Composition of the Floral Odor of *Cucurbita maxima* Duchesne (Cucurbitaceae)

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The floral volatiles from two cultivars of *Cucurbita maxima* Duchesne (squash) have been identified by vacuum steam distillation and gas chromatography–mass spectrometry. Spectral data were obtained for 31 major components, and the structures of 22 of these were verified by comparison with authentic standards. The mixtures are comprised of low molecular weight aliphatic alcohols and aldehydes, monoterpenoids, sesquiterpenoids, indole, and aromatic alcohols, aldehydes, and methyl ethers. The aromatics are similar to known attractants of cucurbit-feeding beetles in the chrysomelid genus *Diabrotica* (corn rootworms and cucumber beetles), and indole has previously been determined to be an attractant of these insects.

The composition of the floral odor of *Cucurbita spp.* (squash, pumpkins) is of interest as a source of potential attractants for phytophagous beetles in the genera *Diabrotica* and *Acalymma* (corn rootworms, cucumber beetles). These insects are serious pests of cucurbits, grasses, and legumes in North and South America. Adult *Diabrotica* and *Acalymma* are known to aggregate in the blossoms of *Cucurbita spp.*, particularly those of *Cucurbita maxima* Duchesne (Fisher et al., 1984; Andersen, 1984). Andersen and Metcalf (1986) have reported that indole, a constituent of the floral odor of Blue Hubbard and Pink Banana Jumbo cultivars of *C. maxima*, acts as an attractant for the western corn rootworm *Diabrotica virgifera virgifera* LeConte and the striped cucumber beetle *Acalymma vittatum* (F.). This compound has also been isolated from the leaves of corn, an important host plant of *Diabrotica spp.* (Thompson et al., 1974). In other studies, Ladd et al. (1983), Ladd (1984), and Lampman et al. (1986) have found that a number of simple aromatic compounds, including eugenol, phenylacetaldehyde, and veratrole, are attractive in the field to adults of the northern corn rootworm (*Diabrotica barberi* Smith and Lawrence), the southern corn rootworm (*Diabrotica undecimpunctata howardii* Barber), and the western corn rootworm. The presence of these compounds in *Diabrotica* or *Acalymma* host plants has not been established.

It is the purpose of this study to determine the composition of the *C. maxima* floral odor as a first step in understanding the complete role of indole and other floral constituents in the selection of blossoms by economically important *Diabrotica* and *Acalymma* beetles.

MATERIALS AND METHODS

The Blue Hubbard and True Hubbard cultivars of *C. maxima* were used for this study. Blossoms from the Blue Hubbard cultivar were collected from a field plot on the evening prior to anthesis, and the stems were placed in tap water in the laboratory. The following morning, the blossoms had opened and were steam distilled. True Hubbard blossoms were collected the morning of anthesis from plants grown in a greenhouse under natural and artificial lighting conditions (16 h light–8 h dark). The latter blossoms were stored at 0 °C until ca. 40 blossoms were obtained. The maximum storage period for these blossoms was 10 days. In both greenhouse- and field-grown plants, male blossoms greatly outnumbered females, so samples were made up primarily of males (Blue Hubbard, 40 male, 0 female; True Hubbard, 37 male, 3 female).

For distillation, the corollas were separated from the nectaries, shredded, and added to a 5-L flask along with 3 L of distilled water. This mixture was distilled under reduced pressure at 35–45 °C (38–56 mmHg), and a slight flow of nitrogen was bled into the flask during the course of the distillation. In a cooled receiving flask, ca. 2 L of distillate was collected. A dry ice cooled trap placed in the system prevented material from escaping the distillate.

The aqueous distillate and cold trap contents were combined and extracted with freshly distilled anhydrous diethyl ether (400 mL) for 16 h using continuous liquid–liquid extractors. The ether extracts were dried over anhydrous sodium sulfate and concentrated to a volume of 15–20 mL by distillation through a Vigreux column. They were then reduced to a volume of 0.5 mL under a gentle stream of nitrogen.

Electron-impact gas chromatography–mass spectrometry (EI GC–MS) (70 eV) was carried out on Kratos MS 30 and Finnigan TSQ instruments. In both cases, a fused silica DB-1701 WCOT column (J&W Scientific) measuring 25 m × 0.32 mm was used, and helium was the carrier gas. The temperature was programmed from 40 to 200 °C at 5 °C/min, and splitless injection was used. Chemical ionization (CI) GC–MS was carried out on a Finnigan TSQ instrument, using the same column, carrier gas, and temperature-programming conditions as above. Isobutane at 0.3 torr was used as the reagent gas and was ionized at an energy of 70 eV.

GC verification of compound identity was accomplished with an instrument equipped with a flame ionization detector (FID) and fused silica WCOT columns with bonded phases of DB-1701 (25 m × 0.32 mm) or DB-Wax (30 m × 0.32 mm). With the former, the temperature was held at 40 °C for 5 min and then raised at a rate of 6 °C/min to 200 °C, while with the latter a 2-min initial hold at 60 °C was followed by an increase of 4 °C/min to a final temperature of 200 °C. In both cases, injector and detector ovens were set at 230 °C, and splitless injection (1–2 μL) was used. All standards were obtained from commercial sources and were found to be at least 95% pure by GC analysis. Retention indices were calculated by using the homologous *n*-alkane series (Van den Dool and Kratz, 1963), and proportional quantities were obtained by electronic integration of peak area.

RESULTS AND DISCUSSION

The qualitative composition of the two volatile mixtures was quite similar (Table I). In both cultivars, the major components were low molecular weight aliphatic aldehydes

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Table I. Composition of True Hubbard and Blue Hubbard Floral Steam Distillates

no.	compound	retention index DB-1701	prominent ions (EI)[a]	basis for ident[b]	True Hubbard, %	Blue Hubbard, %
1	1-penten-3-ol	777	57, 27, 31, 55, *86*	d		0.96
2	hexanal	890	56, 44, 43, 55	a	0.03	0.19
3	(*E*)-2-hexenal	963	41, 39, 83, 69, 57, *98*	a	2.16	1.54
4	(*Z*)-3-hexen-1-ol	982	41, 67, 55, 82, *100*	a	0.68	26.72
5	1-hexanol[c]	994	56, 43, 41, 55	a	1.89	12.59
6	(*E*)-2-hexen-1-ol[c]	994	57, 41, 43, 67, 82, *100*	a	1.93	27.70
7	benzaldehyde	1086	105, *106*, 77, 81, 67	a	tr	tr
8	1-octen-5-ol	1087	57, 70, 55, 43, 110	b	1.08	1.04
9	unknown (MW 110)	1128	81, 39, 27, 53, 110	b	0.43	0.40
10	unknown (MW 110)	1143	81, 39, 27, 53, 110	b	0.28	0.23
11	phenylacetaldehyde	1185	91, 92, *120*, 65, 39	c	0.11	
12	nonanal	1194	41, 43, 56, 57, 70	a		0.45
13	benzyl alcohol	1214	77, 79, *108*, 107, 51	a	12.82	1.08
14	1,4-dimethoxybenzene	1283	123, *138*, 95	a	34.54	7.73
15	decanal	1298	43, 32, 41, 57, 55	a	tr	0.11
16	*p*-cresol	1312	*108*, 107, 77, 79, 90	a	1.62	0.53
17	β-cyclocitral	1358	123, *152*, 137	b	0.25	
18	(*Z*)-cinnamaldehyde	1380	131, *132*, 103, 77, 51	b	0.16	
19	4-methoxybenzaldehyde	1452	135, *136*, 77, 92, 107	a	1.83	0.41
20	(*E*)-cinnamaldehyde	1462	131, *132*, 103, 77, 51	a	1.66	tr
21	1,2,3-trimethoxybenzene	1463	*168*, 153, 125, 110	a	0.64	0.82
22	4-methoxybenzyl alcohol	1495	*138*, 109, 121, 77, 94	a	3.41	1.25
23	(*E*)-cinnamyl alcohol	1514	92, 91, *134*, 105, 115	a	1.06	0.47
24	1,2,4-trimethoxybenzene	1533	*168*, 153, 125, 110, 93	a	11.24	1.89
25	cadinene or muurolene isomer	1543	161, 105, 119, *204*, 81	b	2.51	0.61
26	indole	1554	117, 90, 89	a	2.56	0.85
27	α-ionone	1567	121, 93, 43, 136, 91, *192*	a	0.40	0.29
28	β-ionone	1628	177, 43, 91, 135, *192*	a	0.83	0.72
29	nerolidol isomer	1668	69, 93, 107, 136, 161, 189	a	3.34	4.77
30	unknown	1699	161, 105, 41, 119, 204	d	tr	
31	dodecanoic acid	1754	73, 60, 57, 129, 85, *200*	a	3.20	tr

[a] In order of decreasing abundance. Molecular ion italicized when present. [b] Key: a = Electron-impact (EI) and chemical ionization (CI) spectrum consistent with structure, identification verified by gas chromatography (GC) on two capillary columns; b = EI and CI spectrum consistent with proposed structure; c = EI spectrum consistent with proposed structure, identification verified by GC on two capillary columns; d = EI spectrum consistent with proposed structure. [c] 1-Hexanol and (*E*)-2-hexen-1-ol were eluted as a mixture on DB-1701. Percentage obtained with DB-Wax capillary.

and alcohols, aromatic compounds, and sesquiterpenoids. Large quantitative differences were seen, however, particularly with the six-carbon alcohols. Blue Hubbard corollas contained much larger quantities of (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, and 1-hexanol. True Hubbard was dominated by 1,4-dimethoxybenzene, 1,2,4-trimethoxybenzene, and benzyl alcohol. This variation could be due to differences in growth conditions or sample preparation or may represent actual genetic variation.

Several components were tentatively identified in both mixtures, but the structures could not be confirmed chromatographically due to a lack of reference standards. Compounds **9** and **10** (Table I) gave identical CI and EI mass spectra that indicated a molecular weight of 110. The fragmentation patterns of both were very similar to that seen in published spectra (McLafferty and Stauffer, 1983) of unsaturated C_8H_{14} branched hydrocarbons. Spectral data for component **17** indicated a molecular weight of 152 and a formula of $C_{10}H_{16}O$. Comparison of the EI spectrum of **17** with published spectra (McLafferty and Stauffer, 1983) for $C_{10}H_{16}O$ monoterpene aldehydes and ketones revealed a strong correlation with the spectrum of β-cyclocitral.

Three sesquiterpenoids were also detected (Table I). One of these (compound **29**) produced a weak fragment at m/e 223 in the CI spectrum, suggesting the possibility of an alcohol with the formula $C_{15}H_{26}O$. Examination of the EI spectrum showed good correlation with that of an isomer of nerolidol (McLafferty and Stauffer, 1983), and cochromatography with a standard mixture of nerolidol isomers verified this assignment. A CI spectrum was not obtained for the trace component **30** of the True Hubbard mixture, but EI spectral and retention data suggested that this was also an alcohol with the molecular formula $C_{15}H_{26}O$. Comparison with literature spectra (McLafferty and Stauffer, 1983) indicated that this compound may be an isomer of cadinol.

Component **25** produced an intense fragment at m/e 205 in the CI spectrum and showed a strong apparent molecular ion at m/e 204 in the EI spectrum. A search of published spectra (McLafferty and Stauffer, 1983) indicated that this compound is a sesquiterpene hydrocarbon and is probably an isomer of either cadinene or muurolene. The major component of a standard mixture of cadinene isomers had a retention index on DB-1701 of 1535, while the retention index of the unknown was 1543.

The identities of three additional compounds were not verified chromatographically. 1-Penten-3-ol was tentatively identified on the basis of EI spectral data alone while the determination of 1-octen-5-ol was supported by CI data. The compound tentatively identified as (*Z*)-cinnamaldehyde produced EI and CI spectra identical with those of (*E*)-cinnamaldehyde.

In past field studies, it has been determined that the northern corn rootworm (Fisher et al., 1984) and the southern corn rootworm (Andersen, 1984) prefer the blossoms of *C. maxima* cultivars over those of the other major cultivated species. The western corn rootworm also prefers *C. maxima* blossoms and is abundant in certain *Cucurbita pepo* blossoms as well (Fisher et al., 1984; Andersen, 1984).

Several of the aromatic compounds found in the floral mixture are structurally similar to the attractants of the northern corn rootworm found by Ladd et al. (1983) and

Ladd (1984) and those of the southern and western corn rootworm found by Lampman et al. (1986) in field screening tests. Also, the floral component indole has been demonstrated to be an attractant for the western corn rootworm and the striped cucumber beetle (Andersen and Metcalf, 1986), and recent field studies have shown that 1,2-dimethoxybenzene, indole, and phenylacetaldehyde synergistically interact to enhance southern corn rootworm attraction to sticky traps (Lampman and Metcalf, 1986).

These data suggest that for the northern corn rootworm, the southern corn rootworm, and possibly the western corn rootworm floral volatiles play an important role in blossom selection. Future studies will attempt to further elucidate the effect on beetle behavior of cucurbit floral components in field trapping tests with single floral constituents and blends of these constituents.

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Composition of Palmarosa (*Cymbopogon martinii*) Essential Oil from Madagascar

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Twelve samples of Palmarosa essential oils from Madagascar were studied by capillary gas chromatography. The analysis using the combination of Kovats indices and gas chromatography–mass spectrometry led to the identification of 69 components. Among them, nine were determined for the first time in Palmarosa essential oil. Statistical analysis shows a high positive correlation between some monoterpenes and a high negative correlation between (*Z,E*)-farnesyl acetate and various monoterpenes and between geraniol and geranyl acetate.

Among the Gramineae family, some species of the genus *Cymbopogon* give by hydrodistillation essential oils of commercial interest. The essential oil of Palmarosa grass (*Cymbopogon martinii* (Roxb.) W. Wats var. martinii) is rich in geraniol and used as perfumery raw material for imparting roselike aroma in soaps and cosmetics products. The United States annual importation ranged from 10 to 20 tons during the last decade. The effect of plant spacing and application of various fertilizers on herb and essential oil yields of Palmarosa has been recently investigated (Singh et al., 1981; Pareek et al., 1983; Rao et al., 1985). Palmarosa grass produced a high oil yield at the flower open stage and early seed formation (Akhila et al., 1984; Pareek et al., 1981). From an improved calcium chloride adduct, geraniol was isolated in pure form from Palmarosa oil (Garg et al., 1975). Siddiqui et al. (1979) described a thin-layer chromatography (TLC) determination of this compound in Palmarosa oil, and for detecting the adulteration of this essential oil with gingergrass oil, Baiswara et al. (1976) described a sensitive TLC method.

Although the hydrocarbon composition of *C. martinii* has been reported first by Naves (1970) and Peyron (1973) and more recently by our laboratory (Gaydou and Randriamiharisoa, 1986), the nature and the composition of the oxygenated constituents remained inaccurate. The existence in Palmarosa oil of some oxygenated compounds such as linalool, α-terpineol, geranial, geraniol, neral nerol, geranyl acetate (Peyron, 1973), nerolidone, and α- and β-betulenols (Naves, 1970) was described, but many constituents of this fraction were unidentified.

In the course of the evaluation of the quality of Palmarosa essential oil from Madagascar, we have investigated the composition and the range of variation of the main constituents of 12 samples representing the production of this country during the years 1979–1982.

EXPERIMENTAL SECTION

Materials. The various samples of Palmarosa essential oil were obtained from freshly cut herb harvested during the years 1979–1982, by industrial steam distillation. The 12 Palmarosa oils investigated were composite samples of three producers located in northwest Madagascar (Nosy-Be, Ambanja, and Mahajanga areas). These oils were supplied by the Service du Conditionnement et du Contrôle de la Qualité des Produits of Antananarivo (Madagascar) who guarantee their authenticities.

Physical and Chemical Constants. Specific gravity and total alcohols (expressed in geraniol) were determined

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