

were not obtained because, although they are found in food products, we could not determine their concentrations in foods and thus could not be certain of a safe level for human consumption. To study the possible additive flavor contribution of these ethers, we compared control orange juice with juice to which we had added eugenol, methyleugenol, and *trans*-methylisoeugenol at half their threshold levels and compared to a control sample. The taste panel distinguished the test flavor sample at the 99.9% confidence level. Concentration of each phenolic ether in experimental orange oil was estimated from GLC peak areas. These oils were estimated to contain 240 ppm methyleugenol, 180 ppm isoelemicin, 120 ppm eugenol, 120 ppm *trans*-methylisoeugenol, 60 ppm elemicin, and 24 ppm *cis*-methylisoeugenol.

Although none of the ethers was present at the flavor threshold level, their flavor effects are additive and the combined effect was apparently sufficient to account for off-flavor of processed single-strength orange juice reported by Moshonas et al. (1976). One compound in particular, eugenol, was present in orange juice at approximately its flavor threshold.

Increased amounts of phenolic ethers were present when oranges were treated with higher concentrations of abscission agents. In one comparison, oil from Pineapple oranges treated with 20 ppm cycloheximide had higher levels of these ethers than oils from comparable fruit treated with 10 ppm cycloheximide, as indicated by the relative GLC peak areas; however, the absolute quantities of these compounds present were not determined.

In practice, several factors tend to minimize any effect on flavor of processed citrus products that the abscission agent may produce. By normal processing practices, juice from fruit treated with abscission agents would be mixed with much larger volumes of juice from untreated fruit so that flavor effects would be reduced by dilution. Also, preparation of concentrated juice tends to reduce, but not eliminate, flavor effects (Moshonas et al., 1976). As mechanical harvesting of citrus becomes more widely adopted, these factors that minimize flavor effects will diminish; thus, it will be increasingly important to carefully control the concentration of abscission agents and to

minimize the time required for fruit loosening after spraying. A new generation of abscission agents, composed of mixtures of the chemicals reported in this study, are effective at lower concentrations than those of the individual agents. Even though preliminary qualitative results show that the mixtures also induce formation of the phenolic ethers, hopefully the levels are low enough that flavor effects are minimal.

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Volatile Components of Corn Silk (*Zea mays* L.): Possible *Heliothis zea* (Boddie) Attractants

Robert A. Flath,* Ralph R. Forrey, Janie O. John, and Bock G. Chan

Volatile components of corn silk were concentrated by vacuum co-distillation with water, followed by ether extraction of the distillate. The concentrate was examined by large bore capillary gas chromatography-mass spectrometry, and 63 compounds were identified. Alcohols comprise the major portion of the concentrate, both in number and in quantity, with 2-heptanol being the major constituent. A highly odorous compound, geosmin, was found among the volatiles. Preliminary attractancy testing with gravid *Heliothis zea* (Boddie) moths yielded inconclusive results.

The manner in which predator insects locate host plants is still subject to controversy (Kennedy, 1977), but it is generally held that some sort of insect response to volatiles emitted by the host plant is involved in causing the insect

to locate the host. Other contributing factors suggested have included differences in infrared radiation between the host plant and its surroundings, plant color, and perhaps shape or texture of plant parts. In dealing with plant volatiles as possible predator attractants, it would be desirable to determine what volatiles are present in the plant, whether the total volatiles vary in composition with the segment of the plant inspected, and whether these volatiles have any effect on the insect predator's behavior.

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The relationship between sweet corn and the economically-important predator *Heliothis zea* (Boddie), the corn earworm, was selected for examination. Thompson et al. (1974) have identified 59 components of corn bud essential oil.

EXPERIMENTAL SECTION

Starting Material. Whole ripe sweet corn ears, including husks and silk, were purchased from a local wholesale market. These were mostly the Vanguard variety, with a small quantity of El Capitan. In some subsequent work, corn grown on an experimental farm in the Gilroy, California area was used. Both Style-Pak and Golden Jubilee were obtained from this source.

The commercially obtained corn was husked and the silk placed in polyethylene bags. These were then stored in a freezer until processed (1–4 weeks storage). The Style-Pak and Golden Jubilee silks were usually held for less than 1 week before use.

Concentrate Preparation. The apparatus and method employed (a vacuum co-distillation with water, followed by liquid–liquid extraction with ether) have been previously described (Forrey and Flath, 1974; Flath and Takahashi, 1977). Corn silk (3.3 kg total; 2.4 kg of Vanguard, 0.9 kg of El Capitan) was combined with 4 L of distilled water in a 12-L sample flask. The distillation was carried out at 40 mm pressure and continued for 5 h, yielding 1.8 L of distillate. The distillate had a strong earthy odor. The distillate was extracted with freshly distilled ether in a conventional liquid–liquid extractor for 8 h. After storage overnight in a freezer, the solvent extract was decanted from droplets of water and transferred to a dry flask. The bulk of the solvent was distilled (water bath; maximum temperature, 47 °C) through a 30 cm × 1.2 cm i.d. glass helix-packed, vacuum-jacketed distillation column, leaving approximately 2 mL of liquid in the pot after cooling. This was transferred to a small vial fitted with an 8-cm long air condenser, and most of the remaining solvent was distilled, again using a water bath (maximum temperature, 48 °C). Preliminary gas chromatographic (GC) examination as well as the odor of the residue indicated the presence of some volatile acidic material, so the concentrate was diluted with 10 mL of freshly distilled ether and washed with small portions of 1 N sodium hydroxide solution, then with saturated aqueous sodium chloride solution. The ether solution was then re-concentrated as described above. GC examination of the residue (23 mg) indicated that it was approximately two-thirds solvent, so 8 mg of corn silk material was present (2.4 ppm yield). Similar preparation of concentrates from Style-Pak and Golden Jubilee (two lots of each) corn silk produced concentrate yields of 1.0 and 3.0 ppm (Style-Pak) and 0.6 and 2.4 ppm (Golden Jubilee).

Concentrate Separation and Identification. GC separations were carried out with either a Hewlett Packard 5830A or a 5840A gas chromatograph (flame ionization detector, FID), using large bore wall-coated stainless steel open tubular columns (152 m × 0.76 mm i.d.) coated with methyl silicone oil (SF-96(50)) containing 5% Igepal CO-880.

Component identifications are based upon GC–mass spectrometric (GC–MS) data obtained with a quadrupole type mass spectrometer (Electronic Associates Quad 300 mass filter; Finnigan Corporation 3000-1B electronics) connected via a silicone rubber membrane type separator to the gas chromatograph. Tentative identifications were always substantiated by GC relative retention time determinations for each component. Using a standard temperature program sequence (25 °C initial temperature;

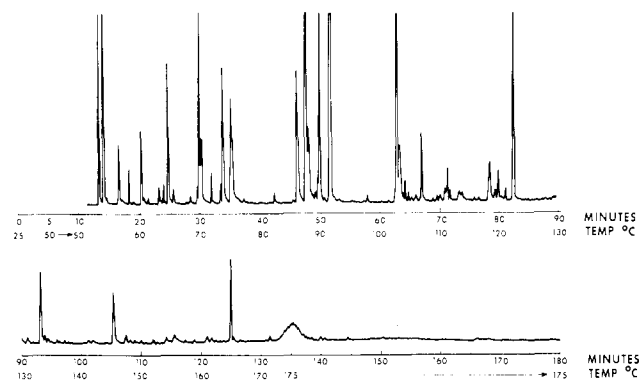


Figure 1. Chromatogram of Vanguard–El Capitan corn silk volatiles. Wall-coated stainless steel open-tubular column (152 m × 0.76 mm i.d.) coated with methyl silicone oil (SF 96(50)) containing 5% Igepal CO-880. Temperature-programmed: 25 °C starting temperature; 5 °C/min to 50 °C; 5 min isothermal; 1 °C/min to 175 °C; 60 min final isothermal period. The broad baseline displacement at 135 min is a column artifact and does not represent a corn silk component.

programmed at 5 °C/min to 50 °C; held at 50 °C for 5 min; programmed at 1 °C/min to 175 °C; held at 175 °C for 60 min); experimental retention indices were determined for each peak of interest in the concentrate chromatogram, relative to the homologous series of normal hydrocarbons from C₆ to C₁₆, which was co-injected. The experimental retention index for an authentic sample of each component tentatively identified from mass spectrometric data was similarly determined, and the appropriate indices were compared for confirmation of the identification.

Area percent values from the GC integration are used without correction for FID response variation (Flath and Takahashi, 1978).

Bioassay. Corn silk concentrate solutions, either in freshly distilled ether or in purified *n*-pentane containing 1% paraffin wax, were applied to an inert substrate—either fluted filter paper disks or clean cheesecloth rectangles which were subsequently wrapped around yellow paper cylinders. The solution concentrations were adjusted to yield concentrations of corn silk volatiles on the substrates in the range of 1–100 ppm, after solvent evaporation. Suitable blanks were prepared as well, using solvent alone. The substrates were suspended in Plexiglas chambers containing female *H. zea* moths reared to the egg-laying stage. The number of samples, including blank, submitted to the moths at one time ranged from two to seven. After exposure for a period of time, typically 48 h, the number of eggs laid upon each of the different sample and blank substrates was counted.

RESULTS AND DISCUSSION

Nonacidic components identified in the Vanguard–El Capitan commercially grown corn silk are listed by compound type in Table I. The retention time values refer to the chromatogram in Figure 1. The sixty-three corn silk volatiles represent approximately 80% of the total chromatogram area. The area percent values in Table I are only provided as a guide to the make-up of a typical corn silk concentrate. The other concentrates were found to be qualitatively quite similar, with some quantitative variation.

Alcohols are by far the major group of compounds found. Several homologous series are present, as well as several branched primary alcohols and a number of unsaturated compounds. 2-Heptanol is present at an unusually high level, comprising 18% of the total corn silk volatiles (0.42 ppm in the silk). 1-Hexanol, a much more common major

Table I. Volatile Corn Silk Components (Nonacidic)

alcohols		2,4-hexadienal (<i>t,t</i> -?)	52.50 (tr)
ethanol	13.13 ^a (3) ^b	<i>trans</i> -2-heptenal	58.10 (tr)
1-propanol	16.49 (1)	2,4-decadienal (<i>t,c</i> -?)	108.85 (tr)
1-butanol	23.26 (tr) ^c	2,4-decadienal (<i>t,t</i> -?)	111.91 (tr)
1-pentanol	33.60 (tr)	esters	
1-hexanol	47.87 (12)	ethyl acetate	18.27 (tr)
1-heptanol	63.03 (6)	γ -nonalactone	117.26 (tr)
1-octanol	78.25 (tr)	ethyl cinnamate	131.34 (tr)
1-nonanol	93.17 (2)	diethyl <i>o</i> -phthalate	147.50 (tr)
1-decanol	107.36 (tr)	ethers	
2-pentanol	25.68 (tr)	2-pentylfuran	64.99 (tr)
2-hexanol	37.41 (tr)	1,8-cineole	71.25 (tr)
2-heptanol	51.98 (18)	(diethyl ether, solvent)	13.87
2-octanol	67.17 (1)	hydrocarbons and aromatics	
2-nonanol	82.51 (5)	limonene	71.52 (1)
2-decanol	97.16 (tr)	<i>p</i> -xylene	45.80 (tr)
2-undecanol	111.21 (tr)	<i>o</i> -xylene	49.75 (tr)
<i>trans</i> -2-hexen-1-ol	48.94 (tr)	1,2,4-trimethylbenzene	65.46 (tr)
<i>trans</i> -2-octen-1-ol	78.51 (1)	1,2,3-trimethylbenzene	69.60 (tr)
<i>trans</i> -3-hexen-1-ol	45.71 (tr)	1,2-dimethyl-4-ethylbenzene	78.20 (tr)
<i>cis</i> -3-hexen-1-ol	46.39 (4)	1,3-dimethyl-4-ethylbenzene	79.43 (tr)
2-methylpropan-1-ol	20.26 (1)	naphthalene	94.37 (tr)
3-methylbutan-1-ol	30.04 (4)	2-methylnaphthalene	109.96 (tr)
2-methylbutan-1-ol	30.39 (2)	1-methylnaphthalene	112.14 (tr)
2-ethylhexan-1-ol	71.88 (tr)	biphenyl	120.85 (tr)
4-hepten-2-ol	50.13 (5)	fluorene	148.60 (tr)
1-penten-2-ol	24.72 (tr)	ketones	
1-hexen-3-ol	35.36 (4)	3-pentanone	24.07 (tr)
1-octen-3-ol	63.80 (tr)	2-methylpentan-3-one	29.75 (tr)
phenethyl alcohol	88.78 (tr)	3,5-octadien-2-one (isomer A)	76.00 (tr)
α -terpineol	95.89 (tr)	2-nonanone	79.43 (tr)
geraniol	105.29 (2)	3,5-octadien-2-one (isomer B)	79.65 (tr)
geosmin	124.85 (2)	β -ionone	133.92 (1)
aldehydes		miscellaneous	
2-methylbutanal	21.43 (tr)	pyrrole	40.01 (tr)
<i>trans</i> -2-hexenal	42.53 (tr)	(chloroform, solvent)	19.01

^a Retention time in minutes, Figure 1. ^b Area percent of component peak, Figure 1; uncorrected for FID response variation. ^c "Tr" signifies an area percent of less than 1.

plant volatile, is present at the 12% level in the concentrate. 2-Ethylhexanol may be a contaminant; exposure of starting material to items containing the common plasticizer dioctyl phthalate might easily introduce small quantities of the alcohol. Such exposure to potential contaminants is always possible when the material passes through commercial distribution channels before purchase. Two other alcohols identified are of particular interest: The first of these is 4-hepten-2-ol, first identified by Buttery (1977) in other portions of the corn plant. The other is geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol), which has been identified in numerous actinomycetes cultures (Gerber, 1968), water supplies (Rosen et al., 1970), red table beets (Acree et al., 1976; Murray et al., 1975), dried beans (Buttery et al., 1976), and soil (Buttery and Garibaldi, 1976). The presence of this compound was strongly suggested by the earthy odor encountered during the preparation of the corn silk concentrate. The only higher plant-related occurrences reported to date for this compound have been in red beets and as an off-odor source in dried beans. The reason for the presence in corn silk is not immediately apparent. The geosmin concentration was found to vary among the different corn varieties as well. In the Vanguard-El Capitan silk, geosmin's concentration is approximately 44 ppb, assuming complete transfer into the volatiles concentrate. GC integration indicates that the Style-Pak silk contains 11 ppb, while the Golden Jubilee has only 1.5 ppb. These last two values are of interest because the two varieties were grown under essentially identical conditions in the same field at the same time. This variation was apparent on smelling the corn silk itself before concentrate preparation. Both the commercial and the Style-Pak silks had pronounced earthy

aromas, while that of the Golden Jubilee silk was a rather nondescript "green", with no noticeable earthy character.

Aldehydic material was found at low concentrations. *trans*-2-Hexenal is very common in disrupted plant tissue, so its appearance is not unexpected. MS evidence for a second 2,4-hexadienal isomer was noted at 53.31 min, but no suitable reference compound could be located. It might be noted that the number and concentrations of aldehydes differ considerably from the situation encountered when corn husk volatiles are concentrated (Buttery, 1977).

Only trace amounts of a few esters were detected. The presence of diethyl *o*-phthalate is somewhat suspicious, because of its widespread use as a plasticizer.

Two compounds with ether linkages were identified, both of which have been frequently identified in plant material. A component at 108.17 min has a mass spectrum (MS) nearly identical with that of estragole (*p*-allylanisole), but its retention time is 13 min too long.

Limonene is the only terpene hydrocarbon clearly present, although MS data suggest the presence of a trace amount of α -muurolene at 137.49 min. No authentic sample was available for verification. Similarly, a sesquiterpene hydrocarbon with a MS resembling that of δ -cadinene is eluted at 139.91 min (trace), but authentic δ -cadinene has a retention time 15 min longer. As usual, a rather diverse group of aromatic components was encountered at low concentration levels. At least seven additional substituted benzenes and naphthalenes are indicated by MS data, but identification was limited by lack of appropriate reference compounds.

Besides the six ketones listed, at least two others appear to be present, both eight-carbon unsaturated compounds. The first of these, at 69.79 min, has a mass spectrum

similar to that of 4,6-octadien-3-one (Buttery, 1978), but sufficient differences exist to make the assignment doubtful. The other, at 71.06 min, appears to be a monounsaturated compound, possibly 2-octen-4-one.

Pyrrole is the only nitrogen-containing constituent fully identified. A peak at 120.85 min contains two compounds, one of which is definitely biphenyl. When the biphenyl MS is subtracted from the combined MS, a MS identical with that of indole remains. However, indole's retention time is approximately 3 min longer. The *m/e* intensities for 116, 117, and 118 are 11, 100, and 11%, respectively, ruling out the related benzyl cyanide and isocyanide, as well as the methyl cyano- and isocyanobenzenes, which typically have 116 intensities equal to 40–80% of the 117 intensity. The preparation of isoindole has recently been reported (Bonnett et al., 1973), but besides showing considerably lower stability than indole, it reportedly has an *m/e* 118 intensity that is 26% of the 117 intensity.

Preliminary bioassay results were largely inconclusive. Some indication of female moth preference for corn silk concentrate during oviposition was noted on occasion, but responses were inconsistent. Future testing design will include correction of several problem areas. The number of samples, including blanks, will be reduced to two, or at most three. The closed chamber, which rapidly became permeated with the silk volatiles, will be replaced with a larger laminar flow chamber, and the question of insect food preference "imprinting" during larval growth stages will be considered.

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Overcrowding Factors of Mosquito Larvae. 11. Biological Activity of 2-Halooctadecanoic Acids and Alkyl 2-Halooctadecanoates against Mosquito Larvae

Yih-Shen Hwang,* H. A. Navvab-Gojrati,¹ and Mir S. Mulla

As part of our studies on the synthesis of active analogues of the overcrowding factors of mosquito larvae, 2-chloro-, 2-bromo-, and 2-iodooctadecanoic acids and their methyl, ethyl, and isopropyl esters were synthesized and evaluated for their larvicidal activity against first instars of the southern house mosquito, *Culex pipiens quinquefasciatus* Say. Based on the evaluation, the structure-activity relationship of the compounds was investigated. 2-Bromooctadecanoic acid and its methyl, ethyl, and isopropyl esters were the most active larvicides over 2-chloro- and 2-iodooctadecanoic acids and their esters. The activity of the 2-halogenated carboxylic acids and esters, except for the 2-bromo analogues, generally declined in the order of acids, methyl, ethyl, and isopropyl esters. The activity did not have clear-cut relationships with the van der Waals radii and electronegativities of the halogen atoms attached to the acids and esters.

In our search for finding active analogues of the overcrowding factors of mosquito larvae, we previously reported the synthesis and structure-activity relationship of 2-alkylalkanoic acids and 3-methylalkanoic acids and their

esters against first-instar larvae of the southern house mosquito *Culex pipiens quinquefasciatus* Say (Hwang, 1976; Hwang et al., 1974a,b, 1976, 1978). These studies revealed that alkanolic acids and esters with certain main-chain lengths and with an ethyl, butyl, or hexyl group at the C-2 position or a methyl group at the C-3 position in the carbon chain manifested a high level of larvicidal activity (Hwang and Mulla, 1976a).

In investigating bromine analogues of the overcrowding factors, we found that 2-bromoalkanoic acids from C-14

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