Volatiles Involved in the Nonhost Rejection of *Fraxinus pennsylvanica* by *Lymantria dispar* Larvae

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Volatiles from green ash foliage or ambient air was trapped by Super Q and their chemical identities determined by GC—MS analysis. Effects of the whole mixture and individual chemical components of green ash volatiles on gypsy moth larval (GML) locomotory behavior were assayed in a T-tube arena. Green ash volatiles (treatment) proved significantly repellent to GML as compared to Super Q trappings of volatiles from the ambient air (control). Such observed effects were due to the combined repellencies of linalool, methyl salicylate, and farnesenes; however, the relatively major component, *trans*-ocimene, proved to be nonrepellent. The significance of such volatiles in GML orientation and host/nonhost decision is discussed.

Keywords: *Gypsy moth; green ash; repellency; volatiles; chemical identification*

INTRODUCTION

Volatile chemicals are recognized as important messengers in communications between plants, insects, and plants and insects. Volatile molecules, e.g., ethylene (Rhoades, 1983, 1985) and methyl jasmonate (Farmer and Ryan, 1990), from one plant may signal and activate inducible genes in other plants. Volatile signals among insects include messengers between sexes (i.e., a sex pheromone) (Kochansky et al., 1975; Landolt and Heath, 1990), between a parasitoid and its herbivore host (i.e., a parasitoid kairomone) (Turlings and Tumlinson, 1992) as a host plant-dependent parasitoid kairomone released in the feeding host herbivore's feces (Ramachandran et al., 1991), between predator and prey (i.e., as a prey defense) (Eisner et al., 1974; Honda, 1990), or between conspecifics (i.e., as an antiaggregation-epideictic pheromone) (Prokopy, 1981). Volatile phytochemicals involved in plant—insect communications may serve as insect attractants (Meyer and Norris, 1967; Hsiao and Fraenkel, 1968; Buttery et al., 1978, 1982a,b, 1985; Finch, 1978; Visser and Ave, 1978; Visser, 1986; Feeny et al., 1989; Lupoli et al., 1990; Henning et al., 1992), insect repellents and deterrents (Jermy, 1966; Gilbert et al., 1967; Rozental and Norris, 1975; Saxena and Prabha, 1975; Ryan and Guerin, 1982; Salama and Saleh, 1984; Hwang et al., 1985; Khan et al., 1987; Henning et al., 1992) or insect toxicants (Hink et al., 1988; Shaaya et al., 1991).

Plant volatiles have been frequently reported as prominent initial cues that affect adult insect orientation and female oviposition (Gilbert et al., 1967; Meyer and Norris, 1967; Jermy, 1976; Visser and Thiery, 1985; Visser, 1986; Feeny et al., 1989; Khan et al., 1987; Liu et al., 1988, 1989; Norris, 1994). However, the roles of such volatiles in the orientation and host/nonhost decisions of immature insects have been investigated less (Saxena and Prabha, 1975; Ryan and Guerin, 1982); this is especially true for gypsy moth larvae (Meisner and Skatulla, 1975). In the current study we investigated the roles of volatiles in gypsy moth larval (GML) rejection of green ash foliage.

Gypsy moth is a generalist that feeds on more than 300 species of trees and shrubs (Doane and McManus, 1981; Odell et al., 1985). Generalist acceptance and rejection of plants as hosts include complex behavioral processes. Such insects need to reject potentially harmful substances, e.g., repellents, deterrents and/or inhibitors, associated with nonhosts and to be variously excited by a wide range of attractants and feedants in selecting superior hosts.

Gypsy moth adult females are rather nonselective regarding their ovipositional sites (Lance, 1983). Weak ovipositional preference may have resulted partially from selective pressures (e.g., limited locomotion, predation and/or parasitism) on this nonflying adult. Such an ovipositional strategy places major pressures on the first-instar larvae regarding dispersal and host—plant acceptance. Ballooning by larvae is the primary, relatively wind-directed, means of species displacement. Such dispersal initially may seem to reduce the importance of remote cues (e.g., volatiles) in nonhost rejection and host—plant acceptance. Volatiles may, however, be especially advantageous to such dispersal in a dense mixed-species forest where the larva may thus distinguish quickly and remotely unfavorable (nonhost) from favorable (host) trees close to its origin. Such quick and remote recognition of unfavorable cues should allow the larva to expend locomotory energy in directions more likely to bring it to acceptable trees; thus, it may

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conserve more of its available energy for growth, development, and reproduction of its genes.

Our present study showed that green foliage releases volatiles which do serve as chemical cues which elicit a negative chemotaxis by gypsy moth larvae. Such an insect decision avoids the necessity for physical contact with nonacceptable plants. This is the first report on the effects of green ash volatiles on gypsy moth larval behavior.

MATERIALS AND METHODS

Insects. Gypsy moth egg masses were obtained from the Otis Air Force Base rearing facility in Massachusetts. If egg masses were not used immediately upon arrival, they were stored at 10 °C in darkness. In preparation for hatch, egg masses were surface-sterilized for 5 min in a 0.1% sodium hypochlorite solution. After sterilization, egg masses were rinsed for 10 min with distilled water and then dried. Thorough drying of egg masses before their introduction onto diet in the rearing arena significantly decreases the incidence of fungal contamination.

Rearing during this study was conducted on a commercial (ICN) wheat germ-based artificial diet in incubators at 25 ± 1 °C with 15:9 (light/dark) hours. Sterilized individual egg masses were placed aseptically into individual plastic dishes (15.5 × 4 cm) along with a piece of diet (1.5 × 1.0 × 1.0 cm). Sterile technique was employed throughout the study. This rearing method has been standardized for use in the gypsy moth-quarantine facility of the University of Wisconsin (UW), Madison. Thirty-five to forty-five-hour-old first-instar GML were used throughout this study because they are much easier to handle experimentally as compared to 68−72-h-old larvae and proved to be behaviorally responsive to the volatiles. Larvae were uniformly starved for 3 h prior to each bioassay.

Plants. Leaves for the studies were collected from green ash (*Fraxinus pennsylvanica* Marsh) trees from outdoor locations that had not been recently exposed to aerial and/or other kinds of pesticide application and which are relatively protected from excessive pollution. One such location is on the UW campus; it involved five green ash trees. Close proximity of this site to our laboratory was advantageous because fresh leaves are required for trapping leaf volatiles.

Trapping Volatiles. Leaves (100 g) were removed from trees and placed, within minutes, in the modified 1000-mL Erlenmeyer flask reservoir of an all-glass device for vacuum trapping of volatiles from plant tissues (Liu et al., 1988, 1989; Ramachandran et al., 1991). The in-line adsorbent trap consisted of a 0.5-cm-diameter by 9-cm-length Pyrex column packed with 0.30 g of Super Q (Alltech Associates Inc., Deerfield, IL). The adsorbent was conditioned before use by rinsing with 5 mL of HPLC grade hexane, drying at 130 °C for 2 h, and storing in a desiccator under vacuum. The components of the all-glass collecting system were connected by ground-glass joints. The air was first drawn by vacuum through calcium chloride desiccant and then vacuum-filtered through activated charcoal; next, the air was drawn through a side inlet into the modified Erlenmeyer flask reservoir containing the leaves and then sucked up through the Super Q trap. The metered vacuumed air flow through the apparatus was 850−950 mL/min for 24 h at 23 ± 2 °C. Every experiment included simultaneous trappings of volatiles (under the same conditions) from both ambient air (control) and foliage (treatment). After termination of a trapping period, contents of the Super Q trap from treatment and control apparatus were separately placed in 7-mL screw-capped vials and extracted in 3 mL of HPLC grade hexane, with vortexing, for 5 min. The resultant hexane extractables of either treatment or control were filtered through Whatman No. 1 paper and stored at −20 °C in darkness. Prior to chemical analyses both treatment and control hexane extractables were concentrated to 20 μL under a slow stream of nitrogen.

Larval Behavioral Responses to Trapped Volatiles in Hexane in a T-Tube Arena. Behavioral responses of gypsy moth larvae to trapped volatiles in hexane were assayed in a leveled 3-cm-i.d. by 15-cm-long glass T-tube arena (Figure 1), which is open to air movement at both ends of the main tube and has a centered 15-mm i.d. sidewall opening for the central introduction of the insect into the arena (Liu et al., 1988, 1989). The arena was divided into three zones: the 6.6-cm control end, designated zone 1; an 1.8-cm-wide area, centered in the cylindrical arena, designated the neutral zone 2; and the 6.6-cm treatment end, zone 3. A digital stopwatch was used to determine the time that the single larva spent in each zone during 5 min. The opening at each end of the cylindrical arena was covered by either a treatment or control-bearing screened two-piece cap which was constructed from clear rigid acrylic tubing (Throne, 1990; Schwartz and Burkholder, 1991). The inner (base) cylinder of the cap was 2.5 cm long (3.6 cm i.d. × 4.4 cm o.d.), and the outer (cover) cylinder was 1.3 cm long (4.4 cm i.d. × 5.1 cm o.d.). Nitex nylon monofilament mesh screen (Tetko, Inc., Elmsford, NY) was sealed over one end of both the base and the cover with a slurry of glue and acrylic shavings. Treatment consisted of 40 μL of hexane extractables from the Super Q trappings of green ash foliage (or of authentic chemical) which were applied to a 4.25-cm-diameter filter paper disk (Whatman No. 1), whereas 40 μL of hexane extractables from the Super Q trappings of ambient air (or of solvent hexane) served as the control and was applied to a 4.25-cm-diameter filter paper. A filter paper disk bearing either treatment or control was positioned between the screen of inner and outer cylinders of the cap on either end of the cylindrical assay arena. Ten replicate assays were conducted for each treatment. After each replicate assay, the T-tube was turned 180 °C to compensate for any effects of otherwise unrecognized stimuli. Bioassays were conducted at 25 ± 2 °C and in 55 ± 7% relative humidity in uniform white-fluorescent lighting and in still air.

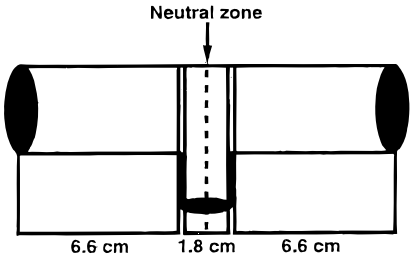

Figure 1. T-tube assay arena. A single starved larva was introduced into the tube through the neutral zone, and its behavior was observed during a 5-min period. Time spent in the neutral zone was subtracted from the summed times (treatment plus control) and was not included in the statistical analysis.

Capillary Gas Chromatography (GC) of Trapped Volatiles. The standardized hexane-extracted, Super Q-trapped volatiles from green ash leaves or control air were analyzed by GC using a Shimadzu GC-14A GC with a DB-17 capillary column (30 m × 0.25 mm, 0.25-μm film thickness, J&W Scientific, Folsom, CA) and a flame ionization detector (FID). The GC was temperature-programmed from 40 °C for 1 min to 220 °C at 5 °C/min. The injector temperature was 220 °C and the detector, 265 °C. A 2-μL aliquot of the extracted volatiles was injected in the splitless mode. Retention time, area, and ratio of each resolved peak area to the total resolved peak area were calculated and recorded by a Shimadzu C-R3A Chromatopac computing integrator.

Capillary Gas Chromatography−Mass Spectrometry (GC−MS) Analysis. Structural identification of the specific chemicals in the biologically active green ash volatiles was determined using a Shimadzu GC-14A GC equipped with a DB-1 capillary column (30 m × 0.25 mm, 0.25-μm film thickness; J&W Scientific) mated via a heated transfer line to a Finnigan MAT ITD 800 series mass spectrometer with ITDS software 4.10. The MS was operated under the electron ionization (EI) mode. A 1-μL aliquot was injected in a split mode using the same temperature program as previously described. The mass spectrum of individual components in the green ash volatiles (or control air components) was compared with mass spectra from reference libraries (i.e.,

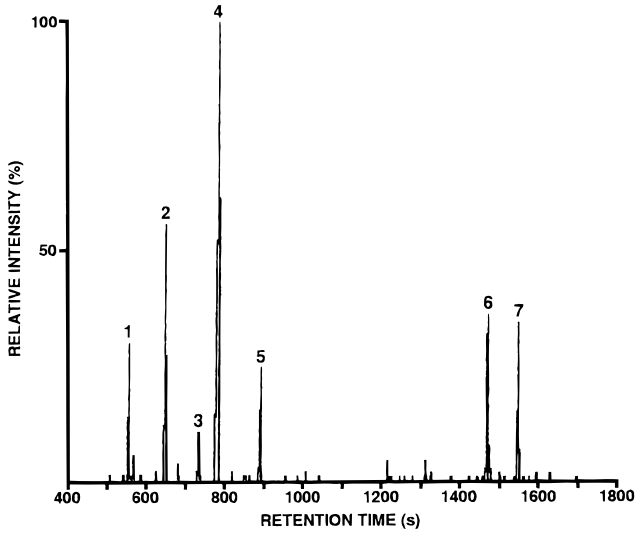

Figure 2. GC−MS resolution of green ash volatiles trapped in June 1995. The chromatogram is a result of one (1-μL) replicate injection conducted in a split mode. Peaks: 1, *cis*-3-hexenyl acetate; 2, *trans*-ocimene; 3, linalool; 4, *trans*-4,8-dimethyl-1,3,7-nonatriene; 5, methyl salicylate; 6, α-farnesene; 7, *trans*-nerolidol.

terpene, NBS, and Wiley 6 libraries) and from authentic compounds. The GC retention time of each resolved volatile compound was further compared with that of the authentic standard on both capillary columns. The authentic chemical was co-injected with the isolated compound on both columns for further confirmation. Co-injection involved a mixture of 1 μL of the authentic chemical with 1 μL of the test (experimental) chemical. The concentration of individual chemicals in a sample was estimated from an experimentally derived regression line relating peak area to known concentrations of the authentic chemical (i.e., by an external standard method).

Authentic Chemicals. Most authentic chemicals were obtained from commercial sources. Linalool, methyl salicylate, and *trans*-nerolidol were obtained from Aldrich Chemical Co. (St. Louis, MO). *cis*-3-Hexenyl acetate was obtained from Sigma Chemical Co. (St. Louis, MO). *trans*-Ocimene was obtained both from the SCM Glidden Durkee Division of the SCM Corp. (Jacksonville, FL) and International Flavors and Fragrances (New York, NY). Farnesene was also obtained from two different commercial sources, TCI America (Portland, OR) and Bedoukian Research (Danbury, CT). *trans*-4,8-Dimethyl-1,3,7-nonatriene was obtained from Dr. John A. Pickett, BEC Department-IACR Rothamsted, United Kingdom. All compounds were purified by GC separation and their identities verified by spectral (i.e., mass spectrometry) and GC retention means.

Statistical Analyses of Data. Insect responses (data) from the studies conducted with the T-tube arena were analyzed using the paired *t*-test. The time (minutes) spent in the treatment zone was subtracted from the time spent in the control zone. A significant difference between treatment and control was determined by using the $P < 0.05$ error limit.

RESULTS

Chemical Analyses of Plant Volatiles. Results of GC−MS analyses of Super Q-trapped green ash volatiles revealed the presence of seven compounds. Those compounds in order of their time of elution on the GC−MS capillary column (i.e., DB-1) were *cis*-3-hexenyl acetate (*cis*-3-HA), *trans*-ocimene, linalool, *trans*-4,8-dimethyl-1,3,7-nonatriene, methyl salicylate (MS), α-farnesene, and *trans*-nerolidol (Figure 2; Tables 2 and 3). Quantitative (i.e., relative quantities of individual peaks) changes were observed over time (e.g., day or month of trapping green ash volatiles).

Larval Behavioral Responses to Trapped Volatiles in Hexane in a T-Tube Arena. When GML were released in the T-tube without the experimental stimulus, they walked the length of the assay arena between the two ends. This resulted in a relatively uniform amount of time spent on either side of the neutral zone. Hexane, the solvent in which the green ash or ambient air volatiles were eluted, did not significantly affect insect behavior when placed alone on both ends of the T-tube arena (Table 1). However, when Super Q trappings of green ash volatiles eluted in 3 mL of hexane and concentrated to 450, 600, and 800 μL were presented to GML, they were significantly repellent ($p = 0.06$; $p = 0.014$; $p = 0.025$; paired *t*-test) as compared to Super Q trappings of volatiles from the ambient air (control). Larvae spent greater time in the control zone (ambient air) of the T-tube and also returned more often to this zone from the neutral zone (Table 1). The difference between the summed times (treatment plus control) shown in Table 1 and the 5-min duration of the test was the time spent in the neutral zone.

Larval Behavioral Responses to Authentic Chemicals Identified in Green Ash in Hexane in a T-Tube Arena. Six authentic chemicals were bioassayed in a standard T-tube assay arena (Table 1). Of those six, linalool elicited highly significant repellency to GML at concentrations of 16 and 20 ng/μL and was marginally significant at 8 ng/μL. Such larvae spent greater time in the control zone (hexane) as compared to the treated zone (linalool) of the T-tube and also returned more often to the control from the neutral zone. The larva was frequently walking, or just being still, on the inner plastic screen which kept it off the control zone filter paper. A mixture of farnesene isomers (including α-farnesene) caused negative GML locomotion at 16, 32, and 64 ng/μL at corresponding *p* values of 0.054, 0.065, and 0.035. MS proved significantly repellent at 8 ng/μL ($p = 0.031$), whereas it was inactive at the other five tested concentrations. *trans*-Nerolidol was marginally significant at 32 ng/μL ($p = 0.088$) but inactive at other assayed dosages. *cis*-3-HA did not cause statistically significant ($p < 0.05$) repellency. Finally, *trans*-ocimene did not significantly affect the observed behavior of GML at any of the four bioassayed concentrations. *trans*-4,8-Dimethyl-1,3,7-nonatriene has not been bioassayed due to the insufficient amount of the authentic sample.

DISCUSSION

Volatile chemicals (those having a relatively high vapor pressure) associated with plants seem to be advantageous to insects over nonvolatile chemicals (those having a relatively low vapor pressure) in that the former can be perceived at a distance from the source (Gilbert et al., 1967; Norris, 1990), whereas the latter are recognized only upon insect arrival on the plant (Kennedy, 1977; Norris, 1990). Regarding the significance of volatiles in GML behavior, Wallis (1959) and Lance (1983) suggested that volatile "positive" messengers (i.e., attractants) may have insignificant effects on GML host-plant selection. This, if true, does not necessarily abolish the importance of volatile "negative" messengers (i.e., repellents and/or deterrents) in nonhost rejection. Such importance is certainly supported by the early work of Meisner and Skatulla (1975), which suggested that volatile etheric oils emanating from leaves of *Pinus silvestris* L. may be deterrent to GML. Our bioassays in the olfactometer indicated that

Table 1. Behavioral Responses[a] of GML to Green Ash Volatiles and to Authentic Samples of Identified Chemicals

	time spent, min ± SE			
treatment[b]	treated side[c]	control side[d]	diff[e]	stats signif[f]
green ash volatiles (μL)				
1200	1.10 ± 0.36	1.67 ± 0.48	0.57 ± 0.76	ns
800	0.96 ± 0.27	2.41 ± 0.36	1.45 ± 0.54	$p = 0.025$
600	1.19 ± 0.17	2.40 ± 0.28	1.21 ± 0.40	$p = 0.014$
450	1.15 ± 0.27	2.27 ± 0.29	1.12 ± 0.52	$p = 0.06$
cis-3-hexenyl acetate (ng)				
4	1.64 ± 0.28	2.02 ± 0.29	0.38 ± 0.51	ns
8	1.64 ± 0.39	2.01 ± 0.47	0.37 ± 0.79	ns
12	1.55 ± 0.38	2.05 ± 0.32	0.50 ± 0.68	ns
16	1.14 ± 0.38	2.30 ± 0.41	1.16 ± 0.73	ns
20	1.93 ± 0.44	1.94 ± 0.35	0.01 ± 0.74	ns
32	1.77 ± 0.41	2.01 ± 0.38	0.24 ± 0.73	ns
trans-ocimene (ng)				
8	2.08 ± 0.37	2.07 ± 0.51	−0.01 ± 0.87	ns
16	1.39 ± 0.52	2.14 ± 0.48	0.75 ± 0.91	ns
32	1.52 ± 0.46	2.55 ± 0.49	1.03 ± 0.92	ns
64	2.13 ± 0.59	1.50 ± 0.49	−0.63 ± 0.99	ns
linalool (ng)				
2	2.25 ± 0.41	1.68 ± 0.41	−0.57 ± 0.79	ns
4	1.86 ± 0.29	1.91 ± 0.33	0.05 ± 0.59	ns
8	1.23 ± 0.23	1.90 ± 0.30	0.67 ± 0.37	$p = 0.1$
16	0.83 ± 0.23	2.87 ± 0.42	2.04 ± 0.61	$p = 0.008$
20	0.68 ± 0.21	2.79 ± 0.44	2.11 ± 0.56	$p = 0.004$
24	1.61 ± 0.35	2.17 ± 0.47	0.56 ± 0.78	ns
32	1.73 ± 0.41	2.00 ± 0.43	0.27 ± 0.80	ns
methyl salicylate (ng)				
4	2.04 ± 0.40	1.30 ± 0.28	−0.74 ± 0.63	ns
8	1.02 ± 0.37	2.74 ± 0.37	1.72 ± 0.67	$p = 0.031$
12	2.09 ± 0.51	1.82 ± 0.55	−0.27 ± 1.04	ns
16	1.31 ± 0.36	1.93 ± 0.45	0.62 ± 0.75	ns
32	2.35 ± 0.46	1.03 ± 0.41	−1.32 ± 0.82	ns
64	1.85 ± 0.48	1.17 ± 0.50	−0.68 ± 0.87	ns
farnesenes[g] (ng)				
8	2.12 ± 0.40	1.82 ± 0.48	−0.30 ± 0.86	ns
16	1.25 ± 0.29	2.61 ± 0.34	1.36 ± 0.62	$p = 0.054$
32	1.21 ± 0.40	2.77 ± 0.40	1.56 ± 0.74	$p = 0.065$
64	1.43 ± 0.22	2.49 ± 0.28	1.06 ± 0.43	$p = 0.035$
trans-nerolidol (ng)				
4	1.74 ± 0.40	1.79 ± 0.32	0.05 ± 0.70	ns
8	1.50 ± 0.37	1.51 ± 0.37	0.01 ± 0.68	ns
16	1.71 ± 0.39	1.58 ± 0.48	−0.13 ± 0.80	ns
32	0.96 ± 0.46	2.77 ± 0.51	1.81 ± 0.95	$p = 0.088$
64	1.37 ± 0.38	2.19 ± 0.53	0.82 ± 0.85	ns
control				
hexane on both sides	2.40 ± 0.52	1.73 ± 0.53	−0.67 ± 1.01	ns

[a] Data represent the mean time (min), during a 5-min test, that the starved GML spent in the indicated side of the T-tube arena. [b] Each assay was replicated 10 times. [c] Treatment consisted of 40 μL of hexane extractables of Super Q-trapped green ash volatiles or authentic chemical applied on a 4.25-cm-diameter filter paper. [d] Control consisted of 40 μL of ambient air volatiles or hexane only on such a filter paper. [e] Differences in the time spent in the control vs treated side (control minus treated) of the olfactometer were analyzed by the paired *t*-test. The difference between the summed times (treatment plus control) shown in Table 1 and the 5-min duration of the test was the time spent in the neutral zone (Figure 1). [f] Differences in means followed by the letters ns were not significant at $p \leq 0.05$. [g] Due to the inability to purchase the pure authentic chemical, a mixture of farnsesene isomers containing α-farnesene (scan number 1450) was bioassayed. Two purchased samples each revealed a GC profile with at least eight peaks which showed very similar mass spectra but different retention times. Great instability of α-farnesene may account for such a result.

GML remotely detected a mixture of green ash volatiles and individual chemicals (e.g., linalool, MS, and farnesenes) (Table 1). In responding, GML frequently lifted their anterior body and turned their head left and right a few times before choosing a direction. Our findings, thus, demonstrated that GML locomotory decisions relating to the nonhost green ash can be influenced by volatiles. Thus, such volatile chemistry may allow larvae to avoid the potentially hazardous, nonacceptable ash without physical contact with that plant. In the mixed-hardwood-species forest or planting containing green ash and the preferred host red oak, caterpillar dispersal therefore may be channeled significantly to red oak and other host species by their avoidance of volatiles emanating from green ash and other nonhost plants.

Super Q-trapped, hexane-eluted green ash volatiles contain several components (Table 2; Figure 2) that have shown a wide range of biological activities including repellency of insects. Linalool, for example, has proven to be antibacterial (Deans and Svoboda, 1989; Gangrade et al., 1989), antifungal (Thakur et al., 1989; Cheng et al., 1990), miticidal (along with MS) (Watanabe et al., 1989), insecticidal (Hink et al., 1988; Shaaya et al., 1991), insect repellent (Ramachandran et al., 1991), and nematocidal (Sangwan et al., 1990). It is, however, also a component of the male pheromone of the cabbage looper (Landolt and Heath, 1990). Linalool together with *trans*-β-ocimene, MS, and *trans*-4,8-dimethyl-1,3,7-nonatriene acts as a predatory-mite kairomone released in spider mite-induced plant volatiles. In addition, it is a component of the spider mite-dispersing pheromone (Dicke et al., 1990). Henning et al. (1992) showed that linalool emanating from alfalfa also acts as a honey bee attractant.

MS has been identified as an insect repellent (Hen-

Table 2. Chemicals Identified in Super Q-Trapped, Hexane-Eluted Green Ash Volatiles

peak no.[a]	chemical compound	major MS ions[b]
1	*cis*-3-hexenyl acetate	39 (25), 43 (100), 53 (6), 55 (11), 67 (63), 68 (11), 82 (41), 83 (69), 95 (2), *142 (4)*
2	*trans*-ocimene	39 (74), 41 (50), 51 (19), 53 (26), 65 (15), 67 (19), 79 (60), 80 (53), 91 (72), 93 (100), 105 (23), 107 (8), 121 (14), *136 (5)*
3	linalool	41 (77), 43 (100), 53 (17), 55 (17), 69 (31), 71 (53), 80 (25), 81 (44), 91 (13), 93 (41), 105 (5), 107 (6), 121 (12), 136 (5), 137 (4)
4	*trans*-4,8-dimethyl-1,3,7-nonatriene	39 (46), 41 (77), 51 (5), 53 (15), 67 (19), 69 (100), 79 (19), 81 (47), 94 (10), 95 (18), 107 (17), 108 (5), 121 (10), 122 (11), 135 (16), *150 (7)*
5	methyl salicylate	38 (15), 39 (43), 50 (6), 53 (18), 63 (29), 65 (35), 92 (100), 93 (18), 120 (99), 121 (35), *152 (56)*
6	α-farnesene	39 (52), 41 (100), 53 (23), 55 (32), 67 (19), 69 (48), 79 (37), 81 (57), 91 (36), 93 (63), 105 (28), 107 (42), 119 (33), 123 (35), 133 (6), 147 (2), 161 (2), *204 (2)*
7	*trans*-nerolidol	41 (100), 43 (78), 53 (17), 55 (27), 69 (57), 71 (33), 79 (18), 81 (23), 91 (14), 93 (40), 107 (27), 109 (9), 121 (14), 123 (7), 135 (4), 136 (10), 147 (2), 148 (2), 161 (9), 189 (2)

[a] Peak numbers as indicated in Figure 2. [b] The two most intense ions within each 14 mass units above *m*/*z* 34. Intensities relative to the most intense ion whose intensity is taken as 100 are shown in parentheses immediately following the *m*/*z* value. Molecular ions (if present) and their relative intensities are listed in italic type.

Table 3. Estimated[a] Quantities[b] of Indicated Chemicals in Super Q-Trapped, Hexane-Eluted Green Ash Volatiles

chemical compound	estimated quantity (ng 100 g^{-1} 24 h^{-1})
cis-3-hexenyl acetate	1316
trans-ocimene	588
linalool	200
methyl salicylate	285
α-farnesene	*c*
trans-nerolidol	338
trans-4,8-dimethyl-1,3,7-nonatriene	*d*

[a] Quantities were estimated by regression equations relating known concentrations of an authentic chemical and corresponding peak areas under the same GC conditions used for analyzing volatile samples. [b] Quantities reported here are based on green ash volatiles trapped in July 1994. [c] Estimate for α-farnesene was not acquired due to inability to purchase pure authentic chemical (see Table 1, footnote *g*). [d] Estimate for *trans*-4,8-dimethyl-1,3,7-nonatriene was not acquired due to insufficient amount of material available.

ning et al., 1992) and a mandibular-gland constituent of ants in the genus *Myrmecocystus*, in which it functions as an alarm pheromone (Lloyd et al., 1989). Campbell et al. (1993) suggested that the addition of MS to the damson-hop aphid aggregation pheromone abolishes pheromonal attractiveness to aphids, whereas Diehl et al. (1991) proposed MS as a component of the aggregation−attachment pheromone in male *Amblyomma variegatum* ticks.

α- and *β*-farnesene function in the territorial-marking behavior of male house mice (Novotny et al., 1990). These compounds also are insect repellents. For example, *trans*-*β*-farnesene disturbs the oviposition of the cabbage root fly when presented at 32 mg/plant (Finch and Jones, 1989). The same authors reported *trans*-*β*-farnesene as a component of the *Myzus persicae* alarm pheromone. Honda (1990) demonstrated that *trans*-*β*-farnesene is a part of the defensive chemistry (namely against insect predators) synthesized *de novo* by papilionid larvae and secreted by eversible glands (osmeteria).

cis-3-HA and *trans*-nerolidol are constituents in the blend of volatiles released upon corn-seedling damage, which attracts the armyworm larval parasitoid *Cotesia marginiventris* (Turlings et al., 1991).

Most of the identified green ash volatiles occur elsewhere in the plant kingdom (Dicke et al., 1990), where they have significant direct (intrinsic) plant-defensive roles or serve to indirectly (extrinsically) attract natural enemies of herbivores feeding on them. Such volatiles are also widely utilized in other animal communication (e.g., alarm, aggregation and sex pheromones, defensive compounds) (see above). Thus, the same volatile molecules apparently are employed by a diversity of organisms for a variety of functions. The qualitative and quantitative mixes of these common volatiles thus largely determine their specific role in a given organismal situation.

The biological activity of some of these compounds is especially influenced by their concentration. Linalool, for example, was repellent to GML only within the 8−20 ng/μL concentration range (Table 1). Above this determined active-concentration range, the GML chemoreception system was probably saturated. The effect of concentration on the biological activity of volatiles is also supported by the finding that the otherwise attractive parasitoid kairomone, 3-octanone, became neutral for *Microplitis demolitor* when the concentration was increased to 100 ng (Ramachandran et al., 1991).

Our bioassay results also clearly indicate that lesser components (e.g., linalool) can contribute significantly to the biological activity of a mixture of volatiles, whereas quantitatively major components, e.g., *trans*-ocimene, may not show significant biological activity (e.g., repellency) over a wide range of concentrations. Burden and Norris (1992) obtained similar results with the soybean antifeedant and antibiotic, coumestrol, which on a molar basis was a very active chemical against the Mexican bean beetle but was only a minor component of the total extractables from the cultivar Davis on the basis of high-performance liquid chromatography.

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Registry No. Supplied by the Author: *cis*-3-Hexenyl acetate, 3681-71-8; *trans*-ocimene, 27400-72-2; linalool, 78-70-6; methyl salicylate, 119-36-8; α-farnesene, 502-61-4; *trans*-nerolidol, 40716-66-3; *trans*-4,8-dimethyl-1,3,7-nonatriene, none.

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