Identification and Bioactivity of Volatile Allelochemicals from Amaranth Residues

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Volatile organic compounds emitted from the residues of the aerial portions of *Amaranthus retroflexus*, *Amaranthus hybridus*, *Amaranthus cruentus*, *Amaranthus spinosus*, *Amaranthus hypochondriacus*, and *Amaranthus palmeri* have been identified by capillary gas chromatography/mass spectrometry after thermal desorption from Tenax and Carbotrap. Seed germination bioassays using carrot, tomato, and onion were conducted at a vapor concentration of 34.4 μmol/L with 31 of the identified compounds. While all the compounds were inhibitory, the most bioactive toward one or more of the test species were 3-methyl-1-butanol, 3-hexen-1-ol, 2-heptanol, pentanal, 2-methylbutanal, 3-methylbutanal, ethyl propionate, ethyl butyrate, ethyl isobutyrate, ethyl 2-methylbutyrate, 2-pentanone, 3-pentanone, 3-methyl-2-butanone, 2-heptanone, and 2-nonanone. The results suggest that these volatiles, usually categorized as components of flavor, fragrance, and/or insect attractant mixtures, are also important factors in allelopathy and soil chemical ecology.

The first recognized volatile chemical inhibitors of plant growth and development were ethylene, hydrogen cyanide, ammonia, mustard oils, essential oils, acetaldehyde, and benzaldehyde (Evenari, 1949). Evidence of phytotoxic volatile emissions from several plant species or their residues has been reported (Heisey and Delwiche, 1983, 1984; Muller et al., 1964; Oleszek, 1987; Patrick, 1971; Vokou and Margaris, 1982); however, the specific bioactive compounds were seldom identified.

Volatile organic compounds have been implicated in the allelopathic effects of residues of Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Bradow and Connick, 1987; Menges, 1987, 1988). Connick et al. (1987) identified many of the volatiles associated with *Amaranthus palmeri* residues and found that 2-heptanone and 2-heptanol were highly bioactive. Bradow and Connick (1988a,b) investigated the inhibitory effects of ketones, alcohols, and other volatiles emitted by *A. palmeri* residues on the germination of onion, carrot, tomato, *A. palmeri*, and other crop and weed seeds. Flath et al. (1984) examined fresh *Amaranthus retroflexus* and found that the headspace volatile profile was strongly affected by the treatment received by the plant tissue, and the chemical composition changed significantly with sample storage time.

Connick et al. (1987) used packed-column gas chromatography/mass spectrometry (GC/MS) to qualitatively analyze *A. palmeri* residue volatiles. For the present study, a more efficient capillary GC/MS method was used to identify volatiles associated with residues of weedy (*A. retroflexus*, *Amaranthus spinosus*, *A. palmeri*) and crop (*Amaranthus hypochondriacus*, *Amaranthus cruentus*, *Amaranthus hybridus*) amaranth species. Bioassays and statistical analyses were extended to include previously untested compounds, and we report on the bioactivity of all of the identified compounds, in the vapor state, on the germination of carrot, onion, and tomato seeds.

MATERIALS AND METHODS

Amaranth Samples. *A. palmeri*, *A. hybridus*, *A. cruentus*, *A. spinosus*, and *A. hypocondriacus* were grown in small outdoor plots in New Orleans between March and October 1986, and the aerial parts were harvested postflower anthesis, but prior to seed dispersal. Mature *A. retroflexus* plants, freshly cut and shipped frozen by overnight carrier, were provided by Dr. A. D. Worsham, North Carolina State University, Raleigh. Aerial plant parts (leaves, stems, flowers) were cut into 0.5–2.5-cm pieces and placed in the laboratory to air-dry. Representative samples of the air-dried residues were taken after 1 and 15 days for analysis.

Volatiles Trapping Procedure. Volatiles from amaranth residues were collected as previously described (Connick et al., 1987). A sample of amaranth (80 g if air-dried for 1 day or 20 g if dried 15 days) was placed in a 1.2-L glass bottle with Teflon inlet and outlet valves (Kontes No. K-323255). Ultrapurified water (10 mL, Water-1 apparatus, Barnstead) was added, the valves were closed, the bottle was wrapped in aluminum foil to exclude light, and the sample was incubated in a cycle of 10 h at 31 °C and 14 h at 21 °C for 3 days. A glass tube (84-mm length × 9-mm o.d. × 1-mm wall thickness) packed with 0.10 g of Tenax GC (60/80-mesh) porous polymer or 0.30 g of Carbotrap (20/40-mesh; Supelco) graphitized carbon black and preconditioned at 220 °C (24 h, 15 mL/min He) was attached to the exit valve of the sample bottle, and the headspace atmosphere was swept through the tube by purified nitrogen (20 min, 100 mL/min). Tenax and Carbotrap have a low affinity for water, but organic volatiles are efficiently trapped by these solid adsorbents. Volatiles from each amaranth residue (air-dried for 1 or 15 days prior to wetting) were collected on one Carbotrap and two Tenax tubes. Most samples were analyzed the day they were collected; for delayed analyses, the tubes were sealed, kept in a freezer, and analyzed within 3 days. Blanks or controls that were run with only 10 mL of water in the sample bottle gave insignificant background peaks.

GC/MS Analysis. Amaranth residue volatiles were thermally desorbed from sample tubes in an external, closed inlet system (Scientific Instrument Service, River Ridge, LA) interfaced with GC/MS instruments as previously described (Legendre et al., 1979). A Perkin-Elmer Sigma 300 GC/Finnigan MAT ion trap detector (ITD), Model 700, unit as well as a Finnigan MAT GC/MS/DS Model 4000 quadrupole instrument were used to separate and identify the volatile compounds. The capillary GC column in each instrument was 50 m × 0.31 mm (i.d.) coated with a 0.52-μm film of cross-linked 5% phenyl methyl silicone (Hewlett-Packard Ultra-2).

Volatiles were desorbed from a sample tube at 200 °C with a helium flow of 10 mL/min for 3 min onto the head of the capillary column, which was held at −30 °C by dry ice in a wire basket that was placed in the column oven (Dupuy et al., 1985). The injector valve was then switched to the vent position, and the oven temperature was held at −30 °C for 3 min, raised at 6 °C/min to 30 °C, held for 5 min, and raised at 3 °C/min to 250 °C. The GC/ITD helium gas pressure was 12 psi, and the split ratio was 25:1; 5:1 with the Model 4000. The ITD (Version 2.0 software; transfer line at 250 °C) was set for full scan (33–250 amu), 1-s scan time, 10^5 gain plus 200 V, 70-min acquisition. The Model 4000 was run generally as described by Dupuy et al. (1985) except for using the same temperature program as the GC/ITD unit and a 60-min acquisition time.

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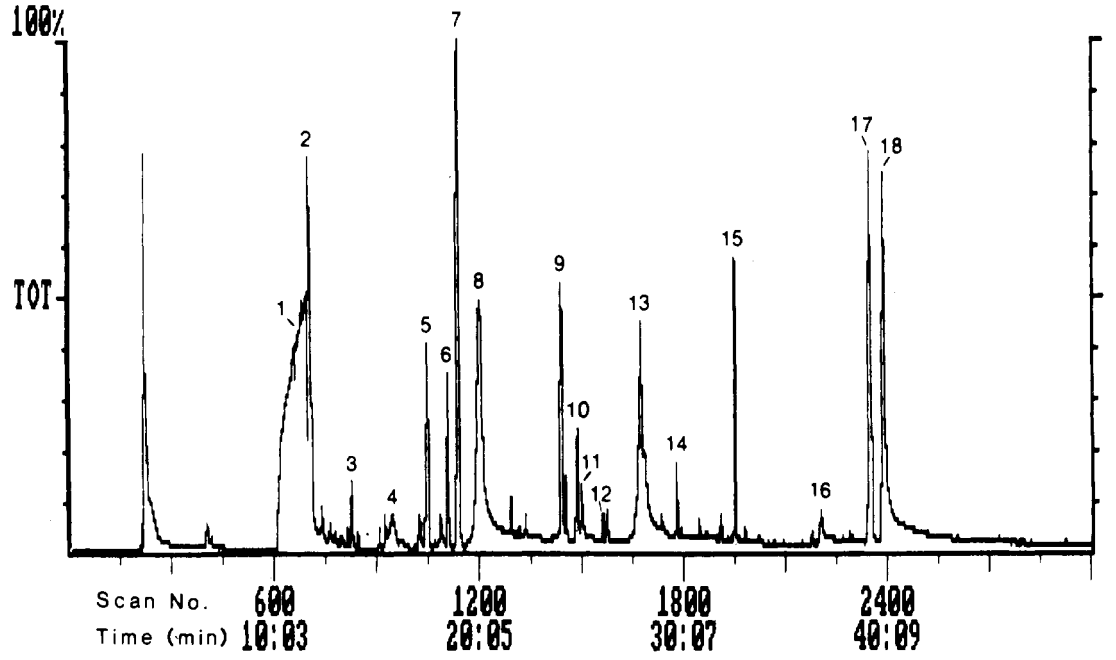

Figure 1. Total ion current capillary GC/ITD chromatogram of Carbotrap-isolated, thermally desorbed volatiles collected from the headspace of 1-day dried *A. cruentus* residue, with representative compounds designated: (1) ethanol, (2) pentane (usually present in residueless controls), (3) methyl acetate, (4) 1-propanol, (5) 2-butanone, (6) 2-methylfuran, (7) ethyl acetate, (8) 2-methyl-1-propanol, (9) 2-pentanone, (10) 3-pentanone, (11) 2-ethylfuran, (12) ethyl propionate, (13) 3-methyl-1-butanol, (14) ethyl isobutyrate, (15) octane, (16) 3-hexen-1-ol, (17) 2-heptanone, and (18) 2-heptanol. Refer to the text for a description of thermal desorption and GC conditions.

All identified compounds from the amaranth residues had been run as authentic samples on the GC/ITD under the same conditions as the unknowns. Their mass spectra were stored in a user-generated library that could be searched rapidly. A Tenax and a Carbotrap tube containing the volatiles collected for each amaranth sample were analyzed with the GC/ITD unit; the remaining Tenax tube was analyzed with the Model 4000 quadrupole instrument. Compounds were considered to be positively identified if their retention times and mass spectra closely matched those obtained for authentic compounds (Aldrich Chemical Co.). An identification in at least two of the three sample tube runs was further required for a compound to be listed as an amaranth residue volatile.

Seed Germination Bioassays. Germination of seeds of carrot, *Daucus carota* L., cv. Danvers Half-Long (Burpee, Warminster, PA); tomato, *Lycopersicon esculentum* Mill., cv. Homestead (Carolina Biological, Burlington, NC); and onion, *Allium cepa* L., cv. Texas Early Grano 502 (Baxter's, Weslaco, TX) were used to compare the bioactivities of each identified amaranth residue volatile with pure water (Water-1) controls. The procedure followed was previously described (Connick et al., 1987). Briefly, a 10-mL glass beaker containing 8.6×10^{-5} mol of authentic compound was placed on a bed of 50 g of pure sand contained in a crystallizing dish. The sand had been moistened with 10 mL of pure water. The dish and contents were placed in the bottom of a 2.5-L desiccator (160-mm i.d.).

Seeds (8 20-seed segments) of a single test species were placed on a porcelain desiccator plate covered with filter paper saturated with pure water. Control tests lacked only the beaker with the volatile organic compound and were repeated at 3-month intervals throughout the duration of the experiment. Desiccators were sealed, wrapped with aluminum foil, and incubated for 3 days under the conditions already described for the plant samples in the volatiles sampling procedure. Because all of the compounds vaporized completely during the incubation period, they were in fact bioassayed in the vapor state and not as aqueous solutions. Radicle protrusion was the criterion for germination.

Statistical Analysis. After normalization by the transformation $[(X + 0.5)^{1/2}]$, germination count data from the 3-day bioassays, 16 replicates, were compared separately for each seed species. One-way analyses of variance were used to examine the differences between the effects of the volatile compounds and the water controls after 3 days, and significant differences between the treatment were determined with Tukey's honestly significant difference procedure with a significance level $P = 0.01$ (Sokal and Rohlf, 1981).

RESULTS AND DISCUSSION

Organic volatiles were trapped for qualitative analysis at room temperature on Tenax and Carbotrap adsorbents by an inherently mild headspace method that concentrated the compounds, minimized the appearance of artifacts, and increased the probability that detected compounds were emitted by amaranth residues in nature (Murray, 1977). The headspace concentration of each component depended on its vapor pressure, air–water partition coefficient, and its adsorption on glass and on the plant residue sample (Buttery et al., 1969, Schreier, 1984). Tenax and Carbotrap performed about equally well, although Carbotrap occasionally gave sharper alcohol peaks. Figure 1 shows a typical chromatogram obtained with the GC/ITD. Incubation of undecomposed residue samples in the presence of water helps to liberate volatiles (Connick et al., 1987; Beute and Rodriguez-Kabana, 1979). Only aerial plant part residues, by far the largest portion of amaranth biomass, were included in this study; however, amaranth roots are also a rich source of volatiles (Connick et al., 1987).

The volatile organic compounds emitted by amaranth residues and their seed germination inhibition activities after a 3-day exposure are listed in Table I. The compounds are principally alcohols, aldehydes, esters, hydrocarbons (including terpenes), ketones, and furans. Small amounts of pentane, acetone, carbon disulfide, methylene chloride, chloroform, hexane, benzene, toluene, and styrene were often found in water-only controls that were handled and analyzed like plant samples, so these compounds are not listed as amaranth residue volatiles.

Alcohols. A variety of C_2–C_7 alcohols (primary, secondary, saturated, unsaturated) were identified. Ethanol, 2-methyl-1-propanol, 3-methyl-1-butanol, and 3-hexen-1-ol [probably the cis isomer (Flath et al., 1984)] were found in all amaranth species tested. 2-Heptanol was a relatively large peak in the chromatograms of all the species except

Table I. Volatiles Emitted by Amaranth Residues and Their Germination Inhibition Activity

compound	rel[a] ret time	amaranth species[b]						germin inhibn[c]		
		palmeri	*hybridus*	*cruentus*	*spinosus*	*hypochondriacus*	*retroflexus*	carrot	tomato	onion
Alcohols										
ethanol	0.282	X	X	X	X	X	X	NS	NS	S
1-propanol	0.412		X	X	X	X	X	S	S	S
2-propanol	0.321	T[d]	T	T[d]	T[d]	X[d]		S	S	S
2-butanol	0.471	T[d]	T	T		X	X	S	S	S
2-methyl-1-propanol	0.518	X[d]	X	X	X	X	X	S	S	S
2-pentanol	0.898		X		T			NS	S	S
3-methyl-1-butanol	0.719	X	X	X	X	X	X	*S	S	*S
3-hexen-1-ol[f]	0.931	X	X	X[e]	X[e]	X	X[e]	*S	*S	(*Z*) *S, (*E*) S
Aldehydes										
pentanal	0.631					X		S	S	*S
2-methylbutanal	0.566	X[e]	T[e]		T[e]	X[e]	X	S	*S	S
3-methylbutanal	0.552	T[e]	T[e]	X[e]	X[e]	X	X	*S	*S	*S
Esters										
methyl acetate	0.345	X	X	X	X	X	X	S	S	S
ethyl acetate	0.487	X	X	X	X	X	X	S	NS	S
methyl propionate	0.512		T							
ethyl propionate	0.667	T[e]	T	T	T	T[e]	T	*S	NS	S
ethyl butyrate	0.842		T		T[e]	T[e]	X	*S	S	S
ethyl isobutyrate	0.763	X[e]	X	X[e]	X[e]	X[e]	X	S	*S	S
ethyl 2-methylbutyrate	0.928	T[e]	T[e]	T[e]		T[e]	X[e]	S	*S	S
Hydrocarbons										
heptane	0.638	T[e]	X	T	T[e]	X	X	S	S	S
octane	0.833	X	X	X	X	X	X	NS	S	S
nonane	1.013	X	T	X	T	X	X	NS	NS	S
α-pinene	1.080		T[e]	T		T[e]		NS	S	S
terpene, MW 136	1.183	T[e]	T	T[e]			T			
terpene, MW 136	1.333	T[e]	T[e]	T[e]	T[e]	X[e]	X[e]			
Ketones										
2-butanone	0.451	X	X	X	X	X	X	NS	S	S
3-hydroxy-2-butanone	0.738		X	X	X[e]		X	NS	S	S
2-pentanone	0.621	X	X	X	X	X	X	S	*S	S
3-pentanone	0.633	X	X	X	X	X	X	NS	*S	S
3-methyl-2-butanone	0.719				T	X		NS	*S	S
2-heptanone	1.000	X	X	X	X	X	X	*S	*S	*S
2-nonanone	1.325			T[e]		X[e]	X	*S	*S	S
Miscellaneous										
dimethyl sulfide	0.325	X[d]	X	X	X	X	X			
2-methylfuran	0.457	X	X	X	X	X	X	S	S	S
2-ethylfuran	0.643	X	X	X	X	X	X	NS	S	S

[a] Relative to 2-heptanone (retention time of compound in sec from injection/retention time of 2-heptanone). [b] Compounds that were identified in residue volatiles and were not also found in controls using water as the only sample. Key: X = detected in residues dried 1 and 15 days; T = found in trace amounts. [c] After a 3-day exposure to 34.4 μmol/L; mean value of 16 replications; 99% confidence level. Key: S = significant inhibition compared with the control; *S = >90% inhibition; NS = not significant. [d] Detected only in residues that were dried 15 days. [e] Detected only in residues that were dried 1 day. [f] Specific isomer (*Z* or *E*) was not determined.

A. retroflexus where it was not detected. 3-Hexen-1-ol was present only in 1-day dried samples of *A. cruentus*, *A. spinosus*, and *A. retroflexus*. 2-Propanol was usually found only in 15-day dried samples.

These alcohols, except ethanol (onion) and 2-pentanol (carrot), inhibited the germination of all the test seeds. Vapors of 3-methyl-1-butanol, 3-hexen-1-ol, and 2-heptanol were particularly effective allelochemicals. The inhibitory properties of some of these alcohols have been described and discussed in detail in an earlier paper (Bradow and Connick, 1988b).

Aldehydes. Three C_5 aldehydes were detected. Pentanal was found only in volatiles from *A. hypochondriacus*, the species richest in aldehydes. 2-Methylbutanal and 3-methylbutanal (found in all species) were usually detected only in 1-day dried samples. Vapors of each of these aldehydes inhibited germination of the three test seeds; 3-methylbutanal was consistently the most potent inhibitor. Acetaldehyde may have been present, but the GC method used does not retain it long enough to allow positive identification.

Esters. Among the group of esters identified, methyl acetate, ethyl acetate, ethyl propionate (trace levels), and ethyl isobutyrate were found in all the amaranths. Methyl propionate and higher molecular weight esters were usually detected in trace amounts and/or in 1-day dried samples only. Ethyl propionate and ethyl butyrate were very inhibitory of carrot germination, and ethyl isobutyrate and ethyl 2-methylbutyrate severely inhibited tomato seeds. No ester was highly inhibitory of onion or of more than one test seed species.

Hydrocarbons. Heptane, octane, and nonane were present as components of all the amaranth headspace volatiles. The terpenes found were α-pinene and two other monoterpenes with the empirical formula $C_{10}H_{16}$. They were usually detected in trace amounts in 1-day dried samples. Only the unidentified terpene with a 1.333 relative (to 2-heptanone) retention index was associated with all the amaranth species. In general, the hydrocarbons were least inhibitory of carrot and most inhibitory of onion germination.

Ketones. The C_4–C_9 ketones that were identified are

all 2-alkanones (methyl ketones), except 3-pentanone. All the amaranths tested emitted 2-butanone, 2-pentanone, 3-pentanone, and 2-heptanone. 2-Heptanone vapors were the most inhibitory of all the test seeds. Carrot was least affected by the ketones of five carbons or less; tomato was the most affected. The inhibitory properties of these ketone volatiles in time- and concentration-dependent bioassays have been reported in detail (Bradow and Connick, 1988a).

Miscellaneous Compounds. Dimethyl sulfide, 2-methylfuran, and 2-ethylfuran were readily detected in all the amaranth species. Only the two furans were bioassayed, and their vapors were generally allelopathic.

Amaranth samples that were dried 15 days usually gave smaller and fewer peaks in their chromatograms compared with the much fresher 1-day dried samples. The amaranths that showed surprisingly little (less than about 10%) drop in volatiles concentration over this time period were *A. cruentus*, *A. hypochondriacus*, and *A. retroflexus*.

The 8.6×10^{-5} mol/2.5 L or 34.4 μmol/L concentrations chosen for the seed germination bioassays were sufficient to indicate which volatile compounds had inhibitory properties and are comparable with the concentration of other common, bioactive volatiles naturally occurring in soil. To achieve a concentration of 34.4 μmol/L of the highly active and ubiquitous 2-heptanone, for example, 12 μL of neat liquid was placed in the beaker within the 2.5-L desiccator. Upon complete evaporation, the 2-heptanone vapor concentration was 4.9 ppm by volume and occupied 0.08% of the desiccator atmosphere (25 °C, 760 mm). In comparison, the carbon dioxide concentration in soil is usually 0.15–0.65%, and soil ethylene can reach 5 ppm in early spring (Russell, 1973). Significant inhibition of seed germination by some of the ketones and alcohols listed in Table I has been reported at concentrations as low as 3.4 μmol/L; furthermore, the effects are additive (Bradow and Connick, 1988a,b).

Most of the rather common volatile organic compounds associated with amaranth residues that were identified and shown to have allelopathic properties in this study have long been recognized as components of flavor, fragrance, and insect attractant mixtures (Buttery et al., 1978; Chen et al., 1982; Flath et al., 1984; Idstein et al., 1984; Maga, 1978; Pyysalo et al., 1977; Schreier, 1984). 2-Heptanone and other flavor-related volatiles had been reported to produce inhibitory and/or stimulatory responses in a variety of weed seeds (French and Leather, 1979).

On the basis of the widespread occurrence in nature of many of the volatiles released by amaranth residues, and their ability to inhibit seed germination and cotton radicle elongation (Bradow and Connick, 1988c) at relatively low concentrations, we believe that these volatiles play an important, but insufficiently studied, role in allelopathy and soil chemical ecology. We strongly recommend that volatile emissions be included in the characterizing bioassays whenever a plant or its residue is suspected of having allelopathic properties.

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Registry No. Ethanol, 64-17-5; 1-propanol, 71-23-8; 2-propanol, 67-63-0; 2-butanol, 78-92-2; 2-methyl-1-propanol, 78-83-1; 2-pentanol, 6032-29-7; 3-methyl-1-butanol, 123-51-3; 3-hexen-1-ol, 544-12-7; 2-heptanol, 543-49-7; pentanal, 110-62-3; 2-methylbutanal, 96-17-3; 3-methylbutanal, 590-86-3; methyl acetate, 79-20-9; ethyl acetate, 141-78-6; methyl propionate, 554-12-1; ethyl propionate, 105-37-3; ethyl butyrate, 105-54-4; ethyl isobutyrate, 97-62-1; ethyl 2-methylbutyrate, 7452-79-1; heptane, 142-82-5; octane, 111-65-9; nonane, 111-84-2; α-pinene, 80-56-8; 2-butanone, 78-93-3; 3-hydroxy-2-butanone, 513-86-0; 2-pentanone, 107-87-9; 3-pentanone, 96-22-0; 3-methyl-2-butanone, 563-80-4; 2-heptanone, 110-43-0; 2-nonanone, 821-55-6; dimethyl sulfide, 75-18-3; 2-methylfuran, 534-22-5; 2-ethylfuran, 3208-16-0.

LITERATURE CITED

Beute, M. K.; Rodriguez-Kabana, R. Effect of Volatile Compounds from Remoistened Plant Tissues on Growth and Germination of Sclerotia of *Sclerotium rolfsii*. *Phytopathology* **1979**, *69*, 802–805.

Bradow, J. M.; Connick, W. J., Jr. Allelochemicals from Palmer Amaranth, *Amaranthus palmeri* S. Wats. *J. Chem. Ecol.* **1987**, *13*, 185–202.

Bradow, J. M.; Connick, W. J., Jr. Volatile Methyl Ketone Seed-Germination Inhibitors from *Amaranthus palmeri* S. Wats. Residues. *J. Chem. Ecol.* **1988a**, *14*, 1617–1631.

Bradow, J. M.; Connick, W. J., Jr. Seed-Germination Inhibition by Volatile Alcohols and Other Compounds Associated with *Amaranthus palmeri* Residues. *J. Chem. Ecol.* **1988b**, *14*, 1633–1648.

Bradow, J. M.; Connick, W. J., Jr. Inhibition of Cotton Seedling Root Growth by Rhizosphere Volatiles. *Proc. Beltwide Cotton Prod. Res. Conf.* **1988c**, 90–91.

Buttery, R. G.; Ling, L. C.; Guadagni, D. G. Volatilities of Aldehydes, Ketones, and Esters in Dilute Water Solution. *J. Agric. Food Chem.* **1969**, *17*, 385–389.

Buttery, R. G.; Ling, L. C.; Chan B. G. Volatiles of Corn Kernels and Husks: Possible Corn Ear Worm Attractants. *J. Agric. Food Chem.* **1978**, *26*, 866–869.

Chen, C.-C.; Kuo, M.-C.; Hwang, L. S.; Wu, J. S.-B.; Wu, C.-M. Headspace Components of Passion Fruit Juice. *J. Agric. Food. Chem.* **1982**, *30*, 1211–1215.

Connick, W. J., Jr.; Bradow, J. M.; Legendre, M. G.; Vail, S. L.; Menges, R. M. Identification of Volatile Allelochemicals from *Amaranthus palmeri* S. Wats. *J. Chem. Ecol.* **1987**, *13*, 463–472.

Dupuy, H. P.; Flick, G. J.; Bailey, M. E.; St. Angelo, A. J.; Legendre, M. G.; Sumrell, G. Direct Sampling Capillary Gas Chromatography of Volatiles in Vegetable Oils. *JAOCS, J. Am. Oil. Chem. Soc.* **1985**, *62*, 1690–1693.

Evenari, M. Germination Inhibitors. *Bot. Rev.* **1949**, *15*, 153–194.

Flath, R. A.; Altieri, M. A.; Mon, T. R. Volatile Constituents of *Amaranthus retroflexus* L. *J. Agric. Food Chem.* **1984**, *32*, 92–94.

French, R. C.; Leather, G. R. Screening of Nonanal and Related Volatile Flavor Compounds on the Germination of 18 Species of Weed Seed. *J. Agric. Food. Chem.* **1979**, *27*, 828–832.

Heisey, R. M.; Delwiche, C. C. A Survey of California Plants for Water-Extractable and Volatile Inhibitors. *Bot. Gazz.* **1983**, *144*, 382–390.

Heisey, R. M.; Delwiche , C. C. Phytotoxic Volatiles from *Tricostema lanceolatum* (Labiatae). *Am. J. Bot.* **1984**, *71*, 821–828.

Idstein, H.; Herres, W.; Schreier, P. High-Resolution Gas Chromatography-Mass Spectrometry and -Fourier Transform Infrared Analysis of Cherimoya (*Annona cherimolia*, Mill.) Volatiles. *J. Agric. Food Chem.* **1984**, *32*, 383–389.

Legendre, M. G.; Fisher, G. S.; Schuller, W. H.; Dupuy, H. P.; Rayner, E. T. Novel Technique for the Analysis of Volatiles in Aqueous and Nonaqueous Systems. *J. Am. Oil Chem. Soc.* **1979**, *56*, 552–555.

Maga, J. A. Cereal Volatiles, A Review. *J. Agric. Food. Chem.* **1978**, *26*, 175–178.

Menges, R. M. Allelopathic Effects of Palmer Amaranth (*Amaranthus palmeri*) and Other Plant Residues in Soil. *Weed Sci.* **1987**, *35*, 339–347.

Menges, R. M. Allelopathic Effects of Palmer Amaranth (*Amaranthus palmeri*) on Seedling Growth. *Weed Sci.* **1988**, *36*, 325–328.

Muller, C. H.; Muller, W. H.; Haines, B. L. Volatile Growth Inhibitors Produced by Aromatic Shrubs. *Science (Washington D.C.)* **1964**, *143*, 471–473.

Murray, K. E. Concentration of Headspace, Airborne and Aqueous Volatiles on Chromosorb 105 for Examination by Gas Chro-

matography and Gas Chromatography-Mass Spectrometry. *J. Chromatogr.* **1977**, *135*, 49-60.
Oleszek, W. Allelopathic Effects of Volatiles from Some Cruciferae Species on Lettuce, Barnyard Grass and Wheat Growth. *Plant Soil* **1987**, *102*, 271-273.
Patrick, Z. A. Phytotoxic Substances Associated with the Decomposition in Soil of Plant Residues. *Soil Sci.* **1971**, *3*, 13-18.
Pyysalo, T.; Suihko, M.; Honkanen, E. Odor Thresholds of the Major Volatiles Identified in Cloudberry (*Rubus chamaemorus* L.) and Arctic Bramble (*Rubus arcticus* L.). *Lebensm. Wiss. Technol.* **1977**, *10*, 36-39.
Russell, E. W. *Soil Conditions and Plant Growth*, 10th ed.; Longman: London, 1973; pp 411, 678.
Schreier, P. *Chromatographic Studies of Biogenesis of Plant Volatiles*; Huthig: New York, 1984; p 4.
Sokal, R. R.; Rohlf, F. J. *Biometry*, 2nd ed.; W. H. Freeman: San Francisco, 1981; pp 208-270, 417-428.
Vokou, D.; Margaris, N. S. Volatile Oils as Allelopathic Agents. In *Aromatic Plants: Basic and Applied Aspects*; Margaris, N., Koedam, A., Vokou, D., Eds.; Martinus Nijhoff: Boston, 1982; pp 59-72.

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Effects of Sex, Age, and Diet on the Triacylglycerol Fatty Acid Composition of Subtropical Boll Weevils, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae)

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Six fatty acids accounted for ca. 96% of the total triacylglycerol acids of subtropical adult boll weevils (*Anthonomus grandis* Boheman). In general, females had larger amounts of the major acids, but proportions of each were similar in both sexes. Oleic and palmitic acids were the most abundant in weevils, regardless of sex, age, and diet. Oleic/palmitic acid ratios were consistently greater in boll-fed than in square-fed weevils, regardless of sex or age. Of the C_{18} fatty acids, the more saturated ones (stearic and oleic) were more abundant in boll-fed than square-fed weevils, and the less saturated ones (linoleic and linolenic) were more abundant in square-fed than boll-fed weevils. Total fatty acid amounts were small the first 4 days after pupal eclosion and then increased with age in square-fed weevils; amounts fluctuated greatly with age in boll-fed weevils. Few qualitative differences between subtropical weevils and those of more northern latitudes were proven.

Triacylglycerols (triglycerides) function in storage and liberation of metabolic energy (Downer, 1985) and are the dominant lipid class in animal fat. Lambremont and Blum (1963) found 23 fatty acids in the body fat of boll weevils, *Anthonomus grandis* Boheman, from Louisiana. Lambremont et al. (1964) reported that eight major acids from the triacylglycerol fraction account for ca. 98% of the total fatty acids in these weevils. However, it has been reported that adult boll weevils from the Rio Grande Valley of Texas are biochemically (Keeley et al., 1977; Guerra et al., 1983) and physiologically (Guerra et al., 1982) different from weevils of more northern latitudes. Guerra and Garcia (1982), Guerra et al. (1984), and Guerra (1986) also concluded that subtropical (Brownsville, TX) and tropical (Tapachula, Mexico) weevils can remain physiologically active and reproductive throughout the year as long as temperatures are mild and host plants are available. Alternatively, they may remain in a "quiescent" metabolic state encapsulated inside dry cotton bolls. For these reasons, we hypothesized that the composition of triacylglycerols from subtropical boll weevils may also be different from that of weevils of more northern origins. In this work, we investigated the effects of sex, diet, and age on the triacylglycerol fatty acids of adult boll weevils from the Lower Rio Grande Valley of Texas. This if the first report of triacylglycerol fatty acids from subtropical boll weevils reared on cotton squares and bolls.

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MATERIALS AND METHODS

The effects of sex, diet, and age on the fatty acid composition of subtropical boll weevils were determined by analyzing the triacylglycerol fatty acids of male and females 1, 2, 3, 4, 7, 14, 21, and 28 days after pupal emergence and fed as adults either cotton squares or bolls.

Weevils from infested cotton fruit were collected during the normal cotton season (June–July) in a field near Mercedes, TX. Squares (buds) and bolls were kept separately in outdoor screen-covered 30 × 30 × 30 cm wooden cages protected from rain. After emergence, adults were put in mixed-sex, same-age groups (ca. 40 insects each) in wide-mouth 1000-mL plastic containers with cheesecloth covers. Adults emerging from squares or bolls were fed squares or bolls, respectively, and were kept outside the laboratory but protected from rain. Weevils reaching their test age were sexed (Little and Martin, 1942; Agee, 1964), weighed (wet), and individually triturated in a 2-mL Teflon capsule containing a plastic cylindrical pestle and 0.5 mL of a 2:1 chloroform–methanol extracting solvent (Folch et al., 1957). The trituration materials and procedures were the same as those described by Albach and Guerra (1984), except that the capsule was shaken only once for 30 s and the contents were decanted into a glass vial without centrifugation. The capsule was rinsed twice with 0.5 mL of the extracting solvent. Extracts were stored at −20 °C until analysis. Triacylglycerols were isolated by a two-step procedure. First, they were separated from more polar lipids with a silica SEP-PAK cartridge (Waters Associates, Milford, MA). Extract was loaded onto a hexane-solvated cartridge, and nonpolar and moderately polar lipids including triacylglycerols were eluted with 10 mL of 1% isopropyl alcohol in hexane. Eluant was filtered through a 0.5-μm Millex-SR filter