening and field evaluation of synthetic volatile blends attractive to adults of the tea weevil, *Myllocerinus 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. 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 *Otiorhynchus sulcatus* to plant volatiles. *Entomol Exp Appl* 102:49-64.

van Tol, R.W.H.M., J. H. Visser, and M. W. Sabelis. (2002).Olfactory responses of the vine weevil, *Otiorhynchus 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 JJAV, 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.

Figure captions

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 electrophysiologically 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 electrophysiologically active compounds. Peak letters correspond to those listed in Tables 3 and 4.

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 electrophysiologically active compounds. Peak letters correspond to those listed in Tables 3 and 4.

Fig. 4 GC-EAD responses toward identified standard compounds after liquid injection at 20.0psi (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.

Supplementary Fig. 1 Non metric multidimensional scaling (NMDS) plot based on the mass spectral integration data for the 16 compounds confirmed with reference standards for each species. The plot shows the Bray-Curtis distance rotated so that the variance is maximized on the first dimension. Numbers indicate compound identity as in Tables 2, 3 and 4. Stress ≈ 0.

Figure 1

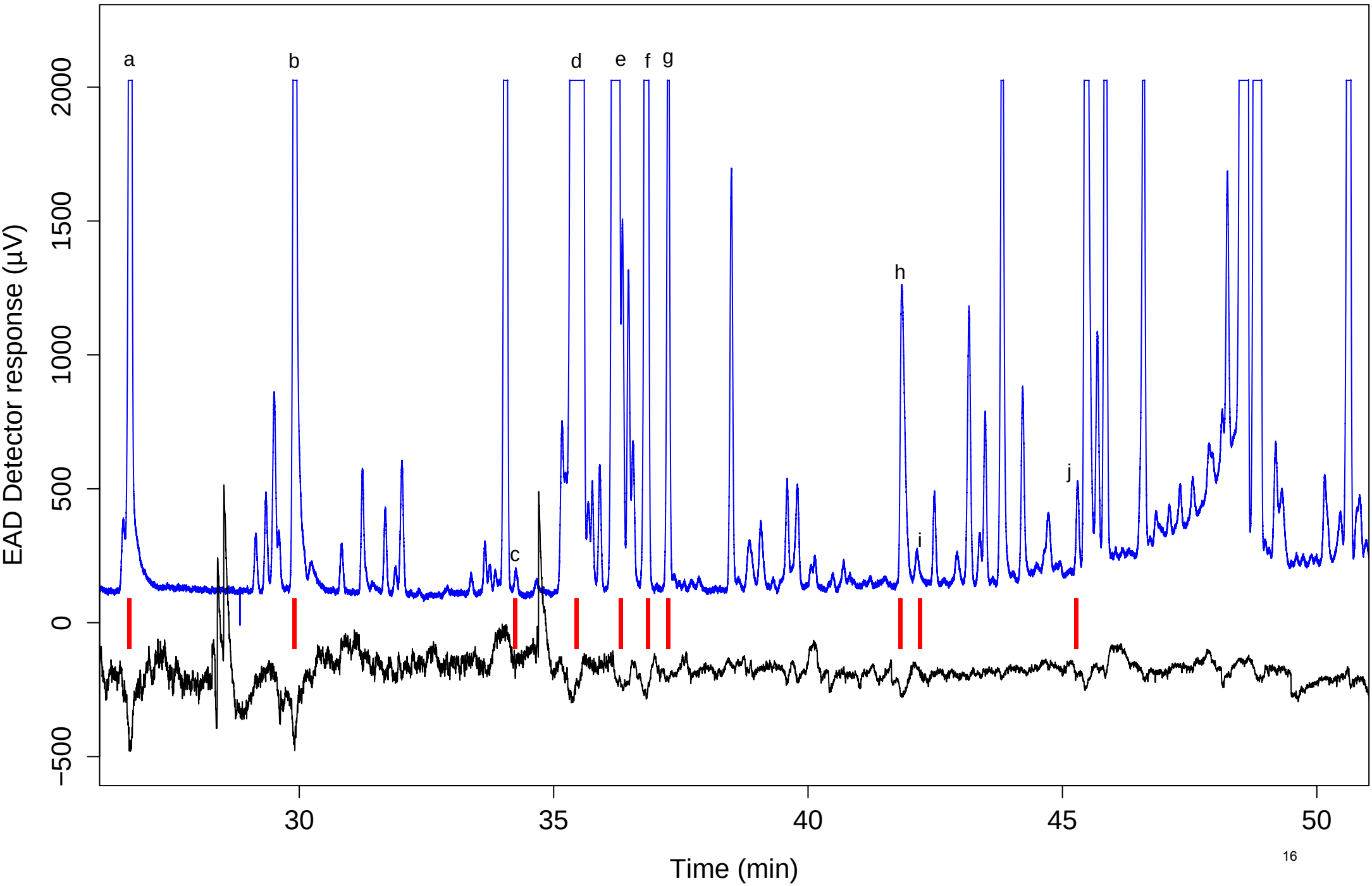

a
b
d
e
f
g
h
i
j
c
EAD Detector response (µV)
2000
1500
1000
500
0
−500
30
35
40
45
50
Time (min)

Figure 2

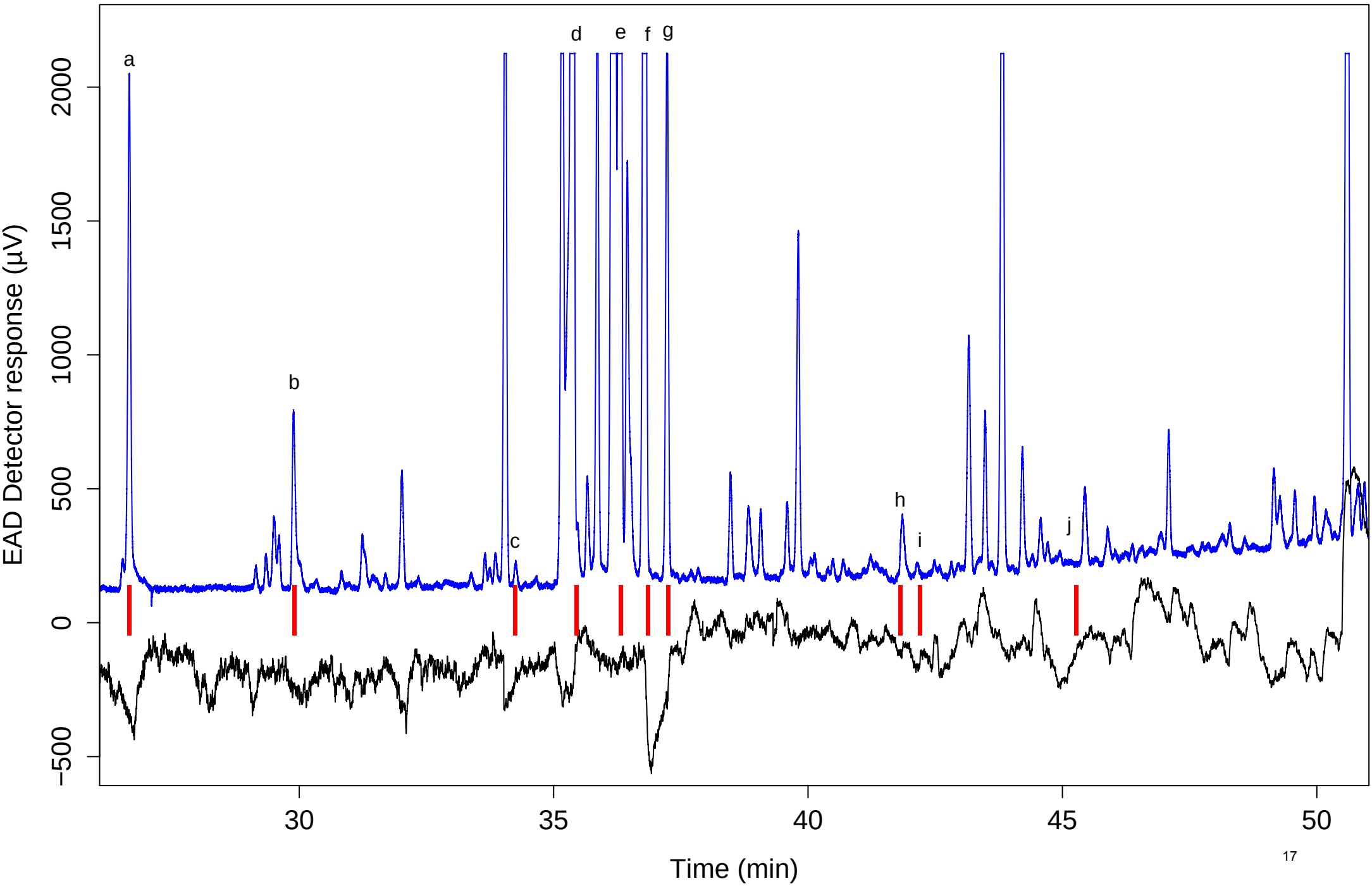

a
b
c
d
e
f
g
h
i
j
EAD Detector response (µV)
2000
1500
1000
500
0
−500
30
35
40
45
50
Time (min)

Figure 3

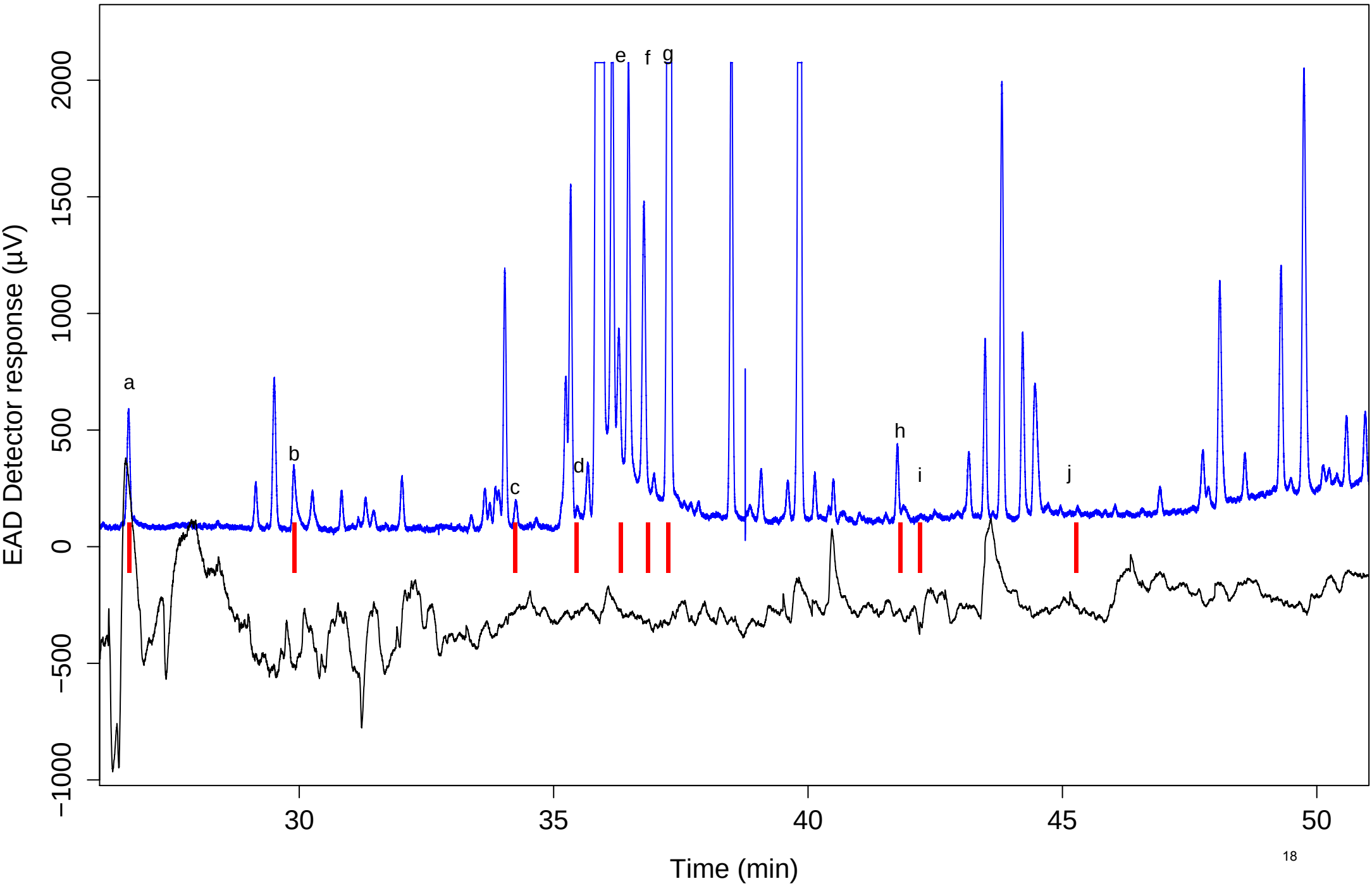
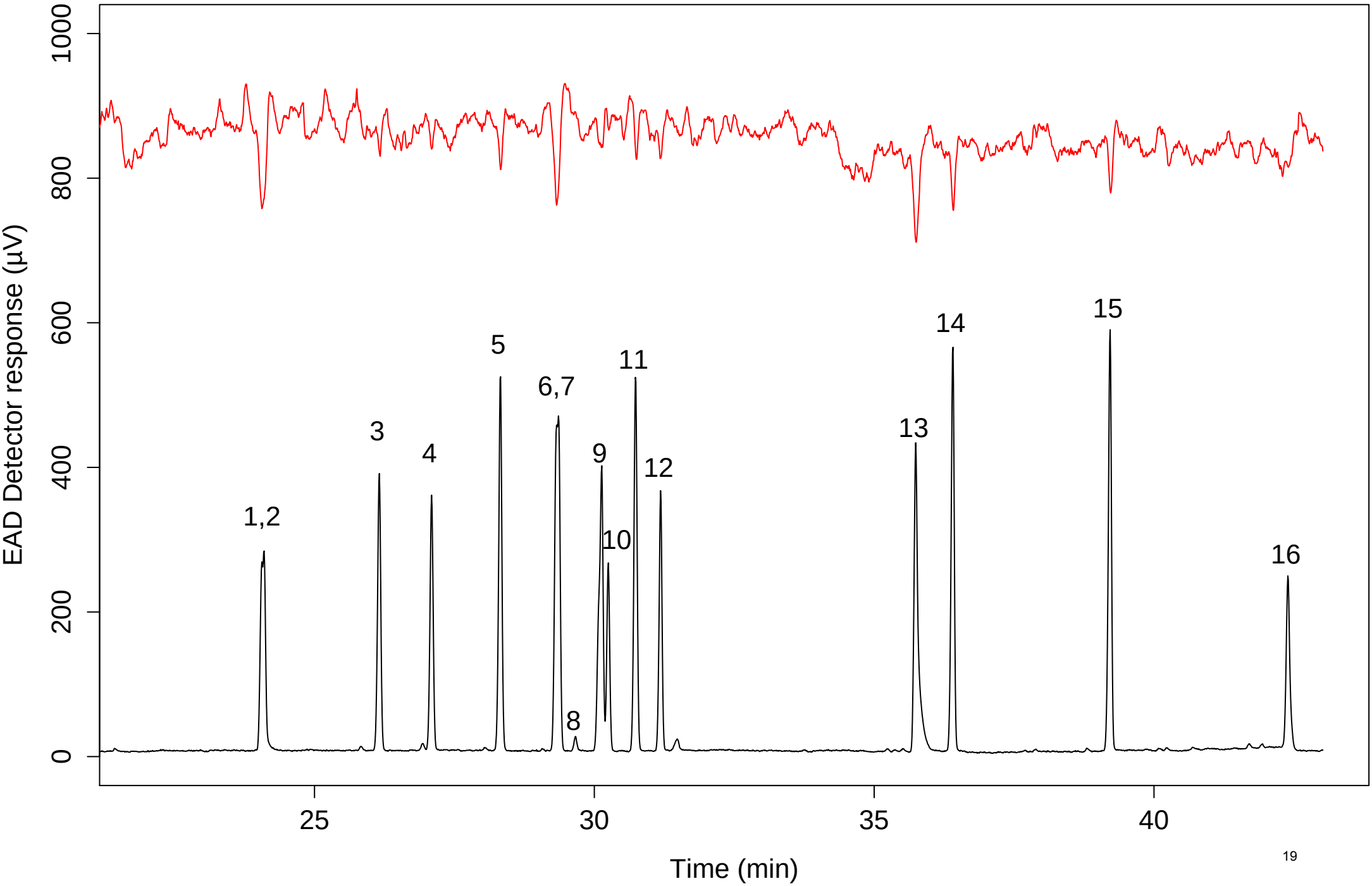

EAD Detector response (µV)
2000
1500
1000
500
0
−500
−1000
a
b
c
d
e
f
g
h
i
j
30
35
40
45
50
Time (min)

Figure 4


EAD Detector response (µV)
0
200
400
600
800
1000
25
30
35
40
Time (min)
1,2
3
4
5
6,7
8
9
10
11
12
13
14
15
16

Supplementary Figure 1

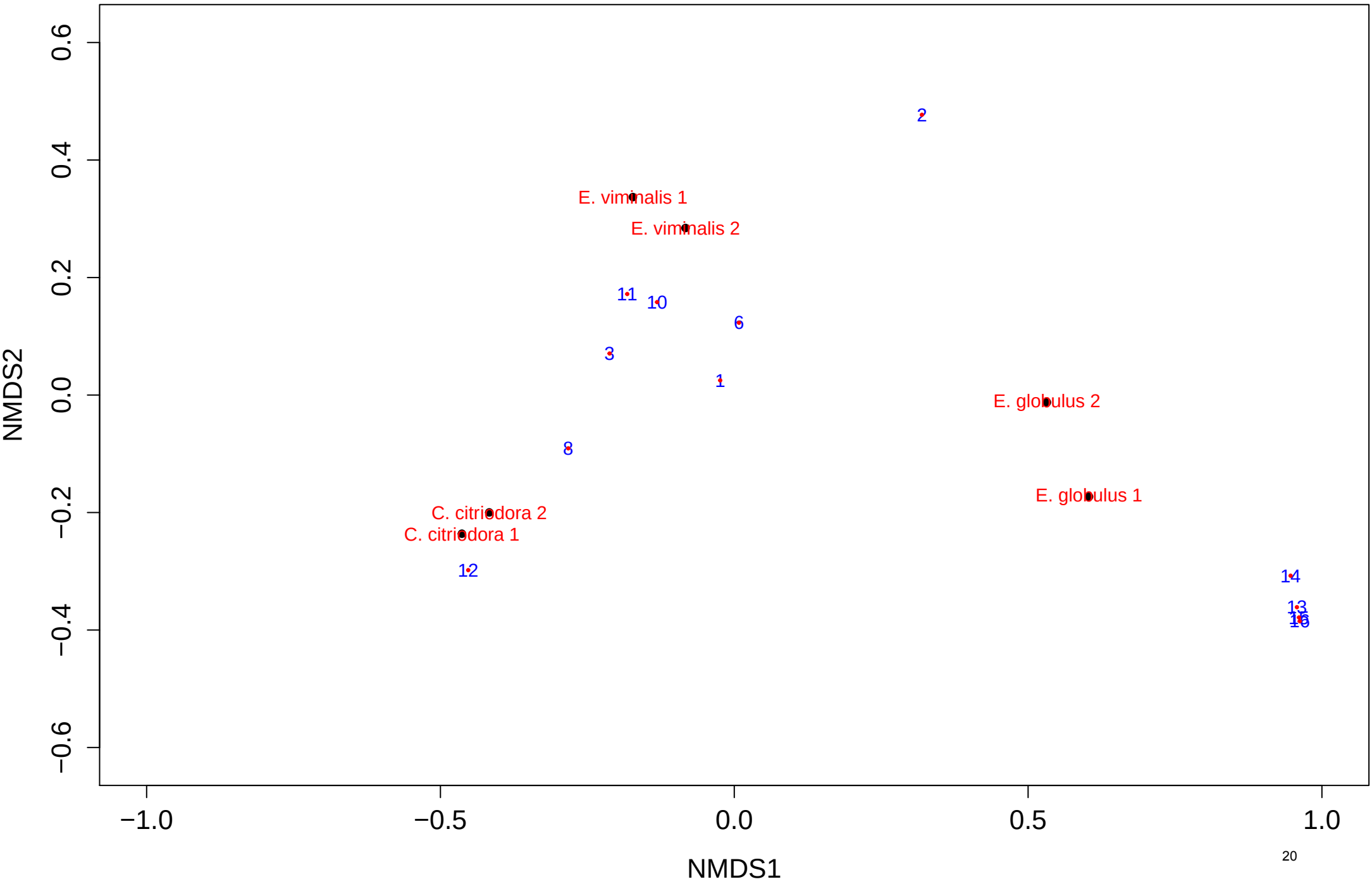


Table 1: Differences between EAG response magnitude to the different leaf treatments. Freshly damaged refers to leaves that were mechanically scraped before recordings were made

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

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

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* KI liquid	GC-EAD* KI thermal	GC-MS* 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	a-pinene	80-56-8	98	959	958	959
4	camphene	79-92-5	99.3	982	980	981
5	b-pinene	127-91-3	99.5	1012	1011	1011
6	(Z)-3-hexenyl acetate acetate	1708-82-3	99	1038	1038	1038
7	3-carene	13466-78-9	96.5	1038	1038	1038
8	a-terpinene	99-86-5	94	1046	1046	1047
9	m-cymene	535-77-3	99	1058	1058	1058
10	limonene	5989-27-5	99.3	1058	1058	1058
11	eucalyptol	470-82-6	-	1074	1073	1074
12	g-terpinene	99-85-4	97	1085	1085	1085
13	2-phenylethanol	60-12-8	-	1209	1209	1226
14	benzyl acetate	140-11-4	99.5	1229	1228	1231
15	ethyl phenylacetate	101-97-3	99	1311	1311	1314
16	terpenyl acetate	80-26-2	-	1411	1411	1413

*Gas Chromatography (GC), Electroantennography 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.

			E. globulus		*E. viminalis*		*C. citriodora*	
Peak[a]	Standard No	Compounds	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	1010	-	-	-	-
d	6	(Z)-3-hexenyl acetate	35.55	1043	35.44	1040	35.31	1037
	7	3-carene	-	-	-	-	-	-
	8	α-terpinene	present	-	35.7	1047	35.65	1045
e	10	limonene	36.32	1062	36.22	1060	36.13	1057
		cymene	present	-	36.35	1063	36.25	1061
f	11	eucalyptol	36.85	1076	36.81	1075	36.75	1073
g	12	γ-terpinene	37.25	1086	37.24	1085	37.25	1086
h	13	2-phenylethanol	41.81	1209	41.83	1209	-	-
i	14	benzyl acetate	42.45	1227	-	-	-	-
j	15	ethyl phenylacetate	45.27	1310	-	-	-	-
	16	terpenyl acetate	48.6	1415	-	-	-	-

[a] Peak letters displayed in table refer to the investigated peaks that were selected from the initial antennal responses as in Figure 2 to 4

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.

			E. globulus (n = 2)			*E. viminalis* (n = 2)			*C. citriodora* (n = 2)		
Peak[a]	Standard No	Compounds[b]	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	1040	21.78 ± 7.80	36.12	1039	19.77 ± 6.05	36.11	1039	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	1047	1.34 ± 0.06	36.43	1047	2.67 ± 0.83	36.41	1047	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	1060	7.15 ± 0.53	36.89	1059	13.81 ± 0.25	36.87	1058	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	1075	14.2 ± 3.47	37.51	1075	38.32 ± 8.09	37.5	1074	11.89 ± 2.29
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	1086	6.98 ± 3.23	37.94	1086	19.53 ± 0.62	37.94	1086	73.86 ± 7.07
h	13	2-phenylethanol m/z **122*** (11), 65 (23), 91 (100), 92 (67)	43.05	1228	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	1231	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	1314	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	1415	40.48 ± 4.91	-	-	0 ± 0	-	-	0 ± 0

[a] Peak letters displayed in table refer to the investigated peaks that were selected from the initial antennal responses as in Figure 1 to 3. [b]Mass fragments are indicated with relative intensities for unknown peaks and standards if not detected in the samples. *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.