ANALYSIS OF VOLATILES FROM MILLET PANICLES AS POTENTIAL FACTORS AFFECTING OVIPOSITION BY THE MILLET HEAD MINER MOTH, *Heliocheilus albipunctella* DE JOANNIS ((LEPIDOPTERA: NOCTUIDAE)

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Abstract. Female moths of the millet head miner, *Heliocheilus albipunctella*, show a marked preference for oviposition on millet panicles at 30% emergence over panicles at other stages, other millet plant parts or sorghum panicles. Volatiles from millet panicles have also been shown to encourage oviposition. In this study, analysis by gas chromatography linked to mass spectrometry (GC-MS) was used to determine the composition of volatiles collected from millet panicles of the 3/4HK variety at 30% emergence, anther, stigma and grain-filling stages, and from sorghum panicles. Nine components were found to account for >80% of the volatiles in most cases and these were identified as 2-hydroxy-3-butanone (acetoin), 1-octen-3-ol (octenol), (3*R*)-(-)-3,7-dimethyl-3-hydroxy-1,6-octadiene (linalool), (2*S*)-*endo*-(-)-1,7,7-trimethyl-2-hydroxy-bicyclo[2.2.1]heptane (borneol), 3-methylbutyric acid (isovaleric acid), hexanoic acid, an unidentified acid, nonanoic acid and decanoic acid. Borneol was the major component in volatiles from millet panicles at 30% emergence, the other components including the carboxylic acids being relatively more abundant in volatiles from other stages. In analyses by GC linked to electroantennographic recording from a female *H. albipunctella* moth, octenol, borneol and isovaleric acid elicited EAG responses. Comparison of the composition of volatiles from four millet varieties, EC87-PCV-1, EC87-PCV-2, HH-VBC-PCV-2 and 3/4HK, showed similar components but marked variation in the relative amounts. It is concluded that differences in composition of the volatiles could affect oviposition preferences for *H. albipunctella* females with borneol favouring and acetoin, the carboxylic acids and possibly octenol discouraging attraction and/or oviposition.

INTRODUCTION

The millet head miner moth, *Heliocheilus albipuntella* de Joannis (Lepidoptera: Noctuidae), is rated as the most damaging pest of pearl millet in the Sahelian region of West Africa (Nwanze and Youm, 1995; Youm and Owusu, 1998). The flight period of the adult moth coincides with the peak of millet emergence and flowering, and gravid females oviposit into the young panicles (N'diaye, 1985).

There is evidence that damage by *H. albipunctella* is less severe on certain millet varieties (Youm and Kumar, 1995). This may be due to asynchrony of flowering and the moths' flight period, non-preference for oviposition, tolerance to feeding, antibiosis and/or physical characteristics of the millet panicle such as the presence of bristles or the compactness of the seed head (Youm and Kumar, 1995).

Chemical cues including plant odour and taste can play an important role in the process of plant selection (Visser, 1986). Laboratory studies by Owusu and Youm (unpublished) showed *H. albipunctella* female moths oviposited almost exclusively on millet panicles in preference to millet stems or leaves or sorghum panicles. Panicles at 30% extension were preferred over panicles at 50% or 100 extension or at flowering or grain-filling stages. Furthermore, methanolic extracts of millet panicles significantly increased oviposition by *H. albipunctella* female moths on artificial millet panicles, suggesting that the extracts contained chemicals that attracted the female moths and/or encouraged oviposition after landing. In a continuation of this work by Owusu and Youm (unpublished) and Green, Owusu and Youm (in manuscript), these results with methanol extracts could not be repeated, but it was observed that levels of oviposition were increased by the presence of millet panicles even if these were not accessible to the moths.

This latter observation suggested that volatiles from the panicles encouraged oviposition, and in the work described here the composition of volatiles from the panicles at different stages of development were compared as were the composition of volatiles from panicles of different millet varieties. A preliminary examination was also made of electrophysiological responses from antennal receptors of the female *H. albipunctella* moth to millet volatiles.

MATERIALS AND METHODS

Collection of volatiles

Panicles from millet growing in the field at ICRISAT Sahelian Centre, Niger, were removed, sealed in aluminium foil bags and frozen. They were transported by air to NRI, Chatham, during which they thawed for up to two days before being refrozen.

For volatile collection, panicles were allowed to thaw over one hour and then placed in a glass column (30 cm x 4 cm). A diaphragm pump (Capex MkII, Charles Austin, UK) was used to draw air through a filter containing activated charcoal (20 x 2 cm, 6-18 mesh), through the column containing the panicle and out through a Pasteur pipette containing Porapak Q (200 mg, 50-80 mesh; Phase Sep, UK) held between plugs of silanised glass wool to trap volatiles. The Porapak was purified by soxhelet extraction with chloroform for 8 hr and each collection filter was washed well with dichloromethane (5 ml) immediately before use.

In a first experiment panicles of the 3/4HK variety at 30% emergence, anther, stigma and grainfilling stages were used. Volatiles were also collected from sorghum panicles at approximately 30% emergence. Volatiles were collected from two separate panicles for each stage from 0-3 hr and 3-24 hr.

In a second experiment, volatiles were collected for 0-24 hr from duplicate samples of panicles at the 30% emergence stage from varieties EC87-PCV-1, EC87-PCV-2 and HH-VBC-PCV-2 and also 3/4HK.

Trapped volatiles were removed from the Porapak filters with dichloromethane (Pesticide Grade; 3 x 0.5 ml) for analysis.

Gas chromatography (GC)

Volatile collections were analysed by GC using a fused silica capillary column (30 m x 0.32 mm i.d.) coated with polar CPWax52CB (Carbowax equivalent; Chrompack UK) and a flame ionisation detector (FID; 220°C). Carrier gas was helium (0.5 kg/cm^2) and injection was splitless (2 µl; 200°C). The oven temperature was held at 60°C for 2 min, then programmed at 6°C/min to 230°C. Data was captured and processed with EzChrom 6.1 software.

GC analyses were also done on a fused silica capillary column (25 m x 0.32 mm i.d.) coated with β-cyclodextrin (Chirasil-Dex CB; Chrompack, UK). Carrier gas was helium (0.5 kg/cm^2) and injection was split (split flow 50 ml/min; 200°C). The oven temperature was held at 120°C.

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analyses were carried out using a Thermoquest Finnigan-MAT Ion Trap Detector (ITD 700) operated in electron impact mode (230°C) coupled directly to the GC. The GC column was a fused silica capillary column (30 m x 0.25 mm i.d.) coated with polar CPWax52CB (Carbowax equivalent; Chrompack UK). Carrier gas was helium (0.5 kg/cm^2) and injection was splitless (2 µl; 200°C). The oven temperature was held at 50°C for 2 min, then programmed at 6°C/min to 230°C.

Compounds were initially identified by comparison of their mass spectra with those in the NBS/NIH/EPA library and the ITD terpenoid library (Adams 1989), and confirmed by comparison of mass spectra and retention times with those of authentic standards (Aldrich, UK).
GC and GC-MS retention times are presented as Kovats' Indices (KI) relative to the retention times of normal hydrocarbons.

Gas chromatography-electroantennography (GC-EAG)

GC-EAG analyses were done using GC column and conditions as described above for GC analyses. The effluent from the GC column was split (approx 1:1) between the FID and a small glass vessel in the GC oven, as described by Cork *et al.* (1991). The contents of the glass vessel were expelled at intervals (3 sec every 17 sec) with nitrogen (200 ml/min) over a female *H. albipunctella* electroantennogram preparation. The latter was prepared from the intact moth by insertion of glass microelectrodes filled with saline into the distal and proximal ends of one antenna. The electrodes were connected via silver/silver chloride electrodes to a AC/DC amplifier in DC mode (UN06, Syntech, Hilversum, The Netherlands), and both GC and EAG data were captured and processed with TurboChrom 4 software (Perkin Elmer-Nelson).

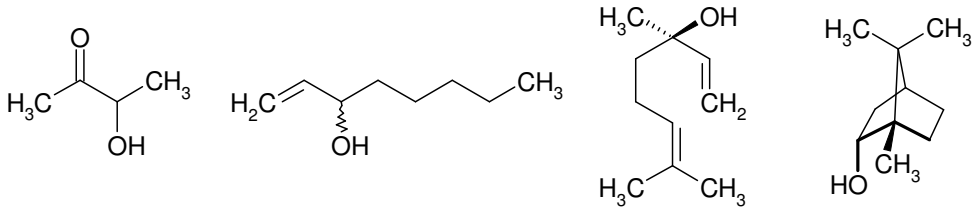
RESULTS

Comparison of volatiles from different stages of 3/4HK

Volatiles were collected from duplicate panicles of 3/4HK at 30% emergence, anther, stigma and grain-filling stages. Volatiles were also collected from sorghum panicles.

Volatiles were collected for an initial period of 0-3 hr and then for 3-24 hr. There was some concern that surface contamination of the panicles might be present, and it was hoped the initial collection would remove these. GC and GC-MS analyses of all the collection showed that no obvious contamination was present, and the profiles of the 0-3 hr and 3-24 hr collections were similar. The complete data set is given in Tables 1(a) and 1(b). As the 3-24hr samples contained more material, summary data is for these collections only.

From GC-MS analyses, 42 components were quantified as representing approximately $\geq$1% of the total volatiles (Tables 1(a) and 1(b)). Of these components, 21 were identified. Comparison of the profiles by eye suggested that nine components represented a high proportion of the total volatiles in most cases, the remainder being made up of small amounts of many of the other components. These major components were 2-hydroxy-3-butanone (acetoin), 1-octen-3-ol (octenol), 3,7-dimethyl-3-hydroxy-1,6-octadiene (linalool), *endo*-1,7,7-trimethyl-2-hydroxy-bicyclo[2.2.1]heptane (borneol), 3-methylbutyric acid (isovaleric acid), hexanoic acid, an unidentified acid, nonanoic acid and decanoic acid (Fig. 1).



acetoin octenol (-)-linalool (-)-borneol

FIG. 1. Structures of the main, non-acidic components of millet panicle volatiles.

The percentages of these nine components in volatiles from the four millet panicle stages and from sorghum panicles are shown in Fig. 2. For the millet volatiles, these accounted for $\geq$84% of the total volatiles.

Volatiles from the 30% emergence stage favoured for oviposition by *H. albipunctella* were dominated by borneol with smaller amounts of linalool and octenol. In the floral and grainfilling stages the relative amount of borneol decreased and significant amounts of acetoin and the carboxylic acids appeared. The latter were even more prominent in the sorghum volatiles.

Analysis on the chiral cyclodextrin GC column separated the enantiomers of borneol ((-) at 15.7 min, (+) at 16.6 min; Fig. 3) and linalool ((-) at 5.94 min, (+) at 6.05 min). The borneol in millet volatiles was the 1*S*-*endo*-(-) enantiomer (Fig.3). The linalool was the 3*R*-(-) enantiomer.

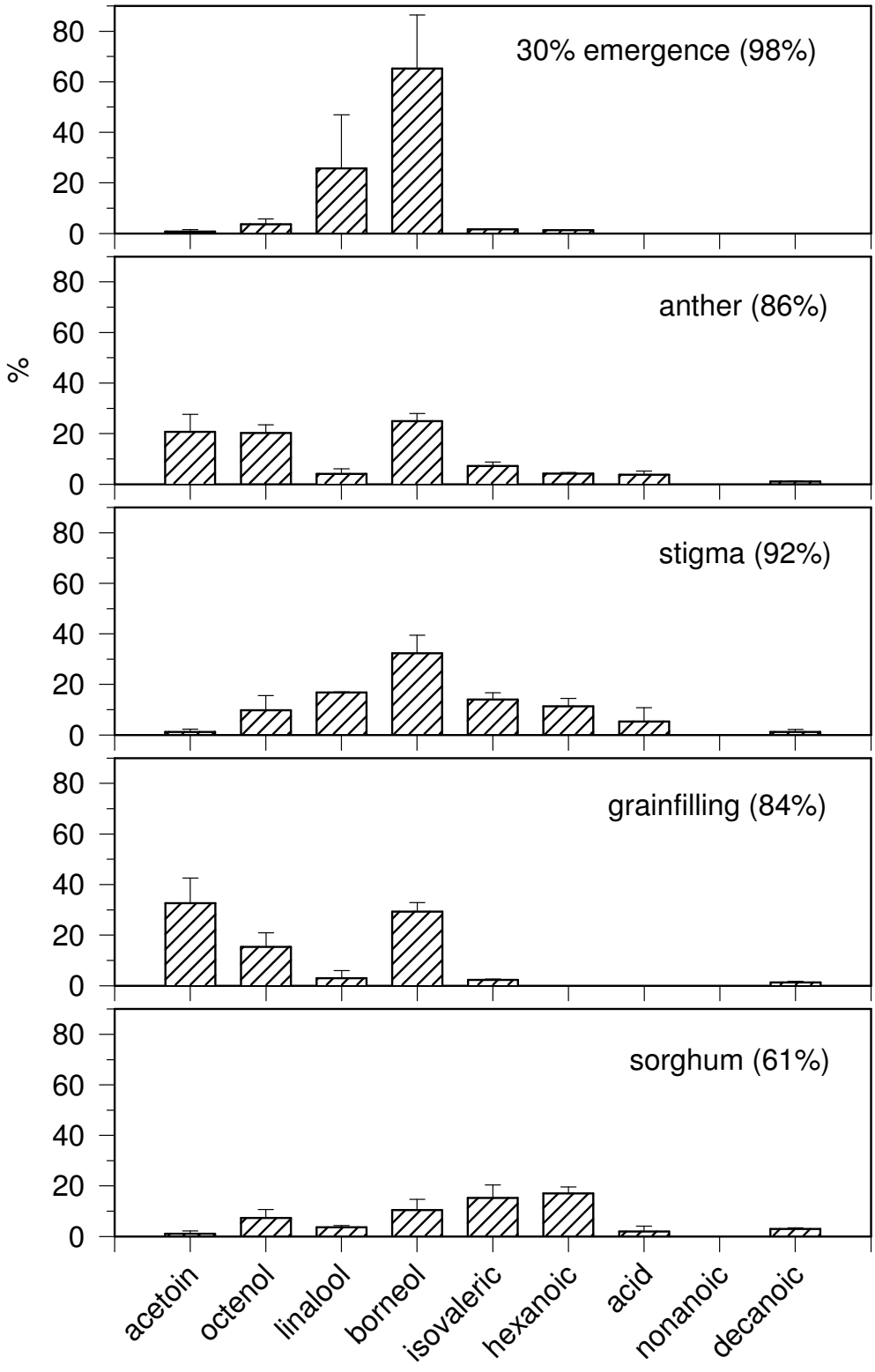


FIG. 2. Percentages of nine components in volatiles from four millet panicle stages and from sorghum panicles (amount of total volatiles accounted for by these components shown in brackets; graph shows mean and spread of results from duplicate samples, 3-24 hr collections).

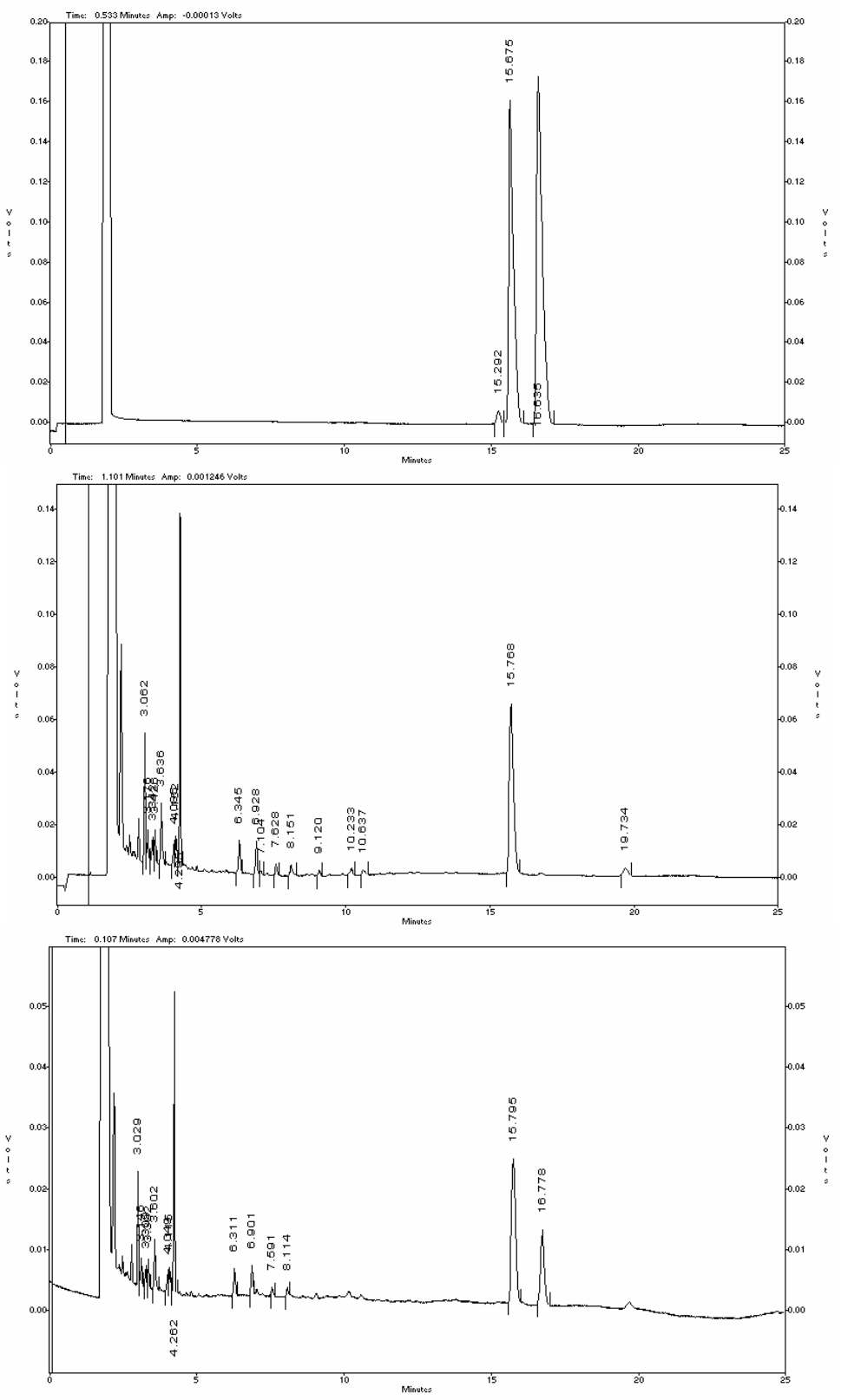


FIG. 3. GC analyses on Cyclodextrin GC column of synthetic (-) and (+)-borneol (upper), borneol in volatiles from grainfilling stage of millet panicle (middle), and millet volatiles and synthetic (+)-borneol (lower).

Comparison of volatiles from panicles of different millet varieties

Data from GC-MS analyses of 0-24 hr collections of volatiles from 30% extension stages of panicles of four different millet varieties are shown in Table 2 and summarised for the nine components referred to above in Fig. 4. Results for the 3/4HK agreed reasonably well with those obtained previously (Fig. 2). In all four varieties, borneol was the major component, although the amount varied with the percentage in volatiles from HH-VBC-PCV-2 > 3/4HK > EC87-PCV-2 > EC87-PCV-1. Proportions of octenol followed essentially the reverse pattern.

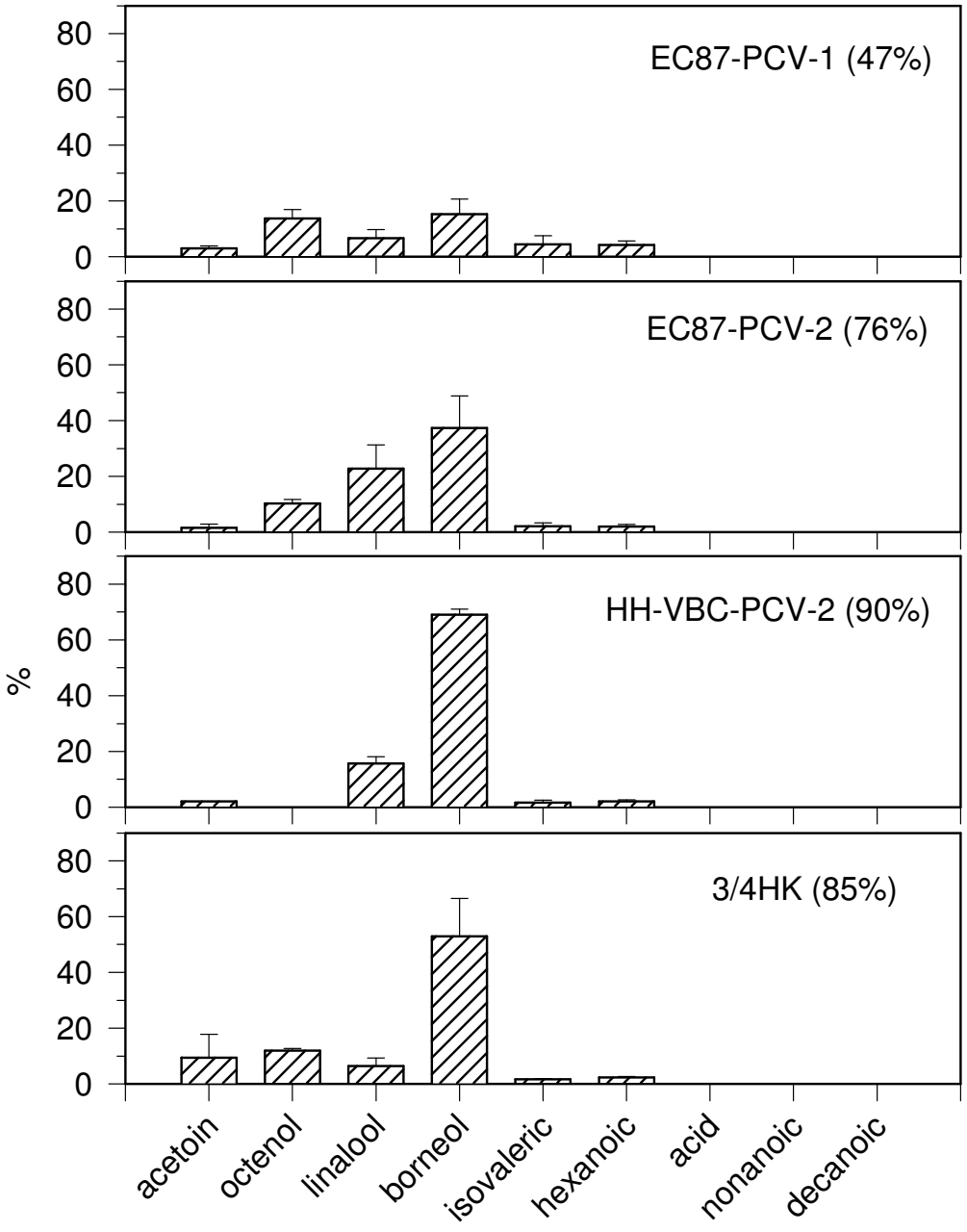


FIG. 4. Percentages of nine components in volatiles from panicles at 30% extension stage of four millet varieties (amount of total volatiles accounted for by these components shown in brackets; graph shows mean and spread of results from duplicate samples, 0-24 hr collections).

Total quantities of volatiles

The relative amounts of volatiles obtained in all the volatile collections were estimated from the total GC integration values obtained. These are shown in Fig. 5, relative to the amount in the first collection from the 30% emergence panicles of 3/4HK. The amounts were variable and showed no consistent pattern, the amount of volatiles obtained from 3/4HK in the second experiment apparently being twice that in the first.

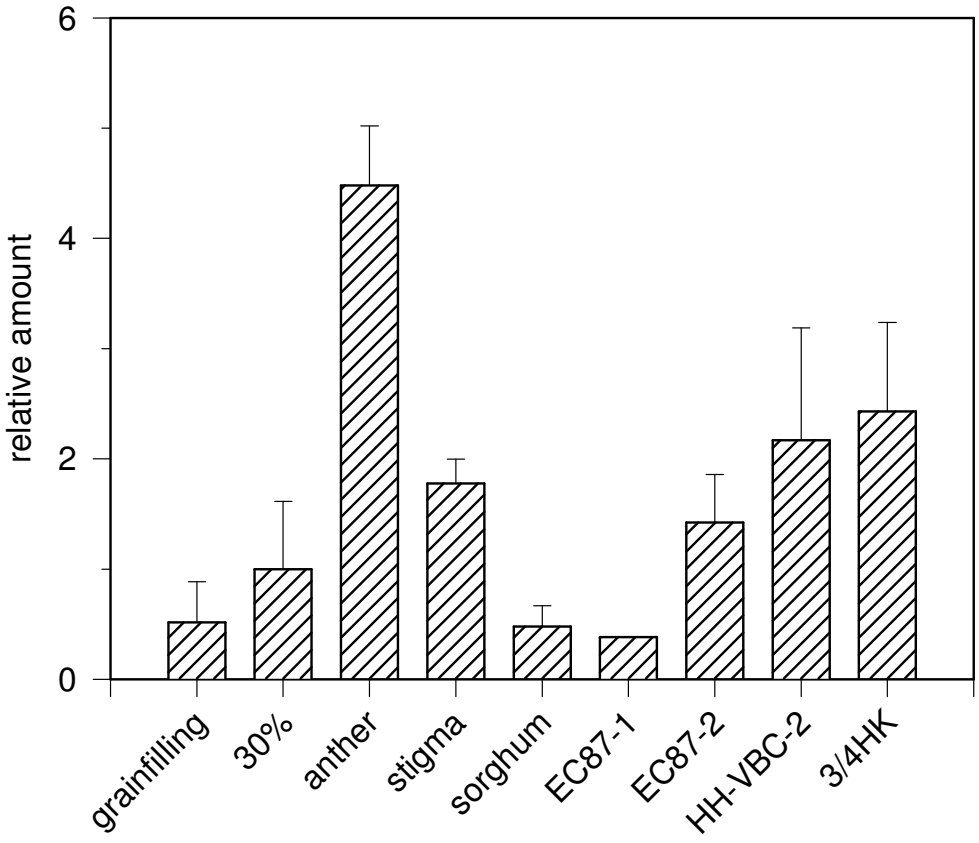


Fig. 5. Total amounts of volatiles collected from millet and sorghum panicles expressed relative to amount from 30% emergence 3/4HK in the first experiment (graph shows mean and spread of results from duplicate samples, 0-24 hr collections).

GC-EAG analyses

A total of 16 GC-EAG analyses was carried out with five of the collections of volatiles from millet panicles against seven female *H. albipunctella* moths. A representative analysis is shown in Fig. 6. Small EAG responses were observed to octenol (15.7 min) and borneol (21.5 min) and possibly to isovaleric acid (20.7 min). Responses to the latter were observed in other runs.

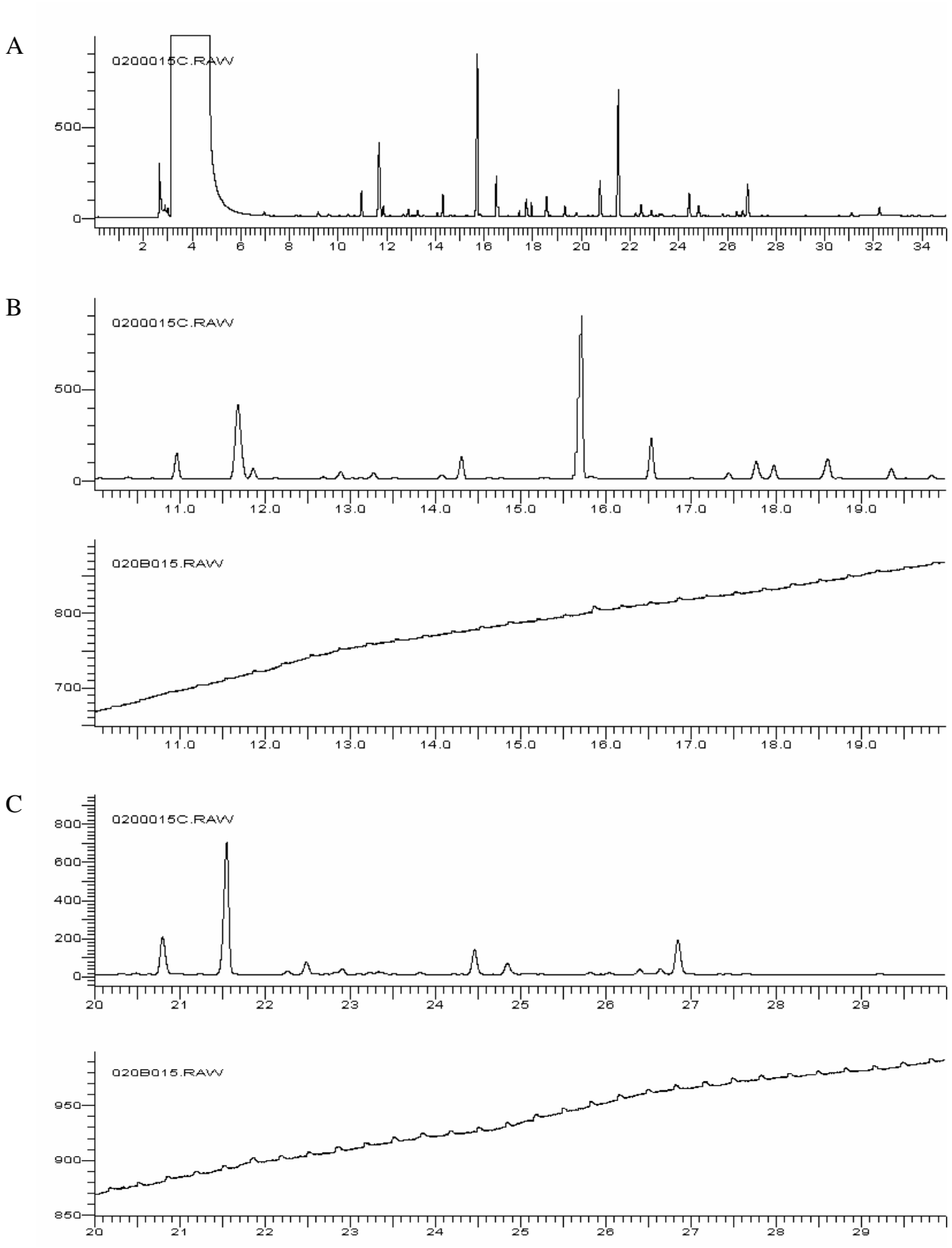


FIG. 6. GC-EAG analysis of volatiles from a 3/4HK millet panicle at the anther stage. (A) complete GC trace; (B) and (C) expanded GC (upper) and EAG (lower) traces.

DISCUSSION

This study arose from observations (Green *et al.*, unpublished) that female *H. albipunctella* moths laid more eggs in the presence of volatiles from millet panicles. In other laboratory studies (Owusu and Youm, unpublished), female *H. albipunctella* showed a high degree of preference for millet panicles at 30% emergence as oviposition sites, over millet panicles at later stages, millet stems or leaves or sorghum panicles. Even in the confines of the laboratory bioassay, it is possible that volatiles played a role in this preference, either attracting moths to favourable stages or discouraging them from ovipositing on less favourable stages.

There is little or no published work on the composition of volatiles from millet plants. Lwande and Bentley (1987) reported eight relatively trivial compounds from sorghum seedlings, and there have been some studies on the composition of odours from sorghum grains (e.g. Jambunathan *et al.*, 1995). In this study, the monoterpene alcohols 1*S-endo*-(-)-borneol and 3*R*-(-)-linalool and the fatty acid oxidation product 1-octen-3-ol were found to be the major components of volatiles from millet panicles. In the 3/4HK variety, borneol constituted over 60% of the total volatiles at 30% emergence of the panicle and this decreased to 20-40% in the floral and grain-filling stages. The proportion of linalool decreased similarly, but the relative amount of octenol tended to increase. In volatiles from these later stages, acetoin was a significant component and there were also significant amounts of several short-chain carboxylic acids. The latter were in fact the major components of volatiles from sorghum panicles, with only small amounts of the three alcohols being present.

Comparison of volatiles from panicles of four different millet varieties at the 30% emergence stage showed similar compounds were present as the major constituents. The highest proportion of borneol was found in volatiles from HH-VBC-PCV-2 at 70% and the lowest in volatiles from EC87-PCV-1 at <20%, with the latter also showing a higher proportion of octenol and the acids *iso*-valeric and hexanoic. In fact, HH-VBC-PCV-2 shows a higher degree of tolerance to *H. albipunctella* in the field than the other varieties, but the origin of this effect is unknown.

Although these studies are far from conclusive, they do show marked differences in the composition of volatiles from millet panicles at various stages of development and particularly between those from millet and sorghum panicles. In that the 30% emergence stage of millet panicles is known to be very much preferred for oviposition by female *H. albipunctella,* it could be hypothesised that high levels of borneol encourage oviposition while relatively more acetoin and carboxylic acids and possible octenol discourage oviposition. Consistent with these ideas were the results of analyses of volatiles by GC linked to EAG recording from a female *H. albipunctella* moth in which borneol, octenol and *iso*valeric acid were found to elicit EAG responses.

It should be noted that acetoin and the short-chain carboxylic acids are found in volatiles from decomposing plant material (e.g. Nagnan *et al.*, 1992). It is thus possible that these were actually artefacts in the studies described here, although the results were remarkably consistent among replicates, the changes in composition followed clear trends and acetoin and the carboxylic acids were not always found in the same proportions. Ideally future work should collect volatiles from intact panicles on the plant or at least freshly excised. Future

work should also determine whether the various plant parts studied here show differing attractiveness to mated female *H. albipunctella* moths in a wind tunnel bioassay and whether blends of synthetic chemicals based on the results obtained in this study do indeed have any attractiveness to female *H. albipunctella* moths.

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