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Short Communication

Simple method for collecting volatile compounds from single insects and other point sources for gas chromatographic analysis

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ABSTRACT

A simple method is described for trapping airborne volatile compounds in the ng range for their subsequent gas chromatographic–mass spectrometric analysis. Volatile compounds are trapped on the inner surface of glass capillaries. The trapped compounds are extracted by rinsing the capillaries with 2–3 μl of solvent, and the extracts are directly analysed. As examples, pheromones are analysed. The method may also be suitable for the collection of volatile compounds from other point sources.

INTRODUCTION

The collection of airborne pheromones and other volatile compounds has been one of the key issues in semiochemical research. In most instances volatile compounds emitted from living organisms have been trapped on Porapak Q, Tenax, active carbon, glass-wool or glass beads [1–6], and then washed off with a relatively large volume (several hundred microlitres or more) of solvent. This necessitates an evaporation stage to remove most of the solvent before analysis, which can result in contamination or loss of components of the volatile compounds trapped. This problem can be overcome by collecting the volatile compounds in glass capillary tubes, which will allow desorption with small volumes of solvents [7,8]. Based on this technique, we describe here a simple, flexible and convenient collection method which makes possible the qualitative analysis of pheromone-like effluents from single insects or other point sources, and which can be carried out also under field conditions. The application of the method to four different compound classes of pheromones is described.

EXPERIMENTAL

Insects

The insect cultures of *Mamestra brassicae* L., *Mamestra oleracea* L., *Heliothis armigera* L. (Lepidoptera, Noctuidae) and *Chiasma clathrata* L. (Lepidoptera, Geometridae) were derived from eggs of female moths collected from the wild. The pupae reared were sexed and the sexes were held separately

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under a reversed 18 h–6 h light–dark photoregime at 25°C in the laboratory. Emerging adults were collected daily and were transferred to 1-l glass jars where they were supplied with a 5% honey solution on cotton-wool. Female moths exhibiting calling behaviour were collected, and deep-frozen at −65°C until used. For performing collections of volatile compounds from live insects, female moths were placed singly in wire containers (100 × 100 × 100 mm) one day prior to collection. The front wall of the container was removable to allow free access to the calling female.

Extracts of volatile compounds

Volatile compounds from calling female moths held at ambient room temperature (20–25°C) were collected in two 20-μl disposable micropipettes (Rudolf Brand, Wertheim, Germany) connected firmly in series by a small piece of PTFE tubing. Air (50 ml/min) was drawn through this set-up using a commercial aquarium pump. The open end of the first capillary was held at a distance of 1–2 mm from the pheromone gland surface of the calling moth. To prevent a possible breakthrough of the volatile compounds collected, the walls of the second capillary were cooled by placing a piece of ice on the outside of the capillary. After a collection period of 20–30 min the capillaries were rinsed with 2–3 μl of solvent by letting the plug of solvent pass two or three times along the total length of the capillaries. The remaining volume of solvent was then taken out from the end of the capillary with a Hamilton microsyringe and was either injected directly into the gas chromatograph or combined with further rinsings and stored for later analysis in a glass ampoule sealed with a flame. A simultaneous blank collection was conducted in a similar way by holding the open end of the capillary at a distance of several centimetres from the calling moths.

Collection of volatile compounds from the pheromone glands of dead females was conducted in a similar manner. The pheromone gland of a dead female moth was artificially held in an exposed position by a pair of fine forceps and the open end of the capillary was held at a distance of 1–2 mm from the gland surface.

For preparation of direct pheromone gland extracts, the terminal segments of the abdomina of females were excised and extracted in small volumes (5 μl per female) of *n*-pentane or *n*-hexane (analytical-reagent grade; Merck, Darmstadt, Germany) for 15 min.

Analysis

Extracts obtained from *M. brassicae* and *H. armigera* were analysed by gas chromatography with flame ionization detection (GC–FID). An HP-5890 gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with an SP-2340 (Supelco, Bellefonte, PA, USA) fused-silica capillary column (30 m × 0.32 mm I.D.) was used. This column was temperature programmed as follows: 60°C for 2 min isothermal, increased at 10°C/min to 100°C, then at 5°C/min to 220°C, followed by an isothermal hold at this temperature. Splitless injections at 210°C were made in 2μl of solvent.

Extracts of *M. oleracea* and *C. clathrata* were analysed by GC–mass spectrometry (MS) using a VG Tribrid double-focusing magnetic sector mass spectrometer (VG Analytical, Manchester, UK) operating in the electron impact (EI) ionization mode (70 eV, 180°C). EI mass spectra (m/z 35–485, 1.16 s per scan) were recorded at nominal resolution. In this instance an SE-54 high-resolution fused-silica GC column (25 m × 0.32 mm I.D.) was used and temperature programmed as follows: 60°C for 2 min isothermal, increased at 20°C/min to 140°C, then at 5°C/min to 280°C, followed by an isothermal hold at this temperature. The samples (2 μl in *n*-hexane or *n*-pentane) were injected on-column at 60°C. Data acquisition was started at 140°C and retention times were measured from this point.

RESULTS AND DISCUSSION

In *H. armigera* the major component in direct gland extracts and collections of volatile compounds from live or dead females (Fig. 1A, B and D) had a retention time identical with that of synthetic (*Z*)-11-hexadecenal. Likewise, small peaks (2–5% of the major component) were detected at the retention times of (*Z*)-9-hexadecenal and hexadecanal in all of the above samples. These three components have been reported as sex pheromone components of *H. armigera* in similar ratios [9].

The major component in both the gland extract and of volatile collection compounds from dead females in *M. brassicae* (Fig. 2A and D) had a reten-

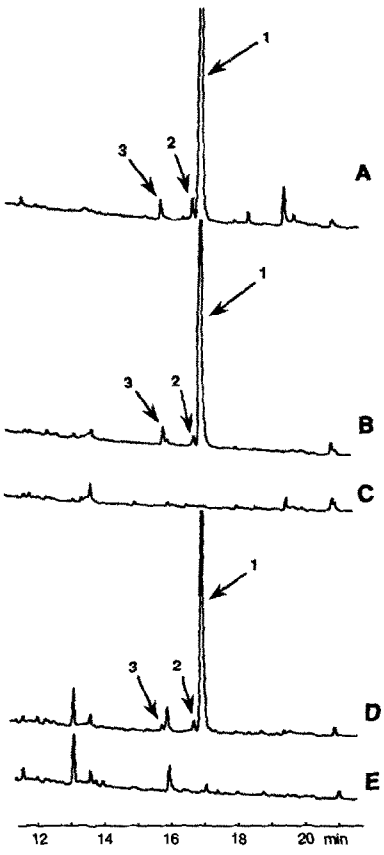

Fig. 1. Chromatograms (FID) of extracts obtained from *H. armigera* females. (A) 0.5 FE (female equivalent) of direct pheromone gland extract; (B) 1 FHE (female hour equivalent) of volatile collection from living females; (C) corresponding blank collection; (D) 0.3 FHE of collection of volatile compounds from dead females; (E) corresponding blank collection. Peaks 1, 2 and 3 coincided with respect to retention time with synthetic (*Z*)-11-hexadecenal, (*Z*)-9-hexadecenal and hexadecanal, respectively.

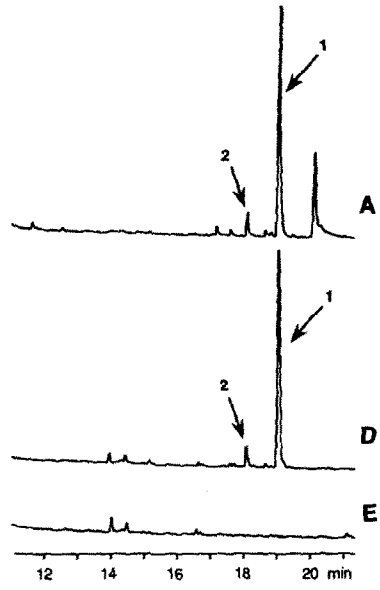

Fig. 2. Chromatograms (FID) of extracts obtained from *M. brassicae* females. (A) 0.7 FE of direct pheromone gland extract; (D) 0.2 FHE of collection of volatile compounds from dead females; (E) corresponding blank collection. Peaks 1 and 2 coincided with respect to retention time with synthetic (*Z*)-11-hexadecenyl acetate and hexadecyl acetate, respectively.

tion time identical with that of (*Z*)-11-hexadecenyl acetate. A smaller peak (*ca.* 5%) was detected at the retention time of hexadecyl acetate. Both compounds have been described as sex pheromone components in this species [10].

In *M. oleracea* (Fig. 3A and D), the presence of (*Z*)-11-hexadecenyl acetate and (*Z*)-11-hexadecenol was verified by comparison of the retention times and mass spectra in both the gland extract and the collection of volatile compounds from dead females. The acetate-to-alcohol ratios were 4:1 and 1.6:1 in the gland extract and in the collection of volatile compounds, respectively. The same two compounds have been reported as sex pheromone components of *M. oleracea* [11].

The presence of (6*Z*,9*Z*)-6,9-*cis*-3,4-epoxyheptadecadiene, previously identified as the main component of the sex pheromone of *C. clathrata* [12], was detected by GC–MS analysis of both gland extracts and collections of volatile compounds from living females (Fig. 4A and B).

In all four species studied the presence of known pheromone components was detected in both collections of volatile compounds and direct gland extracts. The only significant qualitative difference observed was that some peaks present in the gland extracts were not found in the collections of volatile compounds (Figs. 1A, B and D; 2A and D and 4A and B). The four species studied produce four different molecular types of pheromones: long-chain monounsaturated acetates (*M. brassicae, M. oleracea*), alcohols (*M. oleracea*), aldehydes (*H. armigera*) and epoxides derived from polyunsaturated hydrocarbons (*C. clathrata*). The present method for the collection of volatile compounds seems to be

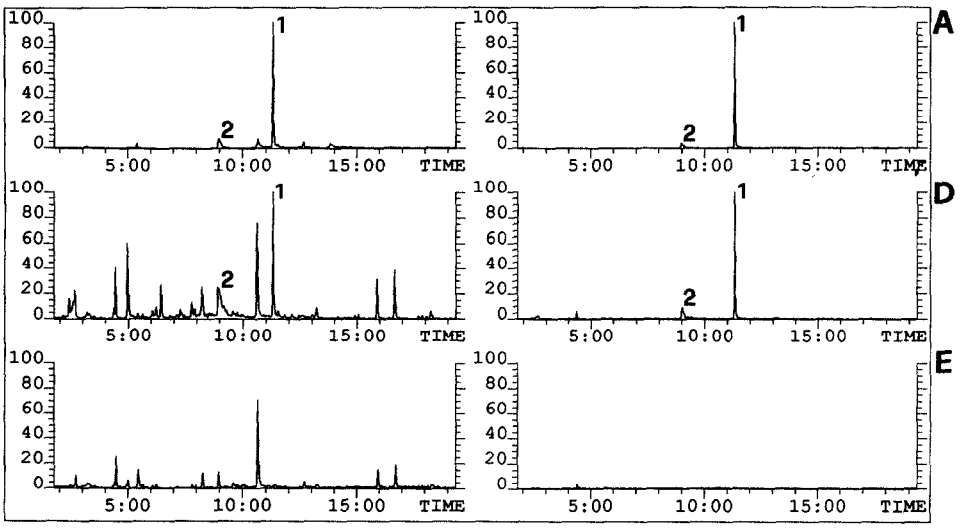

Fig. 3. Reconstructed total ion chromatograms (left) and mass chromatograms (*m/z* 222) selective for hexadecenyl acetates ($M^+ - 60$) and alcohols ($M^+ - 18$) (right) of extracts obtained from *M. oleracea* females. (A) 0.7 FE of direct pheromone gland extract; (D) 1.0 FHE of collection of volatile compounds from dead females; (E) corresponding blank collection. Peaks 1 and 2 coincided with respect to retention time and mass spectrum with synthetic (*Z*)-11-hexadecenyl acetate and (*Z*)-11-hexadecenol, respectively. Time in min.

capable of collecting pheromones with all these structures.

Gland extracts similar to those analysed here are frequently used in identification projects of unknown pheromones. In this study, comparable amounts of the main pheromone components were collected from collections of volatile compounds from living or dead females, and gland extracts (Table I). Consequently, extracts collected in capillaries by the present method could be used for the analysis

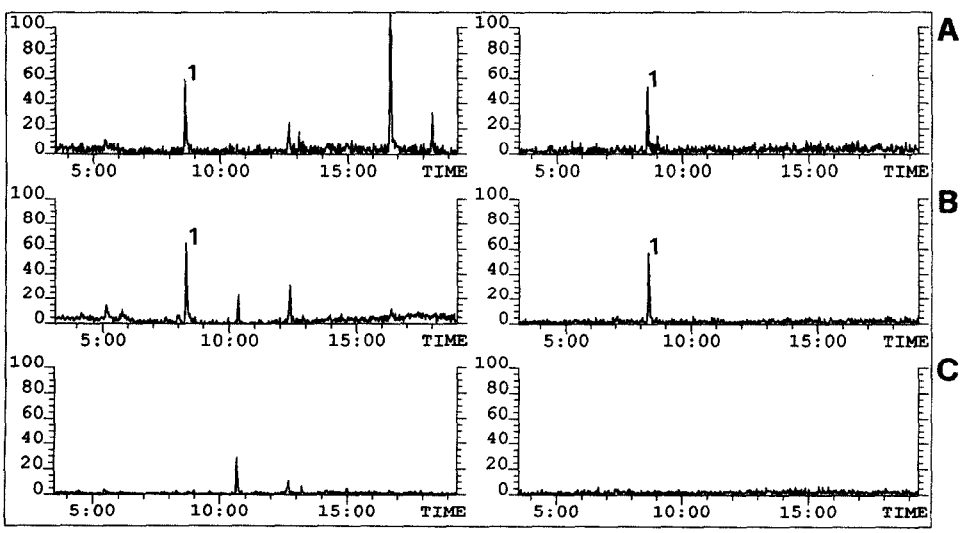

Fig. 4. Reconstructed total ion chromatograms (left) and mass chromatograms (*m/z* 178) selective for (6*Z*,9*Z*)-6,9-*cis*-3,4-epoxyheptadecadiene (right) of extracts obtained from *C. clathrata* females. (A) 1.0 FE of direct pheromone gland extract; (B) 1 FHE of collection of volatile compounds from living females; (C) corresponding blank collection. Peak 1 coincided with respect to retention time and mass spectrum with synthetic (6*Z*,9*Z*)-6,9-*cis*-3,4-epoxyheptadecadiene. Time in min.

TABLE 1

AMOUNTS OF MAJOR PHEROMONE COMPONENTS IN GLAND EXTRACTS, AND IN COLLECTIONS OF VOLATILE COMPOUNDS FROM LIVING OR DEAD FEMALE MOTHS OF FOUR LEPIDOPTERAN SPECIES

Species	Collection of volatile compounds		Gland extract (ng/FE)[a]
	From living females (ng/FHE)[a]	From dead females (ng/FHE)[a]	
H. armigera	7.1 ($n=3$)	15.0 ($n=1$)	8.1 ($n=5$)
M. brassicae	24.7 ($n=4$)	24.9 ($n=9$)	63.8 ($n=6$)
M. oleracea	n.a.	5.9 ($n=6$)	16.0 ($n=6$)
C. clathrata	4.9 ($n=3$)	n.a.	3.2 ($n=5$)

[a] Amounts for (*Z*)-11-hexadecenal, (*Z*)-11-hexadecenyl acetate and (6*Z*,9*Z*)-6,9-*cis*-3,4-epoxyheptadecadiene are given for *H. armigera*, the two *Mamestra* species and *C. clathrata*, respectively. FE = Female equivalent; FHE = female hour equivalent; n.a. = not analysed; n = number of females analysed.

of unknown pheromones. Such extracts may represent more reliably the actual ratios of pheromone components released. In this study there was a clear difference between the ratios of (*Z*)-11-hexadecenyl acetate and the corresponding alcohol in extracts obtained by collection of volatile compounds and by gland extraction (Fig. 3A and D). The difference observed may reflect the difference in volatilty of an acetate *vs.* an alcohol. It is interesting that in *H. armigera* or *M. brassicae*, where all pheromone components are aldehydes or acetates, respectively, no significant difference in ratios was observed between collections of volatile compounds and gland extracts (Figs. 1A, B and D and 2A and D).

Peaks in the collections of volatile compounds originating from contaminants in the laboratory atmosphere did not seem to interfere severely with the analysis of the pheromone components (Figs. 1–4B *vs.* C and D *vs.* E). This is possible because the volatile compounds are collected in the close vicinity of their source, and their concentration in the air sampled (ng/l) is therefore likely to exceed the concentration of general contaminants present in the atmosphere. In instances where such contamination hinder the analysis, a curtain of clean air in the vicinity of the source of volatile compounds may be of advantage.

In this study, we did not detect a significant breakthrough of compounds past the cooled part of the micropipette. When collecting more volatile compounds, it may be advantageous to cool the micropipette with dry-ice. On the other hand, this may lead to plugging of the pipette with ice, as water vapour from the air sampled condenses and freezes [7].

The main advantages of the present collection method are its ease of use and simplicity. From a biological viewpoint, it is very advantageous that the insects are not enclosed in small and sometimes sophisticatedly built containers, which could interfere with their normal behaviour, as in earlier methods [7,8]. By directly observing the behaviour of the insects, collection bouts can be restricted to well defined periods, thereby making it possible to analyse volatile compounds emitted while the insects exhibit different types of behaviour.

Although so far the method has been applied only under laboratory conditions, it should easily adaptable to field conditions, if a transferable electric source is provided for the pumps. This would allow collections of volatile compounds from insects in their natural habitats, which would facilitate the interception and identification of chemical messages in natural contexts of their release and reception.

The small volumes of solvents used to rinse off the volatile compounds collected will allow the direct analysis of samples from single insects, and collected in a relatively short period of time.

Apart from collecting volatile compounds from insects, the proposed method could also be used for the monitoring of components emitted from artificial sources, *e.g.*, rubber septa, which are most frequently used as pheromone dispensers in commer-

cial attractant traps. In a preliminary test we collected volatile compounds from a rubber septum treated with a 100-μg dose of a mixture of (*Z*)-7-dodecenyl acetate and (*Z*)-9-tetradecenyl acetate. Whereas the stock solution of these compounds applied to the rubber septum contained 84.7% of the tetradecenyl acetate (as compared with the amount of the dodecenyl compound), rinses of the capillaries contained only 28.9%; such a change to a lower percentage of the less volatile tetradecenyl compound was to be expected. A more accurate knowledge of ratios emitted can help in, among others, optimization of blend ratios of artificial attractants.

The main disadvantage of the proposed collection method is that in its present form it yields only qualitative results. As it operates as an open system, only a portion of the volatile compounds emitted can be collected. The method may be improved by introducing an internal standard, *i.e.*, a volatile source which emits a compound at a known release rate.

Despite the above drawbacks, the proposed method for the collection of volatile compounds is recommended for use first of all in pheromone identification projects, supplementing other sample collection methods, *e.g.*, solvent extraction of glands, GC with solid-sample injection of single pheromone glands [13,14] or in other areas where rapid qualitative monitoring of emitted compounds is required.

Recently, the application of adsorption traps with activated charcoal particles embedded on the inside surface of glass capillary tubes and of capillary traps containing films of a non-polar stationary phase has been described for use in the headspace detection of volatile organic compounds in gas samples [15]. Such capillary traps will evidently have a greater trapping capacity than the empty capillaries used in this study. However, for the time being the application of such adsorption traps will possibly be limited to the few laboratories with the expertise and instrumentation needed for their complicated preparation and use.

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