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ANALYSIS OF AIRBORNE VOLATILES OF COWPEA

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Abstract—Airborne volatiles of cowpea (*Vigna unguiculata*) plants were analysed by drawing air over the plants in a glass chamber, trapping the airborne volatiles on Tenax TA adsorbent, and releasing the trapped volatiles into a GC-MS by heating the Tenax trap. Eleven volatile compounds; hexanal, 1-nonene, α-pinene, β-pinene, (*Z*)-3-hexen-1-ol acetate, hexyl acetate, 1,8-cineole, limonene, (*E*)-β-ocimene, nonanal and α-cedrene, were identified.

INTRODUCTION

Cowpeas (*Vigna unguiculata*) are widely grown in the tropical and subtropical regions of the world for human and animal food [1]. In many developing tropical countries, they provide a cheap source of dietary protein and energy [2]. They are eaten as green seeds and pods, and as dry grains, while the leaves are used as a vegetable [2–4]. Cowpeas are however attacked by a wide range of insect pests which can cause severe crop losses [1].

Volatile compounds originating from the cowpea plant may play a role in the orientation of its insect pests towards the plant and in ultimate recognition of the plant for feeding and oviposition [5, 6]. Knowledge of the voltatile compounds associated with cowpea plants may therefore be useful in studies of insect pest–cowpea plant relationships.

No report in the literature on the volatiles of cowpea has been found by us. We have recently reported on the isolation and identification of airborne volatiles of sorghum (*Sorghum bicolor*) by passing air over sorghum plants, trapping of the airborne volatiles on Tenax TA adsorbent, and desorbing the volatiles directly into a gas chromatograph-mass spectrometer (GC-MS) by heating the Tenax trap in the GC injection port [7]. We now wish to report on the analysis of airborne volatiles of Cowpea (VITA 1 cultivar) plants using a similar but modified method which allows for more efficient desorption of the volatiles from the Tenax trap.

RESULTS AND DISCUSSION

Table 1 lists the 11 airborne volatiles that were trapped and identified from 3-week-old cowpea plants. Unlike in the case of sorghum plants [7], several monoterpenoids and a sesquiterpene were found. α-Cedrene (42.4%), (*Z*)-3-hexen-1-ol acetate (28.8%) and hexanal (9.4%) were the major constituents while 1-nonene, α-pinene, β-pinene, hexyl acetate, 1,8-cineole, limonene, (*E*)-β-ocimene and nonanal were present in minor quantities (*ca* 1–4%).

For insect–plant interaction studies, collection of airborne plant volatiles is preferred to the use of traditional isolation methods such as steam distillation and distillation under reduced pressure since these methods lead to destruction of the plant tissue [6]. Plant tissue destruction may result in enzyme-catalysed oxidation products that are normally not present in the intact plant and that may mask the original plant volatile compounds [8, 9]. Some of the plant volatile compounds may also be degraded by enzyme action.

Table 1. Airborne volatiles trapped from 3-week-old plants of cowpea (*Vigna unguiculata*, VITA 1 cultivar)

Compound	Relative %*
Hexanal	9.4
1-Nonene	1.3
α-Pinene	1.4
β-Pinene	3.1
(*Z*)-3-Hexen-1-ol acetate	28.8
Hexyl acetate	2.1
1,8-Cineole	1.9
Limonene	3.2
(*E*)-β-Ocimene	3.7
Nonanal	2.7
α-Cedrene	42.4

*Based on integration of peaks in the GC-MS total ion current chromatogram without use of internal standards.

A major problem with thermal desorption of analytes from porous polymer traps onto a capillary column is that flow rates of more than 20 ml/min are required for efficient desorption, and capillary column flow rates are typically much lower [10]. The use of a 6-port valve and a second injection port in the present method enabled the desorption of the volatiles from the Tenax trap at a flow rate of 30 ml/min.

Some of the identified cowpea volatiles (Table 1) have been previously reported to play a role in eliciting behavioral responses in some adult phytophagous insects. Thus, hexanal is a component of a blend of plant volatile compounds that was reported to increase trap

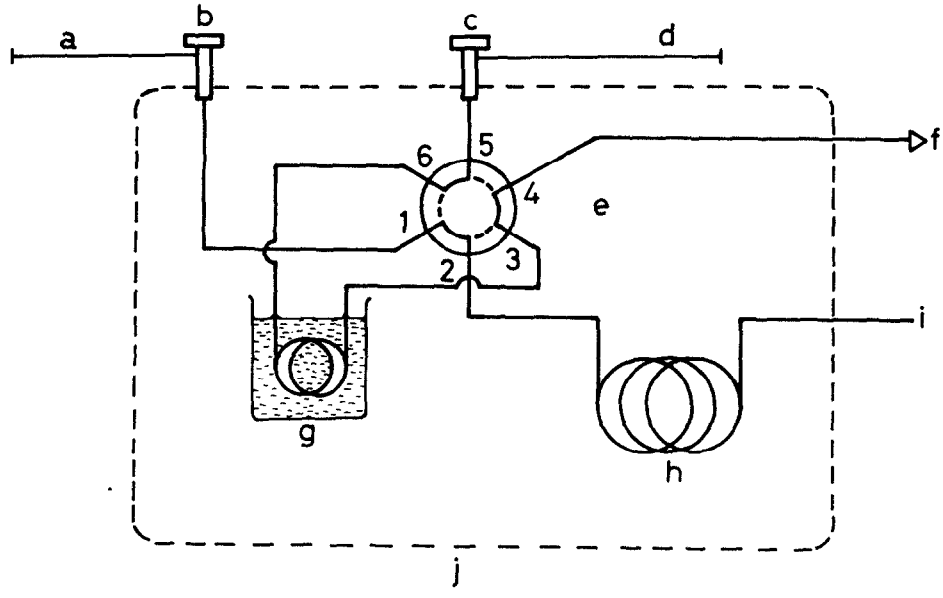

Fig. 1. Diagram of the set-up for desorption of cowpea (*Vigna unguiculata*) volatiles from the Tenax trap and subsequent injection of the desorbed volatiles into the gas chromatograph-mass spectrometer. (a) Helium carrier; (b) injection port A; (c) injection port B; (d) helium carrier; (e) valco valve; (f) to vent; (g) dry ice/acetone mixture; (h) capillary column; (i) to mass spectrometer; (j) gas chromatographic oven.

catches of the adult carrot fly, *Psila rosae* [11]. α-Pinene, in combination with camphene, is attractive to the leafhopper, *Amrasca devastans* [12], while both α-pinene and limonene are components of blends of plant odour compounds that are attractive to the boll weevil, *Anthonomus grandis* [13], and the scolytid beetle, *Dendroctonus pseudotsugae* [14]. α-Pinene and limonene are components of plant volatile blends that are attractive to the pine weevil, *Hylobius abietis* [15, 16], and the beetle *Ips grandicollis* [17], respectively, while α-pinene and β-pinene stimulate oviposition of the Eastern spruce budworm, *Choristoneura fumiferana* [18]. (*Z*)-3-Hexen-1-ol acetate is a component of a blend of plant volatiles that was found to be attractive to adult Colorado potato beetles, *Leptinotarsa decemlineata* [19]. Hexyl acetate is attractive to the Cabbage rootfly, *Delia brassicae* [20]. Plans are underway to test authentic samples of the identified cowpea volatiles with some cowpea insect pests in both behavioral and electrophysiological studies.

EXPERIMENTAL

Plant material. V. unguiculata (VITA 1 cultivar) plants were grown in a greenhouse in Nairobi, Kenya, in October 1987, under 28 ± 5° and 70 ± 10% relative humidity. Seven 3-week-old plants were used. The plants were uprooted and the roots immediately immersed in a beaker of water.

Isolation and identification. Charcoal filtered air was drawn over the cowpea plants in a glass chamber and subsequently through a Tenax trap at a flow rate of 120 ml/min for 3 hr. The Tenax trap consisted of a Hewlett Packard glass splitter sleeve (cup design) for HP5790A Hewlett Packard GC injection port, packed with Tenax TA (60/80 Mesh, Alltech Associates, Inc.), and preconditioned at 350° for 2 hr.

A VG Masslab 12-250 GC-MS-DS equipped with a Hewlett Packard 5790A GC was used for analysis of the trapped volatiles. The GC was modified to include a second GC injection port (Injection port B in Fig. 1) and a 6-port Valco valve (Cat. No. 12576, Chrompack, Middelburg, Netherlands).

Connections to the 6-port valve (Fig. 1) were made using fused silica capillary column tubing (Chrompack, 0.22 mm i.d.) coated with methyl silicone (0.12 μm film). The standard split/splitless injection port (port A) was connected to port 1 of the 6-port valve and injection port B to valve port 5 by means of short capillary column tubings. The sample loop consisted of a 90 cm capillary column tubing connected to valve ports 3 and 6 while valve port 4 was connected to vent.

In the sample desorption mode, the 6-port valve was set such that He at 30 ml/min flowed through injection port B and the sample loop to vent while He flowed through the normal injection port A and through the capillary column to the MS at a linear flow rate of 25 cm/sec. In the sample injection mode, He flowed through injection port A, the sample loop and the capillary column to the MS, while that through injection port B flowed to vent.

For desorption of the trapped volatiles, the 6-port valve was set in the sample desorption mode. The Tenax trap was placed in the cooled (35°) injection port B and the sample loop immersed in a dry ice/Me_2CO mixture. Injection port B was then heated to 200° for 5 min to desorb the volatiles from the Tenax trap. The desorbed volatiles were trapped in the cooled sample loop. Immediately after the desorption period, the valve was switched to the sample injection mode, the dry ice/Me_2CO mixture removed and the column temperature programmed from 35 to 80° at 2.5°/min, then from 80 to 150° at 5°/min and finally from 150 to 250° at 25°/min. Identification of the volatiles by MS was confirmed by comparison of their mass spectra and retention times with those of authentic samples.

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