Corn Leaf Volatiles: Identification Using Tenax Trapping for Possible Insect Attractants

Ron G. Buttery* and Louisa C. Ling

The volatile compounds in the atmosphere around corn leaves were trapped on Tenax adsorbent. The material eluted from the Tenax was analyzed by capillary gas chromatography–mass spectrometry. A total of 24 compounds were identified. Major components included (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, a sesquiterpene tentatively identified as cyclosativene, α-ylangene, an unidentified oxygenated sesquiterpene, 2-heptanone, (*Z*)-4-hepten-2-one, and caryophyllene. Preliminary studies were also made on the Tenax-trapped volatiles from corn husks, silks, and roots.

We have previously carried out studies on the volatiles associated with the leaves (or other plant parts) of alfalfa, oats, and corn [cf. Buttery et al. (1978, 1982)]. It seems reasonable that such volatile compounds might be important odor cues to the insect pests of these plants.

Sample isolation in our earlier studies on corn volatiles (Buttery et al., 1978) and related work by Flath et al. (1978) had been carried out with vacuum steam distillation–continuous extraction. Although, this is considered a mild method of isolation, our work with alfalfa and oat leaves indicated that even vacuum steam distillation produces many volatile compounds (e.g., lipid and carotenoid oxidation products) that apparently result from tissue damage (and the associated enzyme activity) due to either the vacuum or the 50 °C temperature used. Such compounds can completely obscure the original volatiles associated with the undamaged plant material. The enzyme action might also destroy (e.g., oxidise) some of the volatile compounds present in the intact plant.

Tenax trapping of plant volatiles gave much simpler volatile mixtures, which showed little evidence of compounds resulting from tissue damage. We, therefore, decided to reinvestigate the volatiles of the various parts of the corn plant by the Tenax-trapping technique.

EXPERIMENTAL SECTION

Materials. Corn (Stylepak variety) was grown during the summer of 1983 on experimental fields in Berkeley and a different variety (Bonanza) on experimental fields near Davis, CA. With the smaller plants the leaves were obtained by cutting the whole plant off near the base. With large plants the leaves were cut off at the stem.

Isolation Using Tenax Traps. Except for the point at which they were cut from the corn plant, care was taken not to damage the leaves during the isolation. The isolation was generally begun 1–3 h after cutting the leaves from the plant. The method used was similar to that previously described by us (Buttery et al., 1982) for alfalfa except that a 450-g quantity of the whole (intact) corn leaves was placed in a 12-L flask. The Tenax trap consisted of a Pyrex tube packed with 10 g of Tenax (14 cm long by 2.2 cm diameter). Air drawn from outside the laboratory (and purified by passage through freshly activated charcoal) was led into the flask via a Teflon tube. The air passed over the leaves and left the flask through the Tenax trap. The flow rate of the air was 1 L/min and was continued for 24 h. The trapped volatiles were eluted from the trap with diethyl ether. The extracts from two to four isolations were combined and then concentrated to a small volume (5 μL) with a warm water bath and low hold up fractional distillation columns. This combined concentrate was used in a single (splitless) injection for the capillary gas–liquid chromatography–mass spectral (GC–MS) analysis. With preliminary studies on corn husks, silk, and roots, essentually the same isolation technique was used (with minimum damage to the plant material) except that the roots were cut into pieces ca. 4 × 6 cm.

Capillary Gas–Liquid Chromatography–Mass Spectral Analysis (GC–MS). The capillary column was 150 m long by 0.66 mm i.d. and Pyrex glass coated with Carbowax 20-M. The column was held at 60 °C for the first 40 min and then temperature programmed at 1 °C/min to 170 °C and held at this upper limit. The column inlet pressure was 15 psi of He. Coupling to the mass spectrometer (a modified Consolidated 21-620 cycloidal-type instrument) was done with a single-stage silicone rubber membrane molecular separator. Electron ionization voltage was 70 eV. Separate GC–MS runs were made on the concentrates from five different batches of corn leaves. Compounds were considered identified if their mass spectral and GLC retention data were consistent with that of authentic samples run on the same instruments. Most authentic chemical samples were obtained from commercial sources or synthesized by established methods and purified by GLC separation. Authentic sesquiterpenes were isolated from hop or orange essential oils as described previously (Buttery et al., 1982). Identities were verified by spectral (MS and IR) means.

RESULTS AND DISCUSSION

The amount of total volatiles, isolated from the corn leaves with the Tenax trapping method, was in the range of 1–10 parts of volatiles per billion (10^9) parts of leaves (ppb). The total volatiles trapped (for a given weight of leaves) became less as the plants became more mature. Five different samples of leaves were studied from plants ca. 1–4 ft high and by using two corn varieties. The results of the GC–MS analysis of the concentrate from the Tenax are listed in Table I. The range of relative percent of components found (based on GLC peak areas) is also shown. Figure 1 shows a GLC chromatogram of the Tenax-trapped volatiles from leaves of a young corn plant.

The major compounds found include the common six-carbon-type green leaf components such as (*Z*)-3-hexenol (2–17%) and (*Z*)-3-hexenyl acetate (3–14%). The (*Z*)-3-hexenyl acetate and some of the other more volatile components seemed at their highest relative concentration in the younger plants. Their relative concentration became smaller as the plants became older. The unusual seven-carbon compounds (*Z*)-4-hepten-2-one (2–8%) and (*Z*)-4-hepten-2-ol (2–4%) had been identified previously in the

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, California 94710.

Table I. Volatile Compounds Identified in the Atmosphere Associated with Corn Leaves Using Tenax Trapping

peak[a] no.	compound[b]	major MS ions (one each 14 mass units)[c]	Kovats' GLC index[d]	rel %
		Aliphatic Aldehydes		
8	hexanal	44, 56, 72, 82	1040	0.6–2
13	(*Z*)-3-hexenal	41, 55, 69, 83, 98	1100	1.6–2
15	2-methyl-2-pentenal	41, 69, 98, 55, 83	1130	1–2
20	(*E*)-2-hexenal	41, 55, 69, 83, 98	1190	0.5
49	(*E*)-2-nonenal	41, 55, 70, 83, 96, 111	1500	0.5
		Aliphatic Ketones		
17	2-heptanone	43, 58, 71, *114*, 99	1170	2–10
23	(*Z*)-4-hepten-2-one	43, 69, *112*, 55, 94, 83	1230	2–8
		Aliphatic Alcohols and Esters		
31	(*Z*)-3-hexenyl acetate	43, 67, 82, 69, 73	1310	3–14
33	(*Z*)-4-hepten-2-ol	45, 55, 70, 81, 96, *114*	1310	2–4
34	hexanol	56, 43, 31, 69, 84	1340	0.6–1
36	(*Z*)-3-hexenol	41, 67, 55, 31, 82	1370	2–17
		Terpenoids		
16	myrcene	93, 41, 69, 79, 53, 121	1160	1
19	limonene	68, 93, 41, *136*, 53, 79	1180	0.2–1
43	cyclosativene[e]	105, 94, 41, 161, 119, *204*	1445	9–10
44	α-ylangene	105, 119, 161, 93, 41, 81	1450	10–11
45	α-copaene	105, 119, 161, 93, 41, 81	1460	1
52	linalool	93, 71, 41, 55, 80, 121	1545	2
54	β-copaene	161, 41, 91, 105, 120, *204*	1550	1
57	caryophyllene	41, 69, 93, 79, 133, 55	1570	1–20
67	(*E*)-β-farnesene	69, 41, 93, 79, 55, 133	1650	2
73	α-muurolene	105, 161, 93, 81, *204*, 119	1700	0.4–0.7
77	δ-cadinene	161, 134, 41, 105, 119, *204*	1730	2–3
		Aromatic Compounds		
76	methyl salicylate	120, *152*, 92, 65, 39, 53	1730	0.5
		Unidentified Compounds		
83	oxygenated sesquiterpene[f]	69, 81, 41, 53, 94, 137	1790	8–14

[a] Peak numbers in Figure 1. [b] Complete mass spectrum and Kovats' GLC retention index are consistent with that of authentic samples except for cyclosativene (see footnote *e*). [c] The most intense ion each 14 mass units above m/z 34. Ions in descending order of intensity with molecular ion (if listed) in italic type. [d] Kovats' GLC index for the Pyrex Carbowax 20-M capillary column. [e] No authentic sample was available but the mass spectrum is consistent with published spectra. [f] Not able to identify.

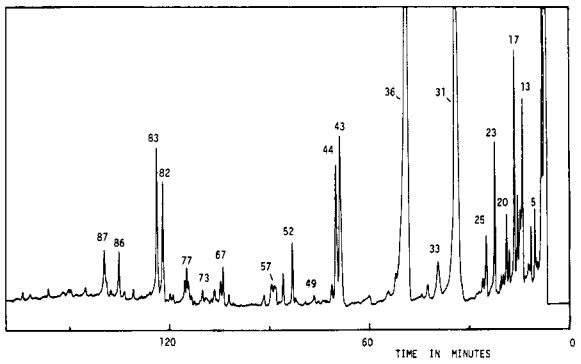

Figure 1. Capillary GLC analysis of the Tenax-trapped volatiles from young corn plant leaves.

vacuum steam distillates of corn husk volatiles by some of us (Buttery et al., 1978; Buttery, 1979). As far as we can determine, (*Z*)-4-hepten-2-one has not been reported as occurring in any other plant and is therefore one of the most unique volatile compounds associated with corn.

After the six-carbon green leaf type compounds, the next major group observed in the Tenax-trapped volatiles is the sesquiterpenes. The major sesquiterpenes in the younger plants are α-ylangene (10–11%), the tentatively identified cyclosativene (9–10%), and an unidentified oxygenated sesquiterpene (8–14%). Caryophyllene (1–20%) was found in very small amounts in the younger plant leaves but occurred in relatively high amounts in the more mature plants. The tentatively identified cyclosativene had the following mass spectrum (two major ions each 14 mass units above m/e 34, intensities in parentheses, molecular ion in italic type): 41 (77), 43 (40); 53 (17), 55 (39); 67 (23), 69 (27); 77 (30), 79 (39); 91 (63), 94 (98); 105 (100), 107 (56); 119 (63), 120 (56); 133 (23), 135 (16); 147 (11), 161 (66); 189 (16); *204* (42); GLC Kovats' index 1445. This was very similar to the mass spectrum obtained by Smedman and Zavarin (1968a,b) for cyclosativene and the GLC retention data obtained for this compound by Anderson and Falcone (1969). The unidentified oxygenated sesquiterpene had the following mass spectrum: 39 (42), 41 (89); 53 (43), 55 (32); 69 (100), 70 (30); 79 (24), 81 (87); 94 (23), 95 (19); 107 (7), 109 (3); 121 (3), 123 (3); 136 (6), 137 (10); 147 (0.6), 149 (0.9); 162 (0.6); 175 (2); 204 (1); molecular ion not detected; GLC Kovats' index 1790.

Preliminary Analysis of Husk, Silk, and Root Volatiles. GC–MS analyses were also carried out on Tenax trap isolated volatiles from corn husks, silks, and roots by using essentually the same isolation procedures as used for the corn leaves.

Corn Husks. GC–MS analysis of the Tenax trap volatile isolates from several samples of corn husks showed that these were qualitatively essentially the same as those from the leaves. The two varieties studied (Stylepak and Bonanza) also showed no obvious qualitative difference in their volatile oils. Quantitatively, in the husks, there were relatively smaller amounts of the six-carbon green aroma type compounds, such as (*Z*)-3-hexenol [and also of (*Z*)-4-hepten-2-ol], and there were relatively larger amounts of the sesquiterpene hydrocarbons when compared to the leaves. The most notable feature of the husk volatiles was that caryophyllene was the major sesquiterpene whereas it was only a very minor component of the young leaves.

Corn Silks. The corn silks gave by far the lowest amount of volatiles, of the different parts of the corn plant, with the Tenax-trap method. The six-carbon green aroma

type compounds such as (Z)-3-hexenol were detected. The compound geosmin was also identified in the Stylepak variety silks but not in the Bonanza variety. Geosmin had been previously identified in the vacuum steam volatile oil of corn silks by Flath et al. (1978). In the present work, sesquiterpene hydrocarbons were also detected in the Tenax-isolated silk volatiles. These appeared to be qualitatively similar to the sesquiterpene hydrocarbons in the leaves, but the levels of these compounds were too low to be able to characterize them with any certainty.

Corn Roots. Corn roots were obtained from relatively mature corn plants. They were cut into pieces ca. 4 × 6 cm to allow them to go through the neck of the flask. Most of the accompanying soil was dislodged in the process of cutting but was not washed off because of the possibility of removing volatiles. The major Tenax-trapped root volatiles were sesquiterpene hydrocarbons. These were qualitatively quite different from the sesquiterpene hydrocarbons identified in the leaves. The major root sesquiterpene could not be identified but had a mass spectrum somewhat similar to that of δ-elemene. The only root sesquiterpenes identified with some certainty (mass spectrum consistent with published data) were (E)-β-farnesene and bazzanene. With corn roots there is the possibility that the volatiles are from the soil rather than the roots. However, in unrelated work, we had previously studied the volatiles from the soil in the general area where the corn was grown and had not detected any sesquiterpenes.

Possible Importance of Volatiles to Insects. Terpene and sesquiterpene hydrocarbons are relatively weak odorants for humans but seem to be much more important to insect olfaction. In a number of plants such as eucalypts, pines, and various "bay" trees, the leaves can contain as much as a few percent of terpenoid hydrocarbons. The concentration found in corn leaves, however, was only of the order of a few parts per billion. Similar low concentrations of terpene and sesquiterpene hydrocarbons were found by us in the leaves of other major crops such as alfalfa, red clover, and oats [cf. Buttery et al. (1982, 1984)]. Such low concentrations may be thought to be insignificant, but alfalfa sesquiterpenes were found to be attractive to an alfalfa insect pest (Kamm and Buttery, 1983). In work by other workers, caryophyllene has been shown to be attractive to the boll weevil (Minyard et al., 1969) and (E)-β-farnesene has been shown to be an alarm pheromone of some aphids (Wohlers, 1981). With many leafy parts of plants the terpene and sesquiterpene hydrocarbons may be located on the surface of the leaves in the hydrophobic cuticular waxy layer. The insects' first contact with the plant could be with this cuticular material.

Some testing of the volatile compounds identified in the present work are being carried out by using electroantennagram methods with the corn earworm (*Heliothis zea*), and the results of these testing studies will be reported elsewhere (Light, 1984).

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Registry No. Hexanal, 66-25-1; (Z)-3-hexenal, 6789-80-6; 2-methyl-2-pentenal, 623-36-9; (E)-2-hexenal, 6728-26-3; (E)-2-nonenal, 18829-56-6; 2-heptanone, 110-43-0; (Z)-4-hepten-2-one, 90605-45-1; (Z)-3-hexenyl acetate, 3681-71-8; (Z)-4-hepten-2-ol, 34146-55-9; hexanol, 111-27-3; (Z)-3-hexenol, 928-96-1; myrcene, 123-35-3; limonene, 138-86-3; cyclosativene, 22469-52-9; α-ylangene, 14912-44-8; α-copaene, 3856-25-5; linalool, 78-70-6; β-copaene, 18252-44-3; caryophyllene, 87-44-5; (E)-β-farnesene, 18794-84-8; α-muurolene, 10208-80-7; δ-cadinene, 483-76-1; methyl salicylate, 119-36-8.

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