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Sweetpotato Volatile Chemistry in Relation to Sweetpotato Weevil (*Cylas formicarius*) Behavior

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Abstract. **The sweetpotato weevil (SPW) [*Cylas formicarius elegantulus* (Summers) (Coleoptera: Curculionidae)] is the single most devastating pest of the sweetpotato [*Ipomoea batatas* (L.) Lam.] worldwide. Attempts to develop host-plant resistance have been only moderately successful due in part to deficiencies in parent and progeny selection methods. Host-plant phytochemicals play critical roles in insect behavior, modulating a cross-section of key behavioral decisions. Thus, identification of the phytochemicals the female weevil uses in decision making could greatly facilitate development of host-plant resistance. The volatile chemistry of the sweetpotato was studied in relation to the host-finding behavior of the female weevil. Critical biologically active volatiles were determined via isolation (Tenax trapping), fractionation (gas chromatography–thermal conductivity detector), identification (gas chromatography and gas chromatography–mass spectroscopy), and bioassay (olfactometry). Differences in volatile chemistry among sweetpotato clones that may relate to differences in resistance or susceptibility to the female SPW were assessed. Volatile extracts from storage roots (site of oviposition) and aerial plant parts were attractive to female SPW, the former being substantially greater. In total, 33 compounds were identified from storage roots and aerial plant parts, including 23 terpenes. Three oxygenated monoterpenes (nerol, Z-citral, and methyl geranate), found in storage roots but not aerial plant parts, were identified as attractants. The sesquiterpene volatile fraction was repellent to female SPW with α-gurjunene, α-humulene, and ylangene active in the concentration range emanating from storage roots. The aerial plant parts emanated a higher composite concentration of sesquiterpenes than storage roots. Differences in the relative attraction among four sweetpotato cultivars to female SPW was inversely correlated with the composite concentration of headspace sesquiterpenes. Selection of clones with decreased volatile attractants and/or increased deterrents using an analytical means of quantification may significantly facilitate developing resistance to the SPW.**

Biologically active volatile phytochemicals represent an important means of communication within plants and between plants and other organisms in their environment (Charron et al., 1995). Plant volatiles, for example, inhibit growth of certain fungi (Song et al., 1996) and bacteria (Deng et al., 1993), and inhibit gemination of seeds (Gardner et al., 1990) and pollen (Hamilton-Kemp et al., 1991). Phytochemicals also play critical roles in insect behavior, modulating host finding, feeding, and oviposition responses for certain species where they may function as attractants, repellents, feeding and oviposition stimuli, deterrents, and toxicants (Metcalf and Metcalf, 1992; Tumlinson, 1988). The interaction between plants and insects is further complicated by the fact that volatile compounds with opposing functions may emanate from a single plant. For example, *trans*-2-nonenal emanating from carrot (*Daucus carota* L.) is a repellent, and at a sufficiently high concentration, an insecticide for the carrot fly (*Psila rosae* F.) (Chamberlain et al., 1991; Guerin and Ryan, 1980). However, bornyl acetate, biphenyl, α-ionone, β-ionone, and 2,4-dimethylstyrene also emanating from the carrot, act as attractants (Guerin and Ryan 1984; Ryan and Guerin, 1982). In some instances, three trophic levels may respond to airborne chemical information. For example, *Nicotiana attenuata* Torr. ex Wats plants under attack by several herbivores release phytochemicals that attract natural predators of the insects, which can reduce the herbivore population up to 90% (Kessler and Baldwin, 2001). Due to their role in behavioral decisions of some insect pests, plant volatiles may represent a possible component in developing host-plant resistance or in other control strategies (e.g., trapping).

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The sweetpotato weevil (SPW) (*Cylas formicarius elegantulus*) is the single most devastating insect pest of the sweetpotato (*Ipomoea batatas*) worldwide, causing damage both in the field and during storage (Sutherland, 1986). The oligophagous SPW consumes most parts of the sweetpotato, while eggs are laid mainly in the basal stem and storage roots (Cockerham et al., 1954). Even very low pre- or postharvest infestations reduce quality and marketable yield (Proshold, 1983). In addition, several extremely bitter tasting and toxic furanoterpenoids are synthesized by the storage root in response to feeding by weevil larvae which render the commodity unfit for consumption (Uritani et al., 1975).

Initial work on volatile phytochemicals emanating from the sweetpotato stemmed from the finding that the SPW could readily orient in the dark to sweetpotato plant parts placed in their vicinity (Nottingham et al., 1989a; Starr et al., 1991). The females are attracted by the volatiles emanating from the leaves and storage roots. In contrast, males are attracted by leaf volatiles but little attracted by those emanating from the storage roots (Nottingham et al., 1989a). This suggested that there are qualitative and/or quantitative differences in the volatile compounds between aerial plant parts and the storage roots. In addition to within plant differences, the degree of attraction is known to vary among cultivars (Nottingham et al., 1989a) and species of *Ipomoea* L. (Starr et al., 1991).

Existing management strategies for the SPW do not provide adequate control (Talekar, 1994) and attempts to develop host-plant resistance have not been successful. To further our understanding of the phytochemicals the female SPW uses in decision making, we studied the volatile phytochemistry of the sweetpotato, the role these compounds play in the behavior of the female SPW, and whether differences in these compounds among clones might account in part for differences in resistance/susceptibility to the SPW.

Materials and Methods

Plant materials. Sweetpotatoes were grown in 1999 at the University of Georgia Horticultural Farm, Athens, (Cecil sandy clay loam soil), using standard sweetpotato production practices (Granberry et al., 1990). Two cultivars susceptible to the SPW, Jewel and Centennial, (Mullen et al., 1981) and two moderately resistant cultivars, Resisto (Jones et al., 1983) and Regal (Jones et al., 1985), were used. Prior to harvest in the fall, medium sized roots (≈100 g) were removed from plants of each cultivar as were vines with leaves, between 0900 and 1000 HR on the day of analysis. The cut ends of aerial plant parts were placed in a test tube of distilled water immediately after cutting so that the leaves remained turgid through the test period. Roots were washed, blotted dry using paper towels, and transported in brown paper bags to the laboratory.

Collection of headspace volatiles. A Tenax trapping system was developed for the collection of headspace volatiles. Air flow through a glass container holding the plant material was generated using a vacuum pump (Airchek Sampler, model 224-44XR; Eighty Four, Pa.) with the flow rate monitored and regulated using a flow meter. An air flow rate of ≈1000 mL·min^{-1} was used which minimized condensation and provided an adequate turnover rate in the chamber. Inlet air was passed through a charcoal filter (Alltech Assoc., Inc., Deerfield, Ill.) (1 cm i.d., 5 cm long Pyrex glass tube with a 3 cm bed of charcoal). Before use, the charcoal filter was cleaned and activated at 200 °C for 24 h by passing purified N_2 (2 mL·min^{-1}) through the trap. The trap was oriented in a vertical position to prevent formation of channels that might allow unfiltered air to pass. An 8-L glass chamber (a modified desiccator) containing 20 storage roots was placed in a water bath (30 °C). All connections in the volatile collection system were ground glass joints or Teflon connectors. Before each collection series, the entire apparatus (e.g., chamber, filter, trapping tubes, and connectors) was washed with deionized water followed by acetone, and was subsequently dried at 100 °C for 24 h.

Volatiles were collected using a Tenax trap (Tenax-TA, 60/80; Alltech Assoc., Inc.) prepared by packing 50 mg of the adsorbent in a Pyrex glass tube (3.5 mm i.d. × 5 cm long), giving a bed length of 3 cm. Silylated glass wool was used to contain the adsorbent at each end. The Tenax was washed with purified hexane (≈300 mL·g^{-1} Tenax), redried with N_2, washed with purified methanol (≈300 mL·g^{-1} Tenax), dried with purified N_2 at 20 mL·min^{-1}, and then heated to 330 °C and held at that temperature for 2 h followed by cooling to 21 °C under continuous N_2 flow. At the end of each volatile sampling period, water retained in the trap was removed by purging with purified N_2 at 40 mL·min^{-1} until dry.

Using a Tenax trap, volatiles from each cultivar were collected from freshly harvested storage roots (20 roots of ≈100 g each) and aerial plant parts (four apical vines with leaves, ≈50 g) for 24 h. The cut ends of aerial plant parts were wrapped in moist cotton which maintained the turgidity of the samples throughout the collection period.

Trapped volatiles were eluted from the Tenax with 1 mL of hexane. Biphenyl (13.3 µg/10 µL hexane) was added as the internal standard for subsequent analyses. The eluted solvent was concentrated to 200 µL by slowly flushing it with purified N_2. During initial experiments, two collection traps were connected in series and subsequently analyzed for breakthrough (loss of volatiles due to insufficient collection by the first trap). Under the conditions used, breakthrough was not a factor.

Analyses of collected volatile Compounds. Volatiles were analyzed using a gas chromatograph (GC) (model 5890; Hewlett Packard, Avendale, Pa.) with a split-splitless injection port temperature of 225 °C and flame ionization detector (FID) temperature of 250 °C. One microliter of extract was injected and separated using a 30 m (0.32 mm i.d. fused silica column coated with DB-5 (0.25-µm-thick bonded stationary phase) (J&W Scientific, Folsom, Calif.). The samples were injected in the splitless mode (50 °C) with a purge time of 0.5 min and held at that temperature for 2 min, after which the oven temperature was increased to 150 °C at 3 °C·min^{-1}, held at that temperature for 1 min, then increased to 235 °C at 15 °C·min^{-1} and held at that temperature for 1 min. The carrier gas was helium at a flow rate of 1.7 mL·min^{-1}. The chromatograph was connected to a HP 3396 Series II integrator to quantify the peak areas.

Fractionation of root volatile extracts. Fractionation of volatile compounds in the extract for bioassaying was accomplished using the same GC column and conditions, but with the column attached to a thermal conductivity detector (TCD). Eight Tenax traps were used to trap the volatiles from different parts of the chromatogram as they exited the detector. The traps were connected to the exit port with a short segment of Teflon tubing. Each trap was inserted through a snugly fitting copper coil through which ice water was circulated to maximize the trapping efficiency of the hot gases emerging from the detector. Trapped volatiles were removed using 1 mL of purified hexane and sealed in small glass vials for subsequent bioassays. Ten-microliter aliquots of the initial volatile sample from storage roots of 'Jewel' were fractionated and collected for bioassaying.

Gas chromatography (GC)–mass spectroscopy (MS) identification of volatile compounds. Samples were analyzed using a HP mass spectrometer (MS 5970) interfaced with a HP 5890 GC. The GC column used was a 30 m [0.25 mm i.d. fused silica column coated with EC-5 (equivalent to DB-5)]. GC and temperature program conditions were as described previously. The GC–MS was programmed with a 3 min solvent delay. Electron ionization was used. MS conditions were: ion source 200 °C; electron energy 70 eV; multiplier voltage 220 V; GC–MS interface zone 300 °C. Ions from 20 to 550 atomic mass units were monitored. The identities of volatile compounds were based on the Wiley Spectral Library (6th ed.) and the National Institute of Standards 75 K library and, when possible, confirmed using authentic standards.

Insect rearing. SPWs were reared on storage roots at 28 °C and 65% relative humidity (RH). Emerging weevils were kept in cages and transferred to fresh roots weekly. Storage roots from the previous week were incubated to provide the next generation of weevils. A cross-section of roots of the cultivars were used as the food source. Female weevils were used in the bioassays when they were 3 weeks old, following the method of Nottingham et al. (1989a).

Bioassay of volatile extracts, fractions, and chemicals. An 8 mm i.d. Y-shaped glass tube olfactometer was constructed with 15 cm long arms at a 70° angle from the 20 cm long stem. A low-flow-rate air delivery system, similar to that described by Todd et al. (1977), was used to provide a flow rate of 20 ± 0.5 mL·min^{-1} into each of the two arms of the Y-tube. The volatile extracts, fractions thereof, and authentic standards in hexane were warmed to 21 °C and applied to small 2 cm^2 strips of Whatman No. 1 filter paper. An equivalent volume of solvent was used as the control. Air-dried treated filter papers were folded lengthwise in a W shape and then inserted into the connectors between the air delivery tubes and the ends of two arms of the Y-tube. Female weevils, held without food for 24 h, were placed inside the base of the Y-tube (starting position) with the apparatus placed horizontally on a bench within a con-

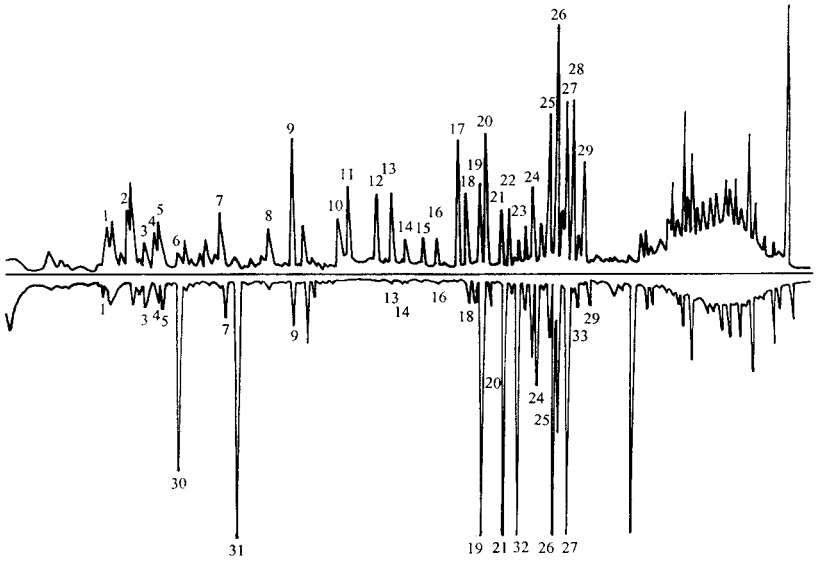

Fig. 1. Total ion chromatograms of the volatile extracts of storage roots (**top**) and aerial plant parts (**bottom**) of 'Jewel' sweetpotato (peak numbers correspond to compounds listed in Table 1).

trolled environmental chamber. The time interval was recorded from when the insect left the start position until reaching the end of one of the arms. Upon moving into the air stream, the female weevil selected one or the other arm of the Y-tube based upon volatile chemistry of the air. A (+) was scored for selection of the extract and a (–) for the control. If the weevil did not reach the end of either tube within 10 min, a zero was recorded. Each volatile sample involved 20 individual female weevils, tested singly. After 10 insects, the treatment and control arms were switched and 10 additional insects were tested using freshly prepared filter papers. Ten runs took <1 h and there was no indication of decreasing attractiveness of the extracts over this period. Between experiments (e.g., 20 runs), the Y-tube and connectors were dismantled and washed with a detergent solution, rinsed with deionized water

Table 1. Volatile phytochemicals emanating from storage roots and aerial plant parts of four sweetpotato cultivars over 24 h (μg·kg^{-1} fresh weight).

		Storage roots				Aerial parts
No.	Volatile compound	'Resisto'	'Regal'	'Centennial'	'Jewel'	'Jewel'
1	1,2-Dimethylbenzene[z]	0.45 ± 0.04[y]	0.55 ± 0.04	0.33 ± 0.03	0.40 ± 0.02	1.50 ± 0.10
2	6-Methyl-5-hepten-2-one	0.63 ± 0.03	0.54 ± 0.05	0.61 ± 0.03	0.55 ± 0.05	ND[x]
3	1,4-Dichlorobenzene[z]	0.22 ± 0.02	0.39 ± 0.06	0.13 ± 0.03	0.15 ± 0.04	5.76 ± 1.09
4	p-Cymene	0.23 ± 0.03	0.32 ± 0.02	0.55 ± 0.04	0.35 ± 0.02	1.20 ± 0.38
5	dl-Limonene	0.47 ± 0.03	0.73 ± 0.03	0.44 ± 0.01	0.48 ± 0.03	2.91 ± 0.72
6	Diethylbenzene	0.04 ± 0.02	0.07 ± 0.01	0.16 ± 0.03	0.12 ± 0.05	ND
7	Undecane	0.47 ± 0.03	0.52 ± 0.03	0.61 ± 0.03	0.59 ± 0.03	2.44 ± 0.53
8	Neroloxide	0.23 ± 0.03	0.34 ± 0.03	0.50 ± 0.04	0.23 ± 0.02	ND
9	Naphthalene	0.54 ± 0.03	0.43 ± 0.06	0.51 ± 0.06	0.41 ± 0.06	2.04 ± 0.05
10	Nerol	0.22 ± 0.01	0.35 ± 0.04	0.52 ± 0.07	0.43 ± 0.04	ND
11	Z-citral	0.70 ± 0.06	0.71 ± 0.08	0.53 ± 0.08	0.68 ± 0.08	ND
12	E-citral	0.36 ± 0.03	0.35 ± 0.02	0.55 ± 0.04	0.39 ± 0.07	ND
13	1-Methylnaphthalene	0.33 ± 0.02	0.41 ± 0.05	0.27 ± 0.04	0.17 ± 0.07	0.69 ± 0.04
14	2-Methylnaphthalene	0.05 ± 0.01	0.05 ± 0.01	0.02 ± 0.01	0.08 ± 0.02	0.35 ± 0.03
15	Methylgeranate	0.04 ± 0.01	0.05 ± 0.03	0.02 ± 0.02	0.06 ± 0.02	ND
16	Alkazene	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
17	δ-Cadinene[z]	2.22 ± 0.51	0.66 ± 0.06	2.26 ± 0.36	0.41 ± 0.09	ND
18	α-Copaene	1.32 ± 0.44	0.47 ± 0.03	1.16 ± 0.18	0.20 ± 0.04	0.48 ± 0.04
19	β-Elemene	0.42 ± 0.04	0.24 ± 0.02	1.17 ± 0.17	0.19 ± 0.10	6.57 ± 1.08
20	Cyperene[z]	0.69 ± 0.23	0.44 ± 0.02	0.42 ± 0.05	0.50 ± 0.05	0.54 ± 0.02
21	*trans*-Caryophyllene	2.48 ± 0.50	0.57 ± 0.04	0.36 ± 0.05	0.23 ± 0.03	15.07 ± 1.02
22	Germacrene D[z]	2.37 ± 0.52	1.01 ± 0.30	0.35 ± 0.06	0.23 ± 0.03	0.48 ± 0.05
23	α-Humulene	2.72 ± 0.49	0.56 ± 0.18	0.34 ± 0.04	0.23 ± 0.10	ND
24	cis-α-Bisabolene	2.31 ± 0.18	1.49 ± 0.41	0.78 ± 0.08	0.30 ± 0.01	0.78 ± 0.05
25	Ylangene	0.70 ± 0.23	0.44 ± 0.02	0.52 ± 0.05	0.50 ± 0.04	0.54 ± 0.02
26	β-Selinene[z]	2.83 ± 0.74	1.50 ± 0.56	1.89 ± 0.69	1.04 ± 0.20	1.37 ± 0.25
27	α-Muurolene	0.65 ± 0.07	1.22 ± 0.21	1.12 ± 0.24	0.61 ± 0.16	6.22 ± 0.29
28	α-Gurjunene	4.44 ± 0.60	1.49 ± 0.43	1.27 ± 0.24	0.67 ± 0.06	ND
29	1S, cis-calamenene[z]	1.68 ± 0.23	0.39 ± 0.07	0.92 ± 0.09	0.59 ± 0.06	0.96 ± 0.38
30	β-Ocimene[z]					18.34 ± 1.94
31	(E)-4,8-dimethyl-1,3,7-nonatriene[z]					29.90 ± 2.44
32	Zingiberene[z]					7.14 ± 1.04
33	(E)-β-farnesene[z]					4.46 ± 0.83

[z]Tentatively identified by mass spectroscopy.
[y]Mean ± SD, n = 3.
[x]ND = not detected by gas chromatography.

Table 2. Sweetpotato weevil (SPW) attraction index (AI) of volatiles from the storage roots and aerial plant parts of four sweetpotato cultivars.

Cultivar	Susceptibility to SPW[z]	Root volatiles (AI)[y]	Aerial plant volatiles (AI)[y]
Jewel	Highly susceptible	56.6 a[x] ± 4.2	24.2 a ± 4.5
Centennial	Highly susceptible	31.6 b ± 2.8	19.8 a ± 3.3
Regal	Moderately resistant	35.0 b ± 3.3	18.5 a ± 3.6
Resisto	Moderately resistant	18.3 c ± 2.8	17.7 a ± 3.4

[z]Based on data by Jones et al. (1983, 1985), Mullen et al. (1981), and Nottingham et al. (1989b).
[y]Mean ± SD (n = 3), 20 insects/replication.
[x]Mean separation within columns by Duncan's multiple range test, $P < 0.05$.

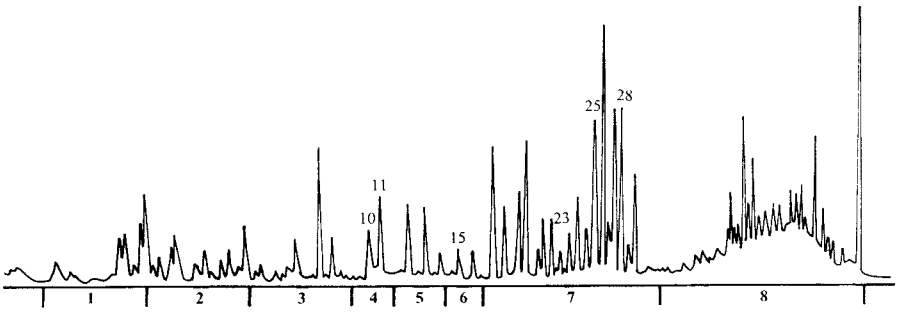

Fig. 2. GC fractionation of storage root volatiles from 'Jewel' sweetpotato into eight subsamples. Peaks labeled are biologically active volatile compounds to female SPW: 10 = nerol, 11 = Z-citral, 15 = methyl geranate, 23 = α-humulene, 25 = ylangene, and 28 = α-gurjunene.

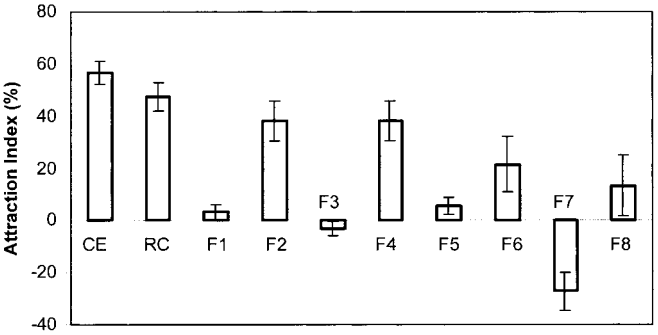

Fig. 3. Attraction index (AI) of GC fractions of storage root volatiles from 'Jewel' sweetpotato (CE = crude extract, RC = recombination of all eight GC fractions, and F1-8 = GC fractions 1 through 8, respectively). Vertical lines = SD (n = 3).

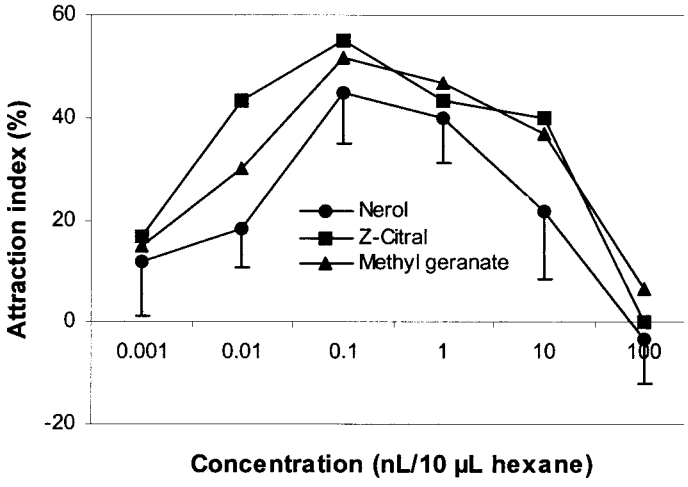

Fig. 4. Effect of concentration on the attraction index (AI) of female sweetpotato weevils to nerol, Z-citral, and methyl geranate. Vertical bars = SD (n = 3).

and then acetone, and subsequently air dried. All experiments were conducted in a controlled environment chamber in white light (cool-white fluorescent bulbs, irradiance 46 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 28 °C and 65% to 75% RH. The Y-tube was carefully positioned using a level in that a slight incline can affect the weevil's behavior. An attraction index (AI) was calculated as (T – C)/20 × 100 for each replication, where T = the number of (+) and, C = the number of (–) scores.

Chemicals. All authentic compounds were purchased from Sigma (St. Louis, Mo.), Fluka (Ronkonkoma, N.Y.), and Pfaltz & Bauer, Inc. (Waterbury, Conn.). Those used for bioassay were purified using the preparative GC method described above and their purities were >99% based capillary GC (FID). A mixture of Z-citral and E-citral from Sigma was fractioned into pure isomers using the preparative GC method.

Statistical analysis. Data were subjected to analysis of variance procedures and means separated by Duncan's multiple range (SAS Inst., Inc. Cary, N.C.)

Results

Volatile compounds emanating from sweetpotato storage roots and aerial plant parts. Representative GC profiles of headspace volatile constituents from 'Jewel' storage roots and aerial plant parts are shown in Fig. 1. The identified compounds and quantitative data from storage roots of the four cultivars and from aerial plant parts of 'Jewel' are listed in Table 1. Compounds identified include hydrocarbons, esters, alcohols, and aldehydes. Among the 33 compounds identified, 23 were terpenes (accounting for ≈70% of the total), while 30 were newly identified from the sweetpotato in this study.

The predominate volatile components were sesquiterpenes which displayed pronounced quantitative differences among cultivars. Four oxygenated monoterpenes [nerol, neroloxide, citral (Z and E) and methyl geranate] were found in relatively low amounts from storage roots of each of the cultivars, but were not detected from aerial plant parts ('Jewel'). Aromatic compounds represented the second most abundant components in the sweetpotato volatiles, including benzene and naphthalene derivatives. No significant differences in aromatic compounds were found among cultivars.

Attractiveness of volatile extracts to female weevils. Ten microliters (comparable to the volatiles emanating from ≈100 g of storage root tissue over 24 h) of Tenax extract (200 µL) from the storage roots and aerial plant parts of 'Jewel' displayed the highest AI in a dilution series (i.e., 1, 10, and 100 µL) test. Subsequent tests used 10-µL aliquots for bioassay and GC fractionation.

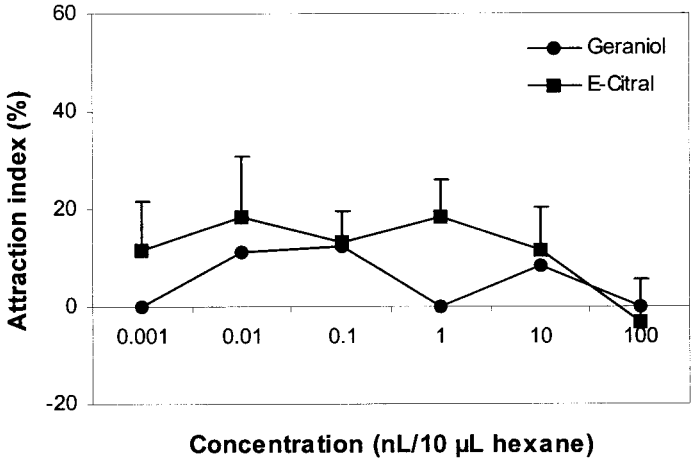

Fig. 5. Effect of concentration on the attraction index (AI) of female sweetpotato weevils to volatile geraniol and E-citral, geometric isomers of nerol and Z-citral, respectively. Vertical bars = SD (n = 3).

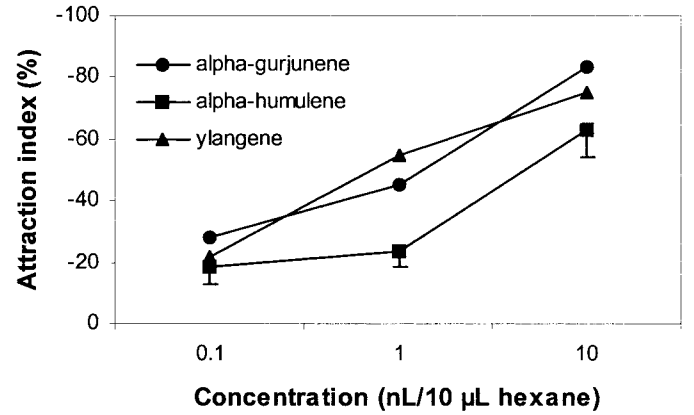

Fig. 6. Dosage responses of the attraction index (AI) of female sweetpotato weevils to the sesquiterpenes α-gurjunene, α-humulene, and ylangene. Vertical bars = SD (n = 3).

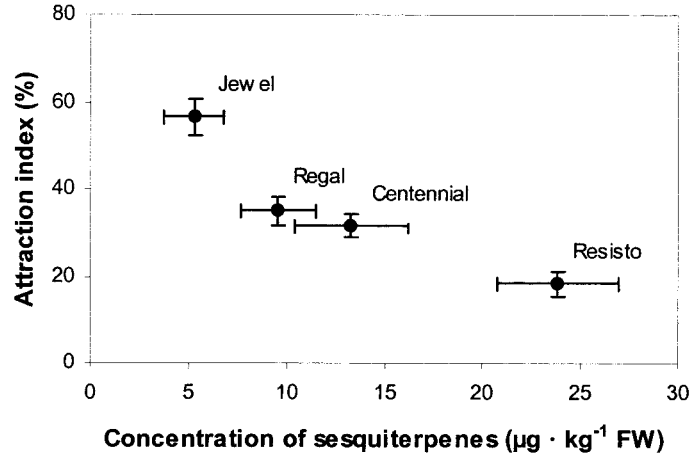

Fig. 7. Relationship between the attraction index (AI) of sweetpotato root extracts and the composite concentration of volatile sesquiterpenes from four sweetpotato cultivars. Vertical and horizontal bars = SD (n = 3).

All of the volatile extracts from storage roots and aerial plant parts of the four cultivars were attractive to female weevils (Table 2). In general, the level of attractiveness of storage root volatiles was higher than volatiles from aerial plant parts. There were no significant differences in the AI among cultivars for aerial plant parts. The volatiles from storage roots of the weevil susceptible 'Jewel' were the most attractive (AI = 56.6), while 'Resisto' volatiles were the least (AI = 18.3) based on resistance estimates by Jones et al. (1983, 1985), Nottingham et al. (1989b), and Mullen et al. (1981). There were no significant differences in attractiveness, however, between 'Centennial' (susceptible) and 'Regal' (moderately resistant) root volatiles. This suggests that other factors, in addition to volatiles, are involved in each cultivar's overall resistance.

Attractiveness of volatile fractions to female weevils. The composite volatile sample was fractionated into eight subsamples using the GC for separation and then retrapping. Fractionation grouped isolates with somewhat similar chemistries (e.g., fraction 4 contained predominately monoterpenes; fraction 7 contained predominately sesquiterpenes) (Fig. 2). Three fractions (2, 4, and 6) were the most attractive to the female SPW. In contrast, fraction 7 was the most repellent to the insects (Fig. 3).

Biologically active volatiles. Two compounds, nerol and Z-citral from fraction 4 and methyl geranate from fraction 6 were found to be attractive to female SPW in a concentration range from 0.01 to 10 nL/10 µL hexane (Fig. 4). Higher or lower concentrations were much lower in attractiveness. Geraniol and E-citral, the geometric isomers of nerol and Z-citral, were attractive to female SPW but to a much lesser degree, indicating a relatively high level of binding site specificity for monoterpenes (Fig. 5). Fraction 2 contained ≈10 compounds of which five were identified. None of the four (obtained commercially) (Figs. 1 and 2), tested singly or in combination, were found to be attractive to female SPW. We were not able to elucidate the structures of other volatiles in fraction 2.

A series of sesquiterpenes were identified in fraction 7 of which commercial standards were obtained for 10. Three (α-gurjunene, α-humulene, and ylangene) were repellent to female SPW at a concentration range of 0.1 to 10 nL/10 µL hexane (Fig. 6). When the total identified sesquiterpene concentration for each of four cultivars was correlated with their respective root volatile extracts' AI, a significant negative relationship was found (Fig. 7). The moderately resistant 'Resisto' emanated ≈5 times the level of α-gurjunene than the more susceptible 'Jewel'.

Discussion

Terpenes, the largest single class of plant chemicals (15,000 to 20,000 have currently been characterized) (Langenheim, 1994), predominate among the secondary metabolites of the sweetpotato. Terpenes play a multitude of important physiological and ecological roles in higher plants ranging from regulating plant growth and acting as accessory pigments in photosynthesis, to attracting insects for pollination and providing effective defense against herbivores and pathogens. We found that sweetpotatoes emanate significant amounts of terpenes in a volatile form and that the storage roots produce quantitatively a lower composite concentration of terpenes on a fresh weight (FW) basis than the aerial plant parts. We also found that storage roots emanated several times more terpene volatiles upon biotic (SPW) and abiotic damage than healthy roots (data not presented).

Terpenes appear to have played a major role in the coevolution of *Ipomoea* and the SPW. For example, the ovipositional stimulant of the female weevil, which is found on the surface of the storage roots, is a pentacyclic triterpene (Son et al., 1989). We likewise have shown previously that the leaves give off several sesquiterpenes (Nottingham et al., 1989a) and weevil larvae within the storage roots induce the synthesis of a series of furanoterpenoids (Uritani et al.,

1975). The close relationship between the weevil and the plant's terpene chemistry suggests coevolution. The importance of terpenes in the interaction between plants and insects is not limited to the Convolvulaceae. Several of the terpenes we have identified as emanating from the sweetpotato are present in other plants and are known to modulate the behavior of insects herbivores. For example, zingiberene found in wild tomato (*Lycopersicon hirsutum f. hirsutum* Humb. and Bonpl.) leaflets is toxic to the Colorado potato beetle (*Leptinotarsa decemlineata* Say) and has been proposed as a possible source of insect resistance for the cultivated tomato (*Lycopersicon esculentum* Mill.) (Carter et al., 1989). Similarly, trans-β-farnesence released from glandular hairs on the foliage of the wild potato (*Solanum berthaultil* Hawkes) repels aphids (*Myzus persicae* Sulzer) (Ave et al., 1987; Gibson and Pickett, 1983) and α-gurjunene, present in the resin of *Dipterocarpus kerrii* King, protects the tree from attack by the termite *Zootermopsis angusticollis* Hagen (Richardson et al., 1989). Lower herbivore damage on legume tree leaves was significantly correlated with higher concentrations of particular components such as caryophyllene (Langenheim et al., 1986) and bisabolene which have been shown to act as an antifeedant for Colorado potato beetle (Gonzalez-Coloma et al., 1995). The homomono-terpene, (E)-4,8-dimethyl-1,3,7-nonatriene, was a rather prominent constituent of aerial plant part volatiles and several plants appear to produce homoterpenes as defensive chemicals (Tollsten and Muller, 1996).

Our results suggest that sesquiterpenes appear to represent one form of resistance of the sweetpotato to the SPW. Varying levels of resistance have been found for sweetpotato genotypes in the field (Mullen et al., 1980a, 1981, 1982; Story et al., 1997; Thompson et al., 1999), and in laboratory bioassays (Mullen et al., 1980b; Nottingham et al., 1987, 1989b). Resistant mechanisms may be due to escape, for example, by having long thin storage roots set deep in the soil and scattered within growing hills (Jayaramaiah, 1975), tolerance (Velusamy and Heinrichs, 1986), antixenosis (nonpreference) (Singer, 1986), antibiosis (Painter, 1951), or a combination of any of these. The latter two types of plant resistance, which involve modification of insect behavior and metabolism, have a chemical basis. Host-plant chemistry can potentially modify any stage of the weevil's behavior from host finding (volatiles) through feeding and oviposition (surface chemicals) to larval development in the roots (internal root chemistry). At least three sesquiterpenes from sweetpotato plants are repellent to SPW and function in the mode of antibiosis. Sesquiterpenes are derived biosynthetically from three isoprene units and share farnesyl pyrophosphate as a common biosynthetic intermediate. There are many more sesquiterpenes known in nature than monoterpenes. The level of chemical diversity in sesquiterpenes, likewise, may impede the weevil's ability to detoxify them.

Coevolution involves an adaptive response by the SPW to members of *Ipomoea* which produce defensive compounds such as terpenes. SPW has evolved to use certain monoterpenes and a triterpene as host-location and oviposition stimulation clues. Metcalf (1987) reported 300 insect species with selected attraction responses to 64 plant volatile compounds. The basis of this relationship lies in the chemoreceptors, located principally on the insect's antennae, which enable them to detect volatiles selectively, thereby triggering such behavioral responses as host finding and oviposition. The present investigation shows that the geometric isomers for two monoterpene attractants identified were significantly less attractive to the SPW. A relatively high level of binding site specificity of the SPW antenna for monoterpenes was apparent. The interaction between plants and insects is typically not modulated by a single compound but by a mixture of volatiles in relative specific proportions. Of the attractants listed by Metcalf (1987), terpenes and aromatic compounds are the most frequently encountered and to a lesser extent alcohols, esters, aldehydes, and acids. Both a single plant attractant (Finch and Skinner, 1982; Hammack, 2001) or a mixture of compounds with synergistic effects (Zhang et al., 1999) have been tested as lures for traps to monitor or manage certain insects. A synthetic female pheromone has already been used as a lure to monitor SPW populations in sweetpotato production fields, but it only attracts males (Heath et al., 1991). The three monoterpenes we have identified might be used as lures for traps for the female SPW if their level of attractiveness is sufficiently high. Furthermore, host-plant volatiles could increase the efficacy of synthetic pheromone traps to catch certain insects (Hardie et al., 1994). The possibility of increasing the attractiveness of the synthetic pheromone to the SPW by adding sweetpotato volatiles warrants further investigation.

Development of insecticide resistance and an increased concern over the potential hazards pesticides pose for the environment increases the urgency of breeding for host plant insect resistance and finding biologically safe control alternatives to conventional pesticides. In developing countries, on the other hand, application of insecticides is neither an economically nor biologically viable way to control SPW. Development of insect-resistant cultivars has been suggested as an essential component in the integrated management of SPW (Martin and Jones, 1986). Currently, sweetpotato breeders select for weevil resistance using insect damage as an index of susceptibility. The behavioral responses of the female weevils, however, are sufficiently complex to mitigate against achieving success. As a consequence, a more pragmatic analytical approach is needed where critical semiochemicals used by the female are identified and assessed analytically, independent of the insect, for making progeny selection decisions. Further simplifying and standardizing the GC procedure for quantifying sesquiterpenes and monoterpenes will make instrumental selection of parent lines and progeny with higher levels of repellents and lower levels of attractants feasible. A cultivar with an agriculturally meaningful level of resistance to the SPW will need multiple forms of resistance. An analytically guided selection approach will allow incorporating multiple forms of resistance simultaneously into new clones, greatly increasing the chances of achieving agricultural useful levels of resistance.

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