Determination of Plant Volatiles 1: Analysis of the Insect-Attracting Allomone of the Parasitic Plant *Hydnora africana* Using Grob-Habich Activated Charcoal Traps

B. V. Burger* and **Z. M. Munro**
Laboratory for Ecological Chemistry, University of Stellenbosch, Stellenbosch 7600, Republic of South Africa

J. H. Visser
Department of Botany, University of Stellenbosch, Stellenbosch 7600, Republic of South Africa

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1 Introduction

Some of the most extraordinary plants known to man, belong to the family of the Hydnoraceae. Two genera of these parasitic plants with probably no more than 10 to 15 species in total, are recognized at present. Of these, one genus, *Hydnora*, is found in Africa distributed along the coastal and marginal lands from Namibia in the west, round the Cape and northwards as far as Ethiopia. *Hydnora africana* Thunb is found in the semidesert Karoo regions of South Africa where it grows exclusively on the roots of different species of *Euphorbia* [1].

The extremely fetid smell of *Hydnora africana* can easily be detected over a distance of more than four meters by the human nose on a windstill day. The characteristic odor attracts a variety of beetles over long distances, apparently to effect pollination. *Hydnora africana* smells of wet, decaying hide, very much like a tannery [2] and the volatiles responsible for the characteristic smell are produced in bait bodies situated on the inner surface of the perianth lobes of the flower. As a first approach to the study of the pollination biology of this plant, the recovery and identification of the volatile insect attractants became necessary.

Since attempts failed to isolate these volatiles by conventional solvent extraction and gas chromatographic separation techniques, it was decided to apply recently developed headspace-analytical techniques [3,4] to this problem. In this paper the results obtained by trapping the insect attracting volatiles of *Hydnora africana* on different trap types at relatively high flow-rates, followed by on-line thermal desorption and GC as well as GC/MS analysis of the volatiles, are discussed.

2 Experimental

2.1 Instrumental

Gas chromatographic analyses were carried out with a Carlo Erba HRGC 5300 (Mega) gas chromatograph equipped with a split/splitless capillary injector, sniff-port and flame ionization detector. Raw data was acquired and stored with a Hewlett-Packard HP 3363A system (Nelson Analytical Inc. interface). Although glass capillary columns are used almost exclusively in our laboratory, a 27 m × 0.32 mm i.d. fused silica column coated with a 0.83 μm film of crosslinked SE-30 was preferred for this work. Helium was used as carrier gas at a linear velocity of 30.7 cm s^{-1} at 40°C and the column was temperature programmed from 30° to 230°C at 2° min^{-1}. Mass spectra were obtained with a Finnigan 4600 gas chromatograph/mass spectrometer (GC/MS) at 70 eV and an Incos data system used in the repetitive scanning mode. The fused silica capillary column was inserted directly into the ion source. Helium was used as carrier gas at a linear velocity of 30.7 cm s^{-1} at 40°C and analyses were carried out using a temperature program of 2° min^{-1} from 30° to 230°C.

Grob-Habich carbon open tubular traps (COTs) [3] were prepared by melting carbon particles (10-35 μm) into the inside surface of Pyrex capillaries (0.3 mm i.d. × 0.8 mm o.d.) in such a manner that the resulting traps had a standard coated section of 60 mm in length. A film-activated carbon open tubular trap with carbon particles smaller than 10 μm (FACOTT-10) and a film-organic polymer open tubular trap with Porapak Q particles smaller than 10 μm (FOPOTT-10) were prepared according to the procedure described by *Burger* and *Munro* [4].

2.2 Trapping of the Volatiles

The soil was removed from around a *Hydnora africana* plant, the perianth lobes of which normally protrude only a few centimeters from the surrounding soil-surface, to expose about 7 cm of the stem of the plant. Crossed slits were cut in aluminum foil which had been pre-cleaned at 500°C, the foil was slipped over the plant to cover the soil-surface and was secured around the stem of the plant with a steel spring. A bell-shaped glass cover with a flanged rim was placed over the plant and the aluminum foil folded upwards around the cover and secured above the flange with another steel spring as shown in **Figure 1**. This arrangement, although clearly not airtight, served to restrict the movement of moisture and volatiles from the soil into the enclosed space around the flower.

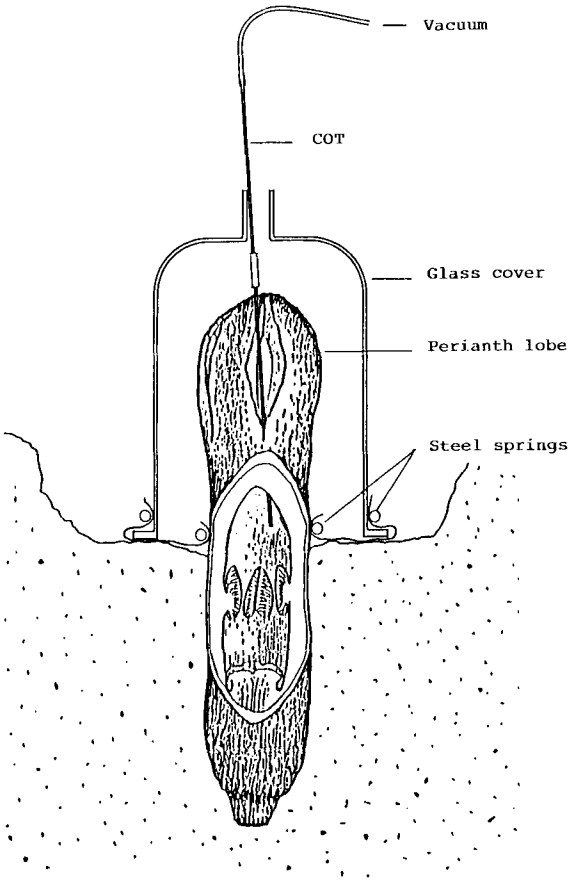

Figure 1

Collection of the insect-attracting volatiles of *Hydnora africana* on two COTs used in series. The positioning of the tip of the first trap inside the flower is shown schematically.

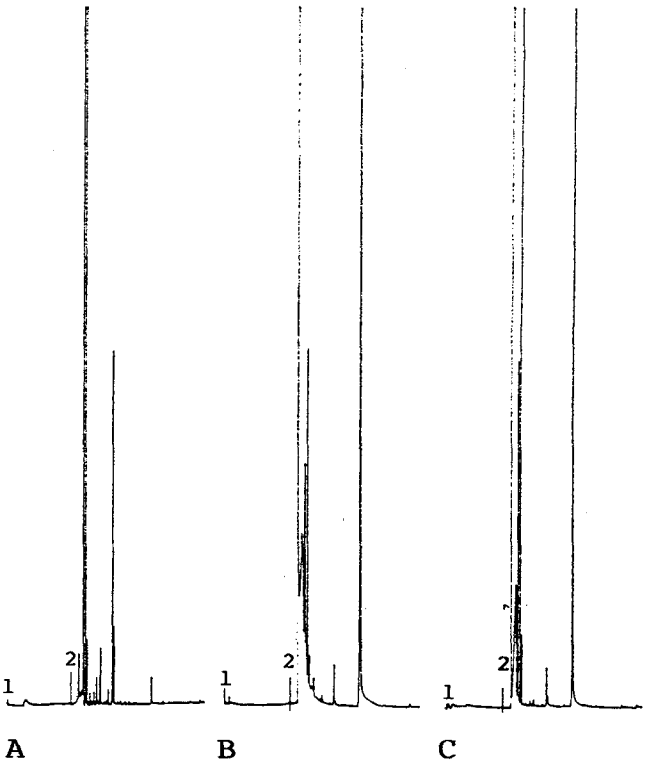

Figure 2

Gas chromatograms (FID) illustrating the break-through of highly volatile constituents of the insect-attracting secretion of *Hydnora africana* flowers on a COT. A = blank run, air sampled for 10 min at 80 ml min^{-1}; B and C = headspace volatiles retained by respectively the first and second COTs used in series, headspace gas sampled for 1 min at 80 ml min^{-1}. Desorption and analytical conditions are given in the experimental section. 1 = desorption started; 2 = temperature program started.

The trap to be used was connected to a vacuum reservoir with a short length of polyethylene tubing. Traps to be used in series were butt-connected with shrinkable PTFE and if a lower flow-rate was required, a suitable length of fused silica tubing was used between the trap and the vacuum reservoir. The flow rates through the traps and restrictions and through various combinations of these elements were determined to allow the measurement of the volumes of air sampled through the traps. Prior to trapping, the COTs were activated for at least an hour at 300°C in the injector of the gas chromatograph at a flow-rate of *ca.* 3 ml min^{-1}. Sampling was carried out by inserting the trap into the flower through one of the three slits between the perianth lobes, as shown in Figure 1, and sucking the desired volume of air through the trap, whereafter the trap was closed with small PTFE stoppers and stored in a screw-cap vial until analyzed. Traps used in series, were stoppered individually.

2.3 Desorption and Gas Chromatography

The desorption and analysis of material trapped on COTs were carried out according to the procedure of *Grob* and *Habich* [3]. However, to avoid thermal decomposition of the volatiles on the activated charcoal, the injector was heated from 30° to 230°C during the desorption stage (9.5 min) of the analysis. The desorbed volatiles were cryofocused on the capillary column with solid CO_2. In analyses with fused silica traps, temperature-programmed desorption and cryofocussing, as described by *Burger* and *Munro* [4], were employed. The desorbed volatiles were analyzed using the analytical conditions given in Section 2.1.

3 Results and Discussion

A number of analyses of material collected from individual *Hydnora africana* flowers revealed considerable quantitative differences in the composition of the volatiles released by these specimens, the flowers of which may have been in different stages of development. These differences that are also detectable by the human nose, complicated the comparison of results obtained with material trapped from different flowers.

Although the long fused silica FACOTTs and FOPOTTs are ideal for the quantitative solventless determination of highly volatile compounds, it was found that relatively little material was collected on the FACOTT-10 and the FOPOTT-10 when trapping for 10 minutes at a maximum flow-rate of 30 ml min^{-1} which could be attained through these traps. Therefore, since the identification of the volatile constituents emitted by *Hydnora africana* flowers was the primary objective of the present study, Grob-Habich COTs, with which much higher flow-rates can be achieved,were used in all further work in spite of the expected early break-through that is

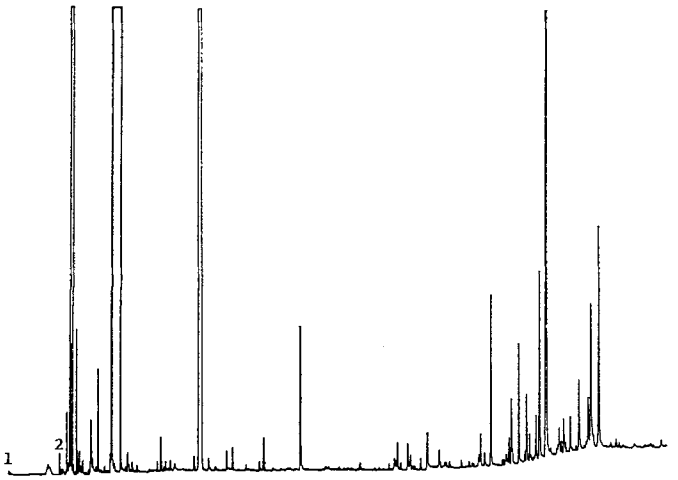

Figure 3

Gas chromatogram (FID) of the headspace volatiles of a *Hydnora africana* flower collected on a COT for 25 min at a flow-rate of 80 ml min^{-1}. Desorption and analytical conditions are given in the experimental section. 1 = desorption started; 2 = temperature program started.

normally found with these traps at high flow-rates and sampling volumes. As expected at a flow-rate of 80 ml min^{-1}, break-through of the highly volatile constituents was already quite pronounced after 80 ml of plant headspace gas had been passed through two COTs used in series (**Figure 2**).

The gas chromatogram shown in **Figure 3** was obtained by trapping the volatiles from a *Hydnora africana* flower for 25 minutes at a flow-rate of 80 ml min^{-1}. Under these conditions some of the less volatile compounds in the headspace gas of the flower were also transported onto the COT. In this analysis the sniff-port detector of the gas chromatograph was utilized to locate any constituents which could contribute to the typical smell of *Hydnora africana* flowers. It was found that 15, mostly relatively volatile constituents had smells strongly reminiscent of, for example raw meat, putrid meat, feces, cabbage, mushroom, and onion.

Table 1

Compounds identified in the headspace gas of a *Hydnora africana* flower.

Peak No.[a]	Compound
127	Ethanal
138	Propanal
186	Carbon disulfide
218	2-Methyl-2-propenal
239	(*E*)-2-Butenal
248	Butanal
512	*S*-Methyl ethanethioate
702	Dimethyl disulfide
993	Hexanal
1740	Heptanal
2136	Benzaldehyde
2298	Dimethyl trisulfide
2497	6-Methyl-5-hepten-2-one
2657	Octanal
3355	*p*-Cresol
3622	Nonanal
3661	Methyl (methylthio) methyl disulfide
3886	Camphor
4536	Decanal
5424	Undecanal
6599	Geranylacetone
6850	Undecanoic acid
7532	Dodecanoic acid
8251	Tridecanoic acid
8985	Tetradecanoic acid
9650	Pentadecanoic acid
9782	1-Hexadecanol
10101	(*Z*)-9-Hexadecenoic acid
10331	Hexadecanoic acid
10904	Heptadecanoic acid
11508	Octadecanoic acid

[a] See Figure 4.

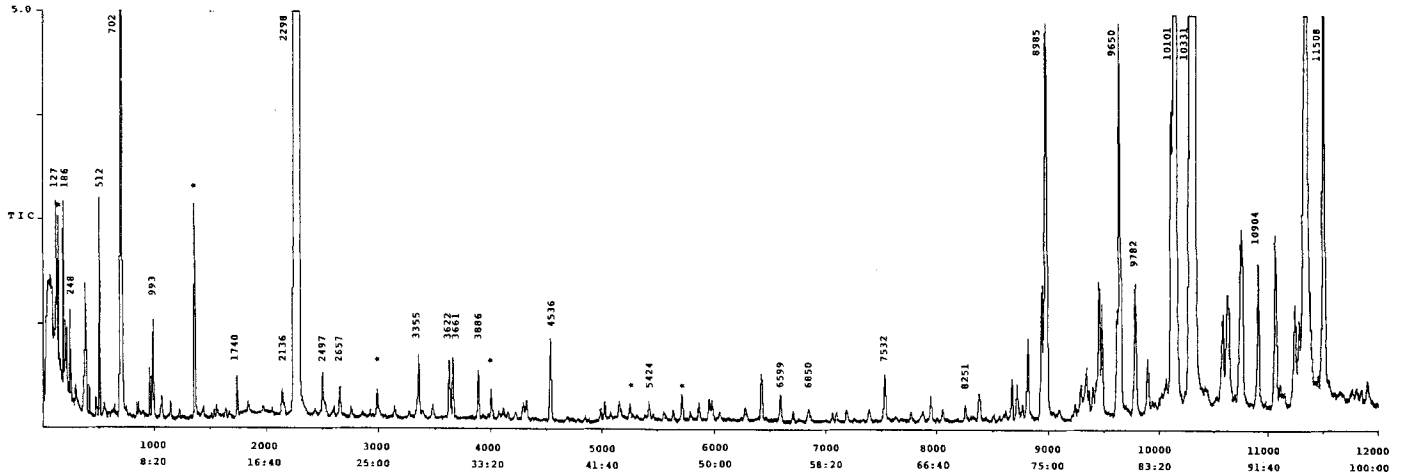

Figure 4

Total ion current chromatogram (TIC) of the headspace volatiles of a *Hydnora africana* flower collected on a COT for 15 min at a flow-rate of 80 ml min^{-1}. Desorption and analytical conditions are given in the experimental section. Background peaks are marked with an asterisk.

For GC/MS analysis the volatiles were trapped off-line on a COT for 15 minutes at a flow-rate of 80 ml min^{-1}. On-line, temperature-programmed desorption with concurrent cryofocusing of the desorbed material, followed by a temperature-programmed GC/MS analysis with the COT still on-line, produced the total ion current chromatogram (TIC) shown in **Figure 4**. The volatile constituents that have so far been identified in the headspace gas of *Hydnora africana* are listed in **Table 1**. The majority of these constituents belong to three compound types *viz.* volatile aldehydes, volatile sulfur-containing compounds, and fatty acids, some of which have relatively low volatilities. The identification of these compounds presented no problem, but a considerable number of sulfur-containing compounds are still unidentified at this stage, as their mass spectra contained insufficient information for positive identification and reference spectra could not be found in the literature or in spectra libraries. Methods for the preparative isolation of these constituents, in order to obtain additional spectral information, are being considered.

Since *Hydnora africana* is a relatively rare plant which cannot be propagated artificially and the flowers are furthermore easily found and consumed by porcupine as soon as they emerge, it was impossible to include other sampling and desorption techniques, such as dynamic solvent effect sampling [5] and solvent desorption of material trapped on COTs [6] in this initial stage of the investigation of the pollination biology of *Hydnora* species. In retrospect, however, since several highly volatile constituents were found in the headspace gas of *Hydnora africana*, starting with solventless techniques appears to have been the correct approach in this investigation.

Acknowledgments

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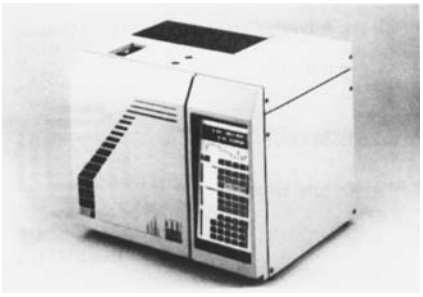

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