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Gas chromatography with mass spectrometric and electroantennographic detection: analysis of wood odorants by direct coupling of insect olfaction and mass spectrometry

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Abstract

A gas chromatography–mass spectrometry–electroantennographic detection (GC–MS/EAD) setup has been designed by adapting a commercially available "Olfactory Detector Port" to the use with an insect antenna. Measurements were performed with antennae of the old house borer *Hylotrupes bajulus*, a widespread insect pest of coniferous timbers. Headspace volatiles from timber of *Pinus sylvestris* were collected and analysed by GC–MS. About 30 compounds were identified in the Kovacs range from 500 to 1200, especially terpenoids and aliphatic alcohols and aldehydes. The antennae of *H. bajulus* responded to nearly half of the detected volatiles with a peculiar sensitivity for α-pinene among the terpenes and for hexanal among the aldehydes.

Keywords: Electroantennographic detection; Detection, GC; *Hylotrupes bajulus*; Wood volatiles; Alcohols; Aldehydes; Terpenes

1. Introduction

The ability of insect antennae to produce electrical potentials when stimulated with volatiles was demonstrated by Schneider [1]. The so-called electroantennogram (EAG) can be recorded with electrodes positioned in the hemolymph at the base and the tip of an antenna. The EAG represents a superposition of receptor potentials of sensory cells with different specifications. When the antenna is stimulated with isolated compounds the amplitude of an EAG signal correlates with the concentration of the applied substance. The high sensitivity and selectivity of insect olfactory receptors combined with a gas chromatographic separation provides a powerful analytical technique [2,3] referred to as GC–EAG or GC–electroantennographic detection (EAD). This technique is predominantly used by chemical ecologists in order to investigate the interrelation between insects and their environment.

The main apparative challenges of this method are the development of an "interface" for the adaptation of the hot and dry GC effluent to the requirements of the insect (i.e. humid and at a biocompatible temperature) and the use of a high-impedance amplifier which allows the recording of the EAD signal with a plotter or a data processor [4]. Examples of recent publications show the usefulness of this method, e.g. in pheromone research [5–7], host finding of parasitoids [8,9], and food location of phytophagous insects [10–12]. Other examples are interactions like chemical mimicry [13,14] or multitrophic interactions [15].

Often EAD is used in parallel to flame ionisation detection (FID) as a GC–FID/EAD technique. When samples of unknown composition are analysed, in addition to the GC–FID/EAD technique an analysis by GC–MS is essential. However, the comparison of chromatograms recorded with two different setups can be very difficult and time consuming. Moreover, modern sampling techniques like solid-phase microextraction (SPME) or thermo-desorption produce samples that are exhausted in the moment of injection and cannot be used for a second injection in another analytical instrument. This raises the demand for a combination of both methods to a coupled GC–MS/EAD technique. The main difficulty of this method is the controlled splitting of the GC effluent between atmosphere (EAD) and vacuum (MS).

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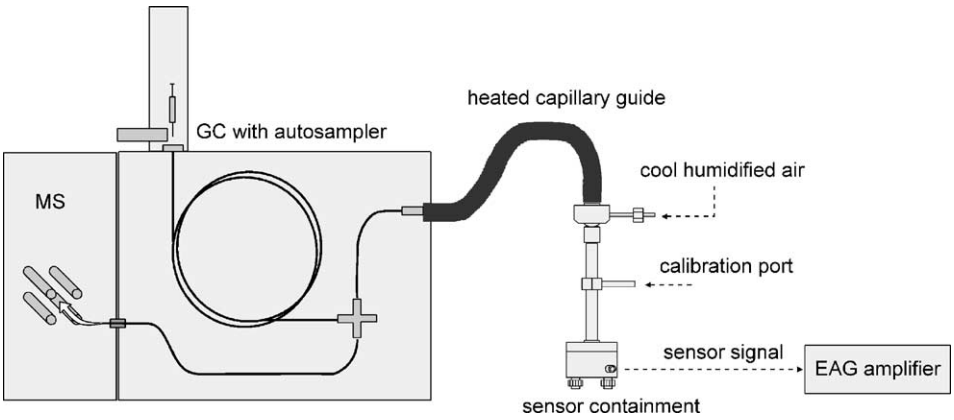

Fig. 1. Schematic drawing of the GC–MS/EAD setup.

A stimulus for solving this problem came from another closely related field of application. In parallel to GC–EAD, the technique of combining gas chromatography with a "sniffing detector" has been developed. This technique, sometimes called gas chromatography–olfactometry (GC–O) is quite similar to GC–EAD, but a human nose is used as detector and the operator is able to record character and intensity of the odours. The main field of application of this technique is found in the production of perfumes, nourishments and stimulants [16–18].

But also other branches of manufacturing industry (e.g. cloths, furniture, cars) realised the importance of the sense of smell for purchase decisions of the consumers. Maybe this demand from economically important industries finally led to the emergence of GC–MS/O as an ultimative method in combining analytical power with the sensory impression of a human operator. Today, devices for the coupling of MS with an olfactory detection are available from several suppliers of analytical equipment. Applications are found for example in the production process of synthetics [19] but also in the monitoring of malodours from agricultural facilities [20]. We now performed the next step by adapting a commercially available GC–MS/O interface to a GC–MS/EAD setup (Fig. 1).

First results with the new method have already been obtained with *Cameraria ohridella*, an invasive pest of horse chestnut trees [21]. As a new challenge we chose the old house borer (also called house longhorn beetle) *Hylotrupes bajulus*. This beetle is a widespread insect pest of coniferous timbers and can cause substantial damage to roof timbering or framework houses. With an origin in Europe the beetle has dispersed into areas of moderate climate all over the world. Adults of *H. bajulus* are found in sizes ranging from 8 to 20 mm. Female beetles lay eggs into cracks of timber or of truncated trees after windblows. The damage is done by the larvae which spend from 2 up to 8 years feeding in the wood before they pupate and emerge as adult beetles. The tunnelling of the larvae often results in considerable financial loss since infested wood in buildings has to be replaced. An understanding of the volatiles relevant for the orientation of *H. bajulus* could help to find new methods for protection of wood and a control of the beetle. *H. bajulus* is very delicate in the choice of sites for mating and oviposition and obviously is guided by olfactory cues [22–24]. Recent behavioural studies supported these results and attached importance to monoterpenoid hydrocarbons as attractants [25]. These experiments were performed with hexane extracts of wood or odorant standards of volatiles that were previously identified as constituents of the coniferous aroma. A direct investigation of the olfactory response of *H. bajulus* to an original odorant sample of its host trees by GC–EAD so far has not been made. The volatile emissions of wood are a complex mixture of terpenes, aldehydes, alcohols and other hydrocarbons which make it inevitable to perform an additional analysis by GC–MS. The use of a combined GC–MS/EAD setup offers the advantage that no toilsome comparison between the chromatograms of two different setups is necessary. The obtained results give a new insight into the chemical ecology of *H. bajulus* which might be used for the development of new methods of wood protection.

2. Experimental

2.1. Sampling of volatiles

Samples for GC–MS/EAD analysis were collected using the closed-loop-stripping-analysis (CLSA) method [26]. Timber of Scots pine (*Pinus sylvestris* L.) was reduced to small cuboids of about 5 cm^3 volume. Of these cuboids 100 g were put into a 500 ml glass flask with a ground neck outlet. The outlet was closed with a PTFE-stopper. Stainless steel capillaries (i.d. 1 mm) were fed through the stoppers. A miniature pump (Fürgut, Tannheim, Germany) circulated air from the flask to an adsorbent trap loaded with 1.5 mg charcoal (CLSA-Filter, Daumazan sur Arize, France). Sampling was performed for 45 min with a flow of 1 l/min. Volatiles were eluted from the charcoal with 75 μl of a mixture consisting of methylene chloride (two parts) and methanol (one part) (both solvents Suprasolv quality, Fa. Merck/VWR, Darm-

stadt, Germany). Samples were stored in an ultra low temperature freezer at −80 °C.

2.2. GC–MS/EAD system

The system (Fig. 1) is based on a GC–MS system produced by Agilent (Palo Alto, USA) and consists of a type 6890N gas chromatograph connected to a type 5973N quadrupole mass spectrometer. The GC is equipped with a type 7163 autosampler and a split/splitless injector. Data acquisition is done with the MS ChemStation software (Agilent). A J&W Scientific HP-5MS column (Agilent) is used (30 m × 0.25 mm i.d., film thickness 0.25 μm). The effluent from the column is splitted into two pieces of deactivated capillary using a Graphpack 3D/2 flow splitter (Gerstel, Mülheim, Germany). One capillary (1 m × 0.1 mm i.d.) leads to the mass spectrometer, the other (1 m × 0.15 mm i.d.) to an "olfactory detector port" (ODP-2, Gerstel). The split and the restriction capillaries were part of the ODP setup and were "factory adjusted" in order to result in an equal split of the gas flow into the two setups.

The ODP incorporates a flexible heating sleeve (length outside GC oven: 35 cm) which guides the capillary out of the GC oven. When the volatiles elute from the end of the capillary they are enveloped by a flow of helium used as a make-up gas in order to prevent contact of the volatiles with the surfaces of the setup. The nose-adapter that normally belongs to the ODP is replaced by a mixing chamber in which the effluent of the capillary is mixed with humidified air [23 °C, 80% relative humidity (RH)]. The humidified air is provided by bubbling synthetic air through a 500 ml washing bottle at a rate of 500 ml/min. The airflow is directed vertically through the flow tube (length: 15 cm, i.d. 6 mm, PTFE) to the insect antenna which is housed in a detector cell made of PTFE. This setup is in the following referred to as EAD interface.

For peak identification the National Institute of Standards and Technology mass spectral library (NIST, Gaithersburg, USA) and the MassFinder 2.1 software together with the library "Terpenoids and Related Constituents of Essential Oils" (Hochmuth, König, Joulain, Hamburg, Germany) are used.

2.3. Analytical conditions

Samples are injected in a quantitiy of 1 μl into the injector in the pulsed splitless mode (pulse pressure 150 kPa until 1.5 min) at a temperature of 250 °C. The GC is operated in the following temperature program: start: 50 °C, hold for 1.5 min, ramp 6 °C/min to 200 °C, hold for 5 min. The chosen temperature program is a compromise between optimal separation conditions and a moderate run time which is essential because of the limited lifetime of the employed antenna.

Helium (purity 99.999%) is used as carrier gas after passing through a combined adsorbent trap for removal of traces of water, oxygen and hydrocarbons ("Big Universal Trap", Agilent). The carrier gas flow is set to 1 ml/min resulting in a gas vector of 24 cm/s. The GC–MS interface is operated at a temperature of 280 °C. The heating sleeve of the ODP is set to 230 °C.

The mass spectrometer uses electron ionisation (EI) at 70 eV and is used in the scan mode with a mass range from 35 to 300 mass units at a scan speed of 2.78 scans per second.

2.4. EAD

Excised antennae of *H. bajulus* are placed in an antenna holder [27] (Professor Koch, Kaiserslautern, Germany) modified from a model designed for a portable EAG system. Within this holder the ends of the antennae are in touch with an electrolyte solution adapted to the insect's hemolymph [28] which provides electrical contact to a pair of Ag/AgCl electrodes (Fig. 2). The antenna holder provides a stable support for the antenna in which its surface is freely accessible to the air flow from the EAD interface.

For amplification of the EAD potentials an electronic setup (Professor Koch) is used that consists of a pre-amplifier, a main amplifier, a frequency filter and an adjustment amplifier. Pre-amplifier (input impedance 100 MΩ) and main amplifier each provide amplification by a factor of 10, resulting in a total amplification of 100. The amplifier has a built-in low pass filter which is set to a cut-off frequency of 1 Hz in order to suppress the ubiquitous mains frequency of 50 Hz.

The amplified signal is recorded by the Agilent GC ChemStation software (which is installed on the data acquisition system in addition to the MS ChemStation software) using the type 35900E A/D converter (Agilent). The A/D converter is connected to the acquisition system via LAN and synchronized by the start signal of the GC. The following steps were taken in order to match the amplifier output to the input signal range of the A/D converter (0–1 V):

(1) The additional frequency filter is used as a high pass filter with a cut-off frequency of 0.01 Hz to suppress the slow drift which is often observed in the EAD signal and can cover several volts in the amplified signal.
(2) Since EAG signals may be positive or negative (depending on the mounting of the antenna in the antenna holder)

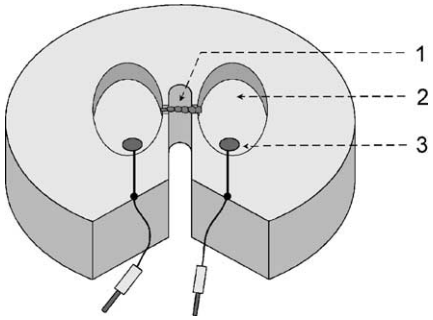

Fig. 2. Antenna holder milled from a Perspex disc (diameter 27 mm, height 12 mm). The antenna (1) is stretched over the central hole of the holder while its ends are in contact with the electrolyte solution reservoir (2). The Ag/AgCl electrodes (3) are connected to micro plugs via silver wires.

it is convenient to use the adjustment amplifier to add a constant voltage of 0.5 V to the amplified, high pass filtered signal. This keeps the signal in the center of the signal input range of the A/D converter (0–1 V) and allows to record positive deflections as well as negative deflections.

2.5. Calibration of the EAD

For a quick calibration of the EAD system a calibration port is installed into the flow tube of the EAD interface (Fig. 1). The calibration port is positioned 4.5 cm below the upper end of the flow tube. Odorant standards are produced using dilution series of the respective compounds in paraffin oil (Uvasol quality, Merck/VWR). Small pieces of filter paper (2 cm^2) are drenched with a small amount of the standard dilution (100 μl). The filter paper is put into a 10 ml glass syringe. Inside the air volume of the syringe the odorant will accumulate in a concentration that is proportional to the concentration of the substance in the solution and its vapour pressure according to Henry's law [29]. By injecting a fixed volume (5 ml) of air onto the antenna a reproducible stimulus can be supplied. Pentanal (97% purity, Sigma–Aldrich, Taufkirchen, Germany), hexanal (98% purity, Sigma–Aldrich) and heptanal (95% purity, Acros, Geel, Belgium) are used as calibration standards.

2.6. H. bajulus

Specimen of the old house borer are obtained from a laboratory rearing of the Federal Institute for Materials Research and Testing (BAM), Berlin. The rearing uses small blocks of pine wood in which individual larvae of the beetle are housed. Under these optimized rearing conditions larval development is shortened to less than 1 year. Adult beetles are used for the experiments at an age of 1–4 weeks after emergence.

3. Results

3.1. Calibration of the setup

Every individual antenna of *H. bajulus* has a unique sensitivity for the volatiles emitted from pine timber. In addition, the sensitivity will change in the course of time. Considering the limited lifetime of an excised antenna a calibration by injection of standard solutions in the GC would consume too much time. However, in order to compare measurements obtained from different antennae it is important to get information on the sensitivity of the employed antenna. This can be done by injection of volatiles into the calibration port of the GC–EAD interface.

When a stimulus is delivered to the antenna as a short pulse (e.g. as a manual injection within 0.5 s), the EAD potential will show a steep depolarisation followed by a slow return to the baseline which may last for some seconds. The duration of the quenching of the signal varies with the fitness of the

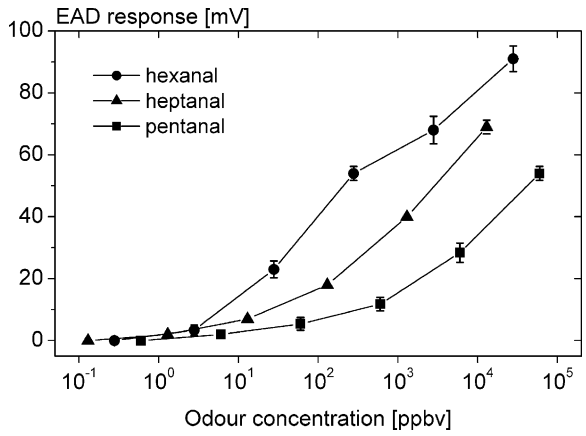

Fig. 3. Calibration of antennae of *H. bajulus* with dilution series of aldehydes. Depicted are EAG amplitudes in response to short (0.5 s) stimulations. Error bars represent standard deviations (n = 5).

antenna and the concentration of the odorant. This implies that the area of a peak recorded in the EAD signal is not a good measure for the amount of the stimulus. However, the amplitude of the deflection correlates with the strength of the stimulus. Fig. 3 depicts the response of antennae of *H. bajulus* to dilution series of three different aldehydes. The results show that among the aliphatic aldehydes hexanal is detected with the highest sensitivity. Experiments with lower (C_1–C_4) and higher (C_8–C_{12}) aliphatic aldehydes show that the sensitivity for aldehydes decreases all the more as the chain length deviates from C_6 (data not shown). A dilution of hexanal in paraffin oil with a concentration of 10^{-6} results in a concentration of ca. 2.8 ppbv.

Antennae of *H. bajulus* show a good response over a period of a few hours after abscission from the insect. The sensitivity decreases in the course of time, especially in the first hour of the measurements. After this decline the sensitivity remains constant for a considerable time. Often even an increase of sensitivity can be observed, especially if the antenna is not charged with further measurements (Fig. 4). Using the tem-

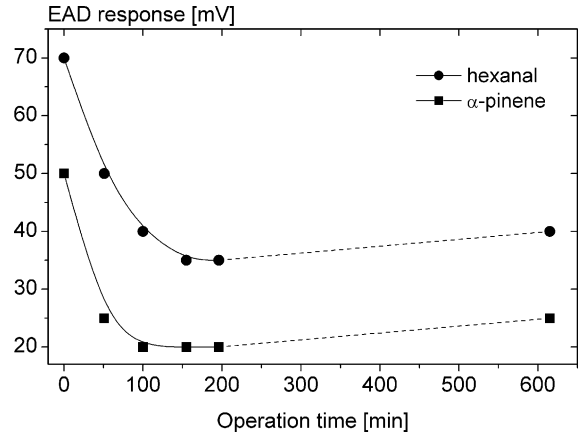

Fig. 4. Response of an antenna of *H. bajulus* to calibration standards (2.8 ppmv hexanal, 5.3 ppmv α-pinene). The dotted line represents a time in which no GC runs were performed.

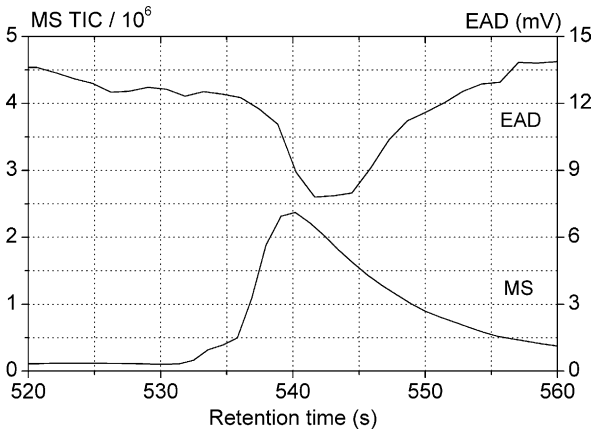

Fig. 5. Detail of a GC–MS/EAD chromatogram recorded with an antenna of a male *H. bajulus* and a sample of volatiles from Scots pine. The EAD peak is delayed about 3 s in comparison to the TIC peak of α-pinene at 9.0 min (540 s).

perature program described above it was possible to record up to 10 chromatograms with one antenna (depending on the performance of the antenna).

3.2. GC–MS/EAD results

The potential measured between tip and base of an antenna normally has a value of several mV and is subject to a slow drift in the course of time. EAD peaks are small deflections in this baseline. The information about total height and drift of the antennal potential is lost in the high pass filter of the EAD amplifier. Thus, the scale of the EAD axis is valid only as a measure for the amplitude of the deflections whereas the absolute value of the potential is irrelevant. For the data presented in Figs. 5 and 6 the position of the baseline has been freely chosen in a way that allows a favourable presentation. All EAD potentials displayed in the following figures represent the amplified (100×) signals of the antenna.

A precondition for the comparability of the chromatograms from MS and EAD is the simultaneousness of the peaks in both signals or at least a constant shift in both signals. The induction of an electrical signal in the antenna is a dynamic physiological process followed by a quenching of the stimulus and a return to the baseline. Thus, the maximum of an EAD peak is not necessarily in coincidence with the maximum of the stimulus. In addition, the peak shape of the EAD signal is modified by the frequency filter. However, the onsets of the peaks obtained by the mass selective detector should match the EAD signals.

In order to demonstrate the simultaneousness of the peaks in EAD and MS signal a detail from a GC–MS/EAD chromatogram is depicted in Fig. 5. The restriction capillaries leading to MS (i.d. 0.1 mm) and EAD (i.d. 0.15 mm) cause a delay of the EAD signal of about 1 s. The EAD signal is further delayed by the passing of the gas through the flow tube of the EAD interface which lasts about 1.5 s. So, the total delay of the EAD signal should amount to 2.5 s compared to the MS signal. Fig. 5 depicts peaks corresponding to α-pinene in both signals. The onset of the peaks as well as the peak maxima are delayed about 3 s in the EAD signal.

With the knowledge of the delay between the signals of EAD and MS an interpretation of complex chromatograms is possible. Fig. 6 depicts a chromatogram of volatiles collected from timber of Scots pine. The peaks with the highest abundances in the MS signal are monoterpenes which are typical coniferous wood volatiles, especially α-pinene (peak 8, t_R = 9.00 min) and 3-carene (peak 14, t_R = 10.95 min). However, the highest deflection in the EAD signal is observed at t_R = 5.85 min and corresponds to hexanal. Further aliphatic aldehydes and alcohols were also detected by the insect antennae. Table 1 shows a summary of the peak assignments obtained from the NIST database and the MassFinder soft-

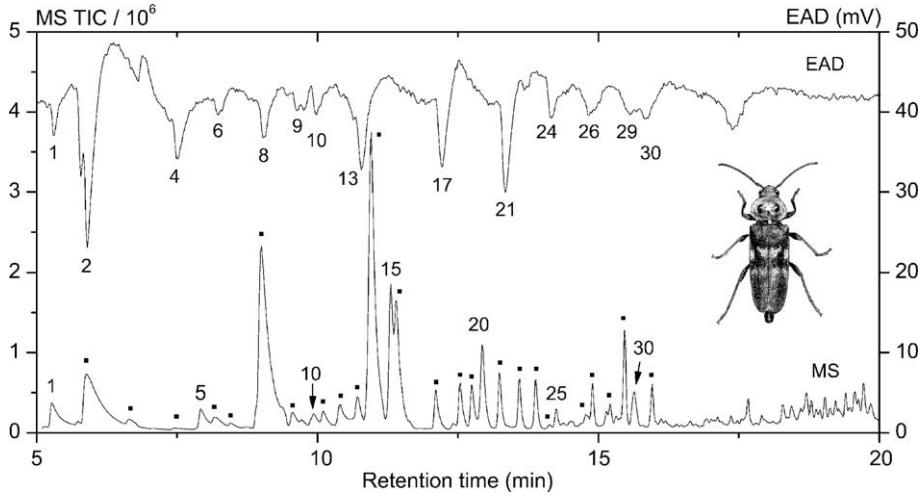

Fig. 6. GC–MS/EAD chromatogram of volatiles sampled from sawed timber of Scots pine (*P. sylvestris* L.). The upper line represents the EAD signal of a male old house borer, the lower line depicts the MS–TIC (total ion current). Numbers assign the peak numbers as listed in Table 1. In the MS chromatogram peak numbers are partly replaced by dots. The absolute height of the EAD signal is shifted in comparison to Fig. 5. Insert: drawing of *H. bajulus*.

Table 1
Volatiles identified in the headspace of pine wood (*Pinus silvestris*)

Peak number	*I*	Substance	EAD
1	779	1-Pentanol	+
2	806	Hexanal	+
3	839	Furfural	+
4	874	1-Hexanol	+
5	894	2-Heptanone	
6	904	Heptanal	+
7	916	Acetic acid, pentyl ester	
8	938	α-Pinene	+
9	960	2-Heptenal	+
10	975	1-Heptanol	+
11	981	1-Hepten-3-ol	
12	993	2-Pentylfuran	
13	1006	Octanal	+
14	1015	3-Carene	
15	1029	*p*-Cymene	
16	1033	Limonene	
17	1061/63	2-Octenal and *p*-Mentha-1,4-diene	+
18	1078	Unknown terpenoid	
19	1086	Benzene, 4-ethenyl, 1,2-dimethyl-	
20	1094	Benzene, 1-methyl-4-(1-methylethenyl)-	
21	1106	Nonanal	+
22	1121	Fenchol	
23	1133	α-Campholenal	
24	1143	*cis*-*p*-Menth-2,8-dienal and unknown ketone	+
25	1148	*trans*-Verbenol	
26	1169	Pinocamphone	+
27	1174	Borneol	
28	1187	Thymol	
29	1198	(+)-α-Terpineol	+
30	1203/05/07	Benzene, 1-methoxy-4-(2-propenyl)- and myrtenal and decanal	+
31	1219	Verbenone	

Peak numbers correspond with assignments in Fig. 6. A "+" in the last column indicates that the respective compound elicits a response in the EAD signal. Retention indices (*I*) are calculated from the chromatograms obtained with a J&W Scientific HP-5MS column (Agilent).

ware. Volatiles which elicit a response from the insect antenna are marked (+).

4. Discussion

The presented data demonstrate that the employed setup is capable of recording GC–MS/EAD chromatograms even from highly complex samples. Odorant samples collected from pine wood using the CLSA method comprise a multitude of volatiles, especially terpenoids, aliphatic aldehydes and alcohols.

The quantitative calibration of the EAD signal is performed by a single-point calibration by injection of stimuli into the calibration port of the EAD interface before and after each GC run. Thus, it is possible to keep track of changes in the sensitivity of an antenna from run to run and to compare chromatograms that were recorded with different antennae. A multi-point calibration with a dilution series is not applied because of the limited lifetime of the antenna. A more time consuming calibration via injection of defined quantities of odorant substances into the injector is possible if a precise quantitative interpretation of the electroantennographic response is necessary.

As a rule, a quantification should be performed using the peak areas of the TIC chromatogram from the mass spectrometer. However, in some cases the insect antenna shows remarkable responses whereas the MS is close to the detection limit, e.g. peak 4 (hexanol) in the shown chromatogram (Fig. 6). The use of the EAD data for a quantification can also be essential when bioactive compounds coelute with other substances, e.g. peak 30 in Fig. 6. In this case, a quantification is possible if the dose-response relationship of the antenna for all involved compounds is known.

An interpretation of the results for the chemical ecology of *H. bajulus* suggests a significance of hexanal, pinene and other aldehydes and terpenes for the host location of the beetle. Previous studies by Fettköther et al. [25] demonstrate that (among other volatiles) α-pinene, α-terpineol, and verbenone induce a behavioural response from *H. bajulus*. This fact is supported by the finding that α-pinene and α-terpineol both elicit a response in the antenna of the beetle (Table 1). The behavioural response to verbenone was observed only for female beetles which is consistent with our results that the male beetle does not perceive this terpenoid. Verbenone is produced by two subsequent oxidation processes from α-pinene and thus might be a measure for the age of naturally degrading wood. It is also a major monoterpenoid emitted

from larval frass of *H. bajulus* [25]. Lindgren and Miller stated that beetles associated with fresh wood are rather repelled by verbenone whereas beetles associated with aged wood are not repelled or even attracted by this terpenoid [30]. *H. bajulus* also has a preference for aged wood [31] which might be a strategy to avoid competition by adapting to a less nutrient diet with a low content of water and soluble sugars [32]. The sexual dimorphism in the response to verbenone might be a hint that females of *H. bajulus* use the scent of larval frass as a clue for an adequate site for oviposition as stated by Fettköther et al. [25].

The EAD chromatograms did not show a reaction of *H. bajulus* to limonene. The behavioural response of the beetle to limonene was only weak [25] which suggests that it perceives limonene only in high concentrations and possibly uses it for a short range orientation.

The finding that aldehydes like hexanal are perceptible to the beetle with high sensitivity is noteworthy. Aldehydes ranging from C6 to C10 were identified in the sample and all of them elicited responses from *H. bajulus*.

The high sensitivity of *H. bajulus* for hexanal is interesting. The relative intensity of hexanal can diverge from the normal pattern when lipoxygenase activity is induced. Hexanal is considered a major indicator of lipid oxidation and is ubiquitous in the biosphere. It is a part of the green leaf odour (GLO) of plants, it is also found in the bark of non-coniferous trees [33] and it contributes to the emissions of meat [34] or flowers [35]. The role of hexanal for other tree-associated beetles is controversial. Some bark beetles show an EAD response to hexanal [36] while others do not [37,38]. A possible deterrent effect is discussed [39,40].

It is not clear if volatiles like hexanal are used by the beetle for its orientation since they may occur in much higher abundances from sources not related to the habitat of the beetle, e.g. green leaves or flowers. However, the fact that an insect is able to detect a volatile does not give an information if the volatile is an attractant, a deterrent, or does not elicit any behavioural response at all. This can only be clarified in behavioural essays. The presented results suggest that in particular the aldehydes ranging from hexanal to decanal should be subject to further studies in order to investigate their influence on behaviour of *H. bajulus*.

The use of the demonstrated technique is not restricted to the investigation of interrelations between insects and plants. Compounds with high significance to an insect are often detected with remarkable sensitivity (sub-picogram range) and/or a wide dynamic range (e.g. 10^6). Phytophagous insects, for example, often use olfactoric cues to get information about the physical condition of a host plant. This implies that the insect (or its antenna) can be used as a sensor for the health of plants [41,42]. Another application is the use of insect antennae to measure pheromone concentrations in the field in the context of the pheromone disruption method [43].

Possible applications for insect antennae as sensors are also found in other disciplines. An example is *Melanophila acuminata*, a beetle that uses burnt trees for oviposition and thus is able to detect the volatiles from burnt wood which makes it a perfect sensor for forest fires [44]. It has also been observed that insects are able to detect anthropogenic compounds which have no direct significance for their normal life. An example is the ability of wasps to detect some kinds of explosives [45]. These findings open up new vistas in solving analytical problems with the help of insect antennae even if the application is not at all associated with the chemical ecology of insects.

5. Conclusions

Recapitulating the presented results it can be stated that the combination of GC–MS with an electroantennographic detection to a GC–MS/EAD setup results in a most powerful analytical tool that combines high sensitivity and specificity of an insect antenna with the analytical power of a mass spectrometer. Experiments with *H. bajulus* revealed that this beetle does not only perceive typical coniferous volatiles like α-pinene and α-terpineol but also has a high sensitivity for aldehydes like hexanal. The role of these compounds and their possible significance for control measures against the beetle have to be clarified in behavioural studies.

Implementations of the presented method are not restricted to questions of chemical ecology but comprise a multitude of applications, e.g. in quality control or plant protection.

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