Volatiles Mediating Plant-Herbivore-Natural Enemy Interactions: Soybean Looper Frass Volatiles, 3-Octanone and Guaiacol, as Kairomones for the Parasitoid *Microplitis demolitor*

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Female *Microplitis demolitor* (Wilkinson) (Braconidae: Hymenoptera) were attracted to Tenax-trapped volatiles from the larval frass of soybean looper, *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae), produced on the foliage of looper-susceptible Davis and looper-resistant PI 227687 soybeans and host Henderson lima beans, in an olfactometer. Linalool (3,7-dimethyl-1,6-octadien-3-ol), guaiacol (2-methoxyphenol), and 3-octanone were identified as major components of such frass volatiles by GC-MS analysis. These compounds were not detected in the volatiles of frass produced on an artificial diet. Such volatiles produced on the artificial diet were not attractive to the parasitoid. Authentic samples of 3-octanone and guaiacol were attractive to female *M. demolitor* in olfactometer tests. These two compounds also elicited higher EAG responses in female *M. demolitor* than linalool and *cis*-3-hexen-1-ol. The quantity of guaiacol in the soybean looper frass produced on PI 227687 was higher (ca. 5 times) than on Davis soybean or lima bean leaves. 3-Octanone was detected in the volatiles trapped from excised foliage of Davis and excised foliage and intact plants of PI 227687 soybeans. Guaiacol and linalool were not detected in soybean foliage volatiles. The significance of volatiles in the host-seeking behavior of *M. demolitor*, the tritrophic interactions among plants, herbivores, and natural enemies, and the role of herbivore frass volatiles in maintaining the natural enemy's host specificity are discussed.

INTRODUCTION

Volatile chemicals apparently serve as messengers in mediating interactions among plants, herbivores, and their natural enemies. Foraging activities of several entomophagous insects have been shown to be especially influenced by volatile chemical stimuli associated with the habitats of their hosts (Vinson, 1976, 1981; Weseloh, 1981; van Alphen and Vet, 1986). Enemies of phytophagous insects have been demonstrated to be strongly attracted to volatiles from plants bearing a feeding host (generally referred to as the plant-host complex) (Drost et al., 1988; Dicke et al., 1990; Turlings et al., 1990, 1991). Volatiles from such a plant-host complex may actually emanate from the plants, the host insect, or host byproducts such as feces, silk, and honey dew (Vinson, 1976; Kennedy, 1984; Mudd et al., 1984; Takabayashi and Takahashi, 1989). Investigations of the relative importance of volatile chemicals from these three sources (plant, host, or host feces) have indicated that plants are the vital source of informational volatiles for a generalist parasitoid, *Cotesia marginiventris* (Cresson) (Turlings et al., 1991). For a specialist parasitoid, *Microplitis croceipes* (Cresson), host feces have been demonstrated to be more important than volatiles from the plants (Elzen et al., 1987; Eller et al., 1988). However, information on specific chemicals in volatile extracts that mediate interactions between phytophagous insects and their natural enemies is limited (Hendry et al., 1973; Auger et al., 1989; Turlings et al., 1990; Whitman and Eller, 1990; Dicke et al., 1990).

A comparison of the electroantennogram responses of a herbivore, *Pseudoplusia includens* (Walker), and a parasitoid, *Microplitis demolitor*, to aliphatic compounds of varying carbon-chain lengths (Ramachandran and Norris, 1991) showed that the herbivore's antennal response peaked at six carbons while that of the parasitoid's peaked at seven and eight carbons. It was suggested that the herbivore's EAG-response profile was adaptive, as six-carbon aliphatic compounds are abundant in the volatiles of its host plant, *Glycine max* (L.) Merr. (Liu et al., 1989). Because seven- and eight-carbon compounds are less common in plants, the authors speculated that the parasitoid's antennal receptors are tuned to perceive volatiles from other sources in addition to plants. It was further suggested that larval frass may be the source of such seven- and eight-carbon compounds for the parasitoid. The objective of the present study was to investigate the chemical nature and parasitoid responses to frass volatiles from herbivore feeding on various host plants, including lines reported as susceptible or resistant to herbivory.

MATERIALS AND METHODS

Insects. The colony of *P. includens* was maintained on pinto bean based diet (Shorey and Hale, 1965) at 27 ± 2 °C and 50 ± 10% relative humidity with a photoperiod of 14 h of light and 10 h of darkness. *M. demolitor* was reared on *P. includens* larvae according to the methods of Herard et al. (1988). Adults of *M. demolitor* (one male and one female) were maintained with honey and moist cotton wool in Petri dishes (3-cm diameter).

Plants. Seeds of a commercial soybean cultivar Davis reportedly susceptible to the soybean looper, *P. includens*, and a plant introduction (PI) 227687 found to be resistant to this insect (Yanes and Boethel, 1983) were obtained from Dr. E. E. Hartwig, Delta Branch Experimental Station, Stoneville, MS 38776. Seeds were germinated in flats of moistened vermiculite in a laboratory growth chamber and plants were grown to the V6-V8 stage in the University of Wisconsin, Madison, biotron controlled-environment facility. Units of 100 g of fully expanded trifoliolate leaves were harvested and used immediately for volatile trapping. Foliage from other such plants was picked and placed directly into polyethylene bags and stored at 10 °C. For trapping chemicals from intact plants, three to five plants were removed from the pots and their roots were cleaned under running water before use. Plants of Davis and PI 227687 soybeans

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Table I. Behavioral Responses[a] of Female *M. demolitor* to Tenax-Trapped Volatiles from Soybean Looper Frass from the Specified Substrate

food substrate on which frass was produced	time spent, s ± SEM: treated	control	diff[f]	statist signif
Davis (biotron)	170 ± 10 (57)	56 ± 7 (20)	114 ± 14	$p < 0.001$
Davis (biotron)[c]	131 ± 23 (44)	80 ± 18 (27)	51 ± 39	ns
Davis (greenhouse)	155 ± 22 (52)	65 ± 11 (22)	90 ± 33	$p < 0.05$
Davis (10×)[d]	141 ± 21 (48)	57 ± 14 (19)	84 ± 33	$p < 0.05$
PI 2276872[b]	135 ± 19 (59)	48 ± 11 (22)	87 ± 26	$p < 0.05$
lima bean[b,e]	137 ± 22 (60)	44 ± 13 (20)	93 ± 34	$p < 0.05$
artificial diet	110 ± 17 (37)	104 ± 12 (34)	6 ± 29	ns
controls				
Tenax control	113 ± 26 (40)	127 ± 29 (42)	−14 ± 54	ns
hexane on both sides	94 ± 31 (33)	58 ± 18 (22)	34 ± 31	ns

[a] Observations were taken for 5 min or [b] 4 min. [c] Volatiles were trapped for only 3 h for this treatment. For all other treatments volatiles were trapped for 24 h. [d] A 10-fold dilution of Davis greenhouse volatiles. [e] Lima bean volatiles were concentrated 2-fold under nitrogen before bioassays. [f] Time spent in the control vs treated sectors was analyzed by the paired *t*-test (Zar, 1984) for statistical significance. Values in parentheses are percentage of the total experimental duration spent on the control or treated side.

and Henderson lima bean (*Phaseolus vulgaris* L.) were also grown in standardized high-light conditions (Neupane and Norris, 1990) in a greenhouse for 6–8 weeks before use in such experiments.

Collection of *P. includens* Frass. Leaves of Davis soybeans, which had been grown in the biotron and stored at 1 °C, were allowed to equilibrate to room temperature in moist filter paper lined Petri dishes (15-cm diameter). Fourth and fifth instar *P. includens* larvae were then allowed to feed on these leaves. Frass was collected every 3 h and transferred to glass jars, which were sealed with parafilm and stored at −10 °C. Frass from greenhouse-grown soybean and lima bean plants was collected in a specially designed apparatus. The roots and lower stem of each such plant were removed with a sharp knife, and the cut end of the plant's top was immersed in 100 mL of distilled water contained in a 150-mL Erlenmeyer flask. This "bouquet" of plant tops (three to five plants) was placed within a plastic container (20 cm tall and 20 cm in diameter). The bottom of the container was replaced with wire gauze (10 gauge). The container rested on the rim of a funnel (20-cm diameter). The stem of the funnel was inserted into a glass frass-collection jar. The jar was placed in a bucket of ice–acetone mixture. Twenty-five fourth or fifth instar larvae were placed on plant tops. Larval frass thus fell through the wire gauze and was collected in the glass jar. Frass was periodically (every 6–8 h) transferred to a glass jar, and the jar was sealed as above and stored at −10 °C until an amount adequate for volatile trapping had accumulated.

Tenax Trapping of Plant and Frass Volatiles. Fully expanded trifoliolate leaves (100 g) or larval frass (42 g from Davis, 34 g from PI 227687, and 21 g from lima bean) was placed in a modified 1000-mL Erlenmeyer flask reservoir (Liu et al., 1989). The Tenax trap consisted of a 0.5-cm diameter by 9-cm length Pyrex column packed with 0.20 g of Tenax GC (Alltech Associates Inc., Deerfield, IL 60015). Using a ground glass jointed all glass system, air was first dessicated by suction through calcium chloride, then vacuum-filtered through activated charcoal, and next passed through a side inlet into the modified Erlenmeyer flask reservoir containing the leaves or frass and then up through the Tenax trap. The vacuum air flow was metered through the trap at 1500–2000 mL/min, and it continued for 24 h (except in one case where volatiles from frass produced on biotron-grown Davis plants were trapped for 3 h; Table I) at 25 ± 2 °C. The Tenax from the trap was placed in a 7-mL screw-capped vial and extracted, using vortexing (2 min), with 3 mL of hexane (HPLC grade). The resultant hexane extractables were filtered through Whatman No. 1 paper, weighed in a screw-capped glass vial, and stored at −10 °C. To collect volatiles from whole plants, the plants were gently removed from pots and the roots were cleaned under running water. The cleaned roots were then inserted into a 250-mL conical flask containing distilled water. This bouquet of plants (three to five plants) was placed inside a glass reservoir (40 cm tall and 20 cm in diameter) and was substituted in the previously described Tenax trapping system instead of the modified Erlenmeyer flask reservoir.

Bioassay of Volatiles with *M. demolitor*. The behavioral response of female *M. demolitor* was assayed in a horizontal open-ended 3 cm i.d. by 15 cm long cylindrical glass tube arena, which had a centered 15 mm i.d. side-wall opening for introduction of the assay insect. The opening at each end of the cylindrical arena was covered by 36-gauge mesh plastic screen (Chicopee Products, Inc., Cornelia, GA 30331) (Liu et al., 1988). The tubular arena was divided into sectors. An area of 2.5 cm in the center of the cylinder (encompassing the centered side-wall opening for introduction of the assay insect) was designated the "start section". The area of the cylinder on either side of the start section was divided into two equal sectors (i.e., proximal and distal). All test insects were 3–5-day-old, mated, gravid females that had no previous ovipositional experience nor exposure to frass or plant volatiles. An insect was introduced into the start section through the centered side opening. Using a stopwatch, parasitoid orientation and movement were recorded in seconds, according to sectors, during 5 min (unless otherwise specified). After each replicate assay, the arena was rotated 180° to correct for effects of otherwise unrecognized stimuli. Eight replicate assays were conducted for each treatment.

Each chemical treatment (5 μL of standardized hexane extractables from the Tenax trappings or a solution of authentic chemicals) was applied to a 3 cm diameter filter paper (Whatman No. 1) disk. The filter paper was pretreated by immersing it in No. 30 white oil, U.S.P. (American Oil Co., Chicago, IL). A disk with 5 μL of hexane extractables from control Tenax trappings (Tenax trapping with an empty reservoir) served as the control for assays with frass volatiles. A white oil treated filter paper disk with 5 μL of hexane served as the control for assays with authentic chemicals. The filter paper disk bearing the chemical treatment and the one bearing the control were positioned oppositely outside the two screened ends of the arena; each positioned disk was secured by a plastic cup (3.5-cm diameter). Each securing cup was uniformly perforated with five (1-cm diameter) holes in its bottom and side to allow air flow.

Bioassays were conducted at 25 ± 2 °C and 65 ± 5% relative humidity with lighting provided by one Sylvania cool-white 34-W fluorescent bulb. The assays were conducted in a chamber from which the air was evacuated for 5 min between consecutive tests.

The statistical significance of differences in the time spent in sectors on the side with the treated filter paper vs the side with the control filter paper was analyzed by the paired *t*-test (Zar, 1984). Percentage of total experimental time spent in the treated vs control arms of the assay tube is also presented.

Electroantennogram Assays (EAG). EAGs were recorded from mated, gravid, naive, female *M. demolitor* (3 to 5-days old) according to the method described by Ramachandran and Norris (1991). Authentic samples of 3-octanone, guaiacol, linalool, and *cis*-3-hexen-1-ol (obtained from Aldrich Chemicals Inc., Milwaukee, WI) were dissolved in HPLC grade hexane at 10 and 1 mg/mL. One microliter of such a solution pipetted on a Whatman No. 1 filter paper (2 × 2 cm) served as the stimulus source (i.e., 1 or 10 μg of chemical on filter paper). Ten micrograms of *cis*-3-hexen-1-ol served as the standard chemical stimulant. *cis*-3-Hexen-1-ol is a common plant volatile and was previously used as the standard in EAG studies on this parasitoid (Ramachandran and Norris, 1991). Stimulation with each of the test compounds was preceded, and followed, by stimulation with the standard. Experiments with 1- and 10-μg doses of the chemicals were performed separately (i.e., with different cohorts of insects). For each chemical and dose, response was recorded from six females. The EAG responses relative to the standard (10 μg of *cis*-3-hexen-1-ol) were analyzed by ANOVA and the means separated by Tukey's honestly significant difference (Zar, 1984).

Capillary Gas Chromatography–Mass Spectrometry (GC–MS) Analysis. Gas chromatography–mass spectrometry was done with a Shimadzu GC-14A gas–liquid chromatograph equipped with a DB-1 capillary column (30 m × 0.25 mm, bonded phase) mated via heated transfer line to a Finnigan MAT ITD 800 Series mass spectrometer (MS) with ITDS software 4.10. The MS was operated under the electron ionization (EI) mode. The GC was temperature-programmed from 1 min at 40 °C to 220 °C at 5 °C/min. Both the injection port and transfer line were at 250 °C.

Analysis was also done on a Finnigan MAT Model 9610 GC

mated via a heated transfer line to a Finnigan MAT quadruple detector with Novel 4 Data General software. This MS was operated in the electron ionization (EI) mode. For molecular weight information, the chemical ionization (CI) mode with methane was used; the upper mass range was 12 195 amu. The temperature program was identical to the previously described analytical procedure. For both analyses, peaks were tentatively identified by matching the sample spectrum with one in the NBS Mass Spectral Library. Frass volatile component identities were further confirmed by matching the experimental spectrum and GC retention time with those of authentic samples (Aldrich).

Capillary Gas Chromatography. The standardized hexane-extracted volatiles were analyzed by capillary gas chromatography (GC) with a Shimadzu GC-14A GC, using a capillary column (OV-17, 25 mm × 0.25 mm, open tubular wall coated, J&W Scientific, Folsom, CA 95630) and flame ionization detector. The temperature program was similar to that previously described. The inlet pressure was 12 psi, and the flow rate was 2.3 mL of He/min. The temperature at the injector was 240 °C and at the detector, 250 °C. The split ratio of the injector was 50:1. A 1-μL sample of the extractables was injected. Retention time, area, and ratio of each peak area were recorded and calculated by a Shimadzu C-R3A Chromatopac computing integrator. Peaks that had been tentatively identified by GC–MS were confirmed by injecting authentic chemicals and comparing the retention times. Alternately, authentic chemicals were co-injected with the sample for confirmation. The concentration of a chemical 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).

RESULTS

Behavioral Responses of Female *M. demolitor* to Tenax-Trapped Volatiles from Soybean Looper Frass. When *M. demolitor* females were released in the static olfactometer without experimental stimulus, they walked the length of the tubular arena, i.e., between the two ends. This uniform searching of the experimental arena resulted in a similar time being spent in the sectors on either side of the start section of the olfactometer. Hexane, the solvent in which the frass volatiles were eluted, did not significantly affect the behavior of the parasitoids when it was placed on either, or both, ends of the olfactometer (Tables I and III). However, in the presence of a stimulus, parasitoids spent a greater time in the sectors on one side of the stimulus and also returned more often to those sectors. A statistically significant difference in the time spent in the control vs treated sections of the olfactometer was observed when volatiles from frass produced by loopers on biotron-grown Davis leaves were used as a stimulus (Table I).

Parasitoids spent a greater time in the frass volatile treated side of the olfactometer, returned more often to these sections from the start section, and noticeably examined and probed the glass surface on the stimulus side with their antennae and ovipositor. The volatiles for this treatment had been trapped in Tenax for 24 h. A similar attraction to such volatiles trapped for only 3 h was noted; however, the differences were not statistically significant ($p > 0.05$) (Table I). A significant attraction to soybean looper frass volatiles produced on greenhouse-grown Davis or PI 227687 soybean or Henderson lima bean was also observed (Table I). Volatiles trapped from frass produced on Davis leaves were attractive to the parasitoids even after a 10× dilution with hexane (Table I). However, volatiles trapped from frass produced on pinto bean based artificial diet did not affect the observed behavior of female parasitoids.

Chemical Analysis of Frass Volatiles. Results of GC–MS analysis of Tenax-trapped, hexane-eluted volatiles indicated the presence of three major compounds.

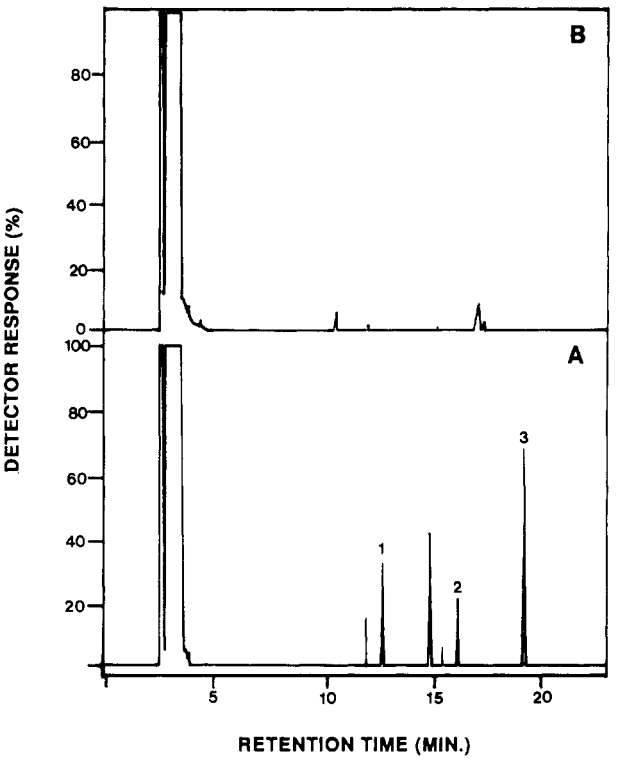

Figure 1. (A) Gas–liquid chromatogram of Tenax-trapped volatiles from soybean looper frass produced on Davis soybean leaves. Plants were grown under controlled environmental conditions in a biotron facility. Leaves were harvested and immediately stored at 1 °C. Such leaves were allowed to equilibrate to room temperature. Third and fourth instar larvae were allowed to feed on these leaves. Volatiles were trapped from 42 g of frass for 24 h and eluted with 3 mL of hexane. One microliter of the extractables was injected into the GLC. 1, 3-Octanone; 2, linalool; 3, guaiacol. (B) Gas–liquid chromatogram of Tenax-trapped volatiles from soybean looper frass produced on a pinto bean based artificial diet.

These chemicals were tentatively identified as 3-octanone, linalool (3,7-dimethyl-1,6-octadien-3-ol), and guaiacol (2-methoxyphenol) by computer matching of the unknown mass spectra with the reference mass spectra of the NBS Mass Spectral Library. The presence of these chemicals in frass volatiles was further confirmed by matching the mass spectrum of the authentic chemical with that of the sample spectrum, by matching of retention times of the sample and authentic chemicals, and by co-injection of known quantities of the authentic chemical with the experimental sample. These three chemicals were identified in volatile samples trapped from soybean looper frass produced on biotron- and greenhouse-grown Davis soybeans, and greenhouse-grown PI 227687 soybeans, and Henderson lima beans (Figures 1A and 2). The quantities of these chemicals in frass samples differed with the substrate on which frass was produced (Figures 1A and 2; Table II). Thus, frass produced on Davis soybean plants grown in the biotron yielded relatively higher quantities of 3-octanone as compared to frass produced on such greenhouse-grown plants (Table II). In contrast, the greenhouse-grown Davis soybeans contained higher quantities of linalool and guaiacol. The quantity of guaiacol in volatiles trapped from PI 227687 was ca. 5 times higher than that from Davis soybeans. The quantities of these chemicals were least in Henderson lima beans. Volatiles trapped from soybean looper frass produced on a pinto bean based artificial diet did not contain these identified compounds (Figure 1B).

Analyses of volatiles trapped from whole undamaged plants vs excised leaves were different (Figure 3) for both

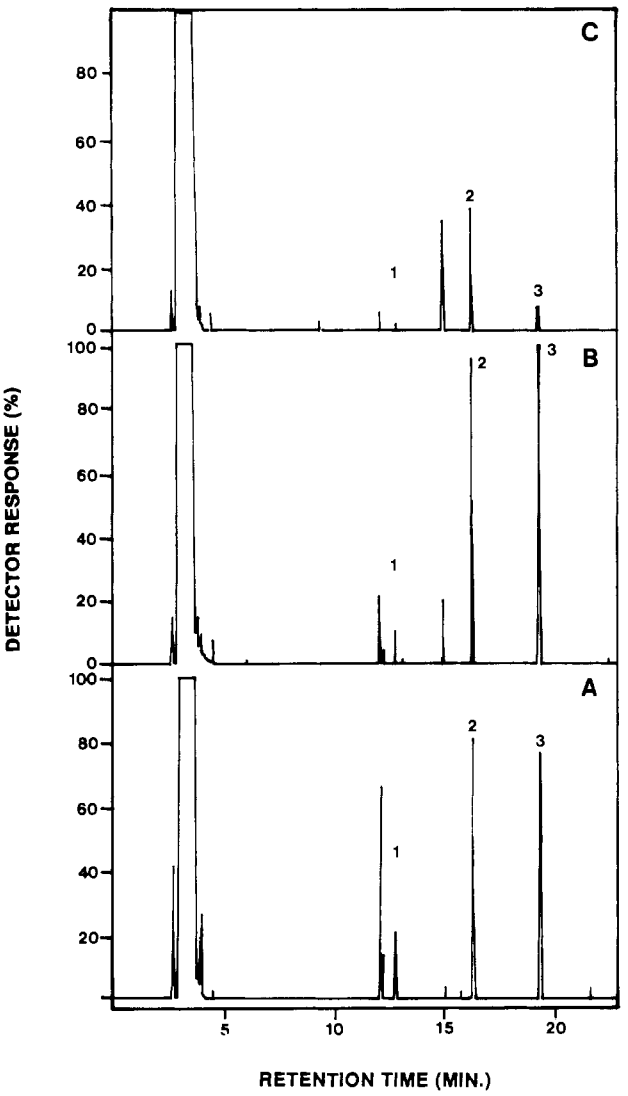

Figure 2. Gas–liquid chromatogram of Tenax-trapped volatiles from soybean looper frass produced on (A) greenhouse-grown Davis. Volatiles were trapped from 42 g of frass for 24 h and eluted with 3 mL of hexane. Hexane extractables were concentrated 3 times, and 1 μL was injected. (B) PI 227687 soybean. Volatiles were trapped from 34 g of frass. Hexane extractables were concentrated 3 times, and 1 μL was injected. (C) Henderson lima bean plants. Volatiles were trapped from 22 g of frass. Hexane extractables were concentrated 4 times before injection. 1, 3-Octanone; 2, linalool; 3, guaiacol.

Davis and PI 227687 soybeans. Fewer peaks were detected in the volatiles trapped from undamaged plants as compared to excised leaves. Among the chemicals identified in the frass volatiles, only 3-octanone was detected in the plant volatiles.

Behavioral Bioassays and Electroantennogram Responses of Female *M. demolitor* to Authentic Chemicals Identified in Frass Volatiles. Of the three chemicals that were bioassayed in the olfactometer, 3-octanone and guaiacol elicited a statistically significant attraction in female *M. demilitor* (Table III). In bioassays with guaiacol, the parasitoids became significantly arrested in the treated side of the olfactometer. This chemical thus appears to have both an attractant and arrestant effect on the behavior of female *M. demolitor*. Linalool did not affect the observed behavior of *M. demolitor* at lower concentrations but repelled the parasitoids ($p < 0.1$) at the highest tested concentration.

At the 10-μg dose the EAG response elicited by each test chemical (relative to a response elicited by 10-μg of *cis*-3-hexen-1-ol) was significantly higher than that with solvent control. The highest response was elicited by 3-octanone, followed by guaiacol and linalool. At the 1-μg dose only the responses elicited by 3-octanone and guaiacol were significantly higher than that for the control (Figure 4). The responses to these two chemicals were statistically similar at the 1-μg dose.

Table II. Estimated[a] Concentration of Indicated Chemicals in the Specified Soybean Plants and in the Soybean Looper Frass from the Given Substrate

	chemical, ng g^{-1} 24 h^{-1}		
treatment	3-octanone	linalool	2-methoxyphenol
plants			
Davis[b]	63		
PI 227687[b]	73		
frass by substrate			
Davis (biotron)	784	657	1112
Davis (greenhouse)	210	970	1674
PI 227687	162	1352	6441
lima bean	c	939	300
artificial diet			

[a] Quantities were estimated by regression equations relating the known concentration of an authentic chemical and its peak area under the same GC conditions used for analyzing volatile samples. [b] Estimated only for volatiles trapped from excised leaves. [c] Peak was not integrated.

DISCUSSION

Volatile chemicals associated with phytophagous hosts have been shown to attract insect parasitoids (Nadel and van Alphen, 1987; Dicke et al., 1990; Turlings et al., 1990, 1991; Whitman and Eller, 1990) and are considered as vital parameters influencing the host-seeking behavior of such entomophagous insects. These chemicals may originate from the plant upon which the host feeds (i.e., synomones) (Whitman, 1988; Ding et al., 1989; Navasero and Elzen, 1989; Sheehan and Shelton, 1989; Martin et al., 1990; Whitman and Eller, 1990; Turlings et al., 1990) or from the host or host byproducts (i.e., kairomones) (Vinson, 1976; Kennedy, 1984; Mudd et al., 1984; Nordlund and Lewis, 1985; Sabelis and van de Baan, 1983; Strand and Vinson, 1983; Takabayashi and Takahashi, 1989). Studies of the relative importance of volatile chemicals from plants, hosts, or host feces as informational volatiles for a generalist parasitoid, *C. marginiventris*, have indicated that plants are vital sources (Turlings et al., 1991), but larval feces have been demonstrated to be a more important source of cues than plants for a specialist parasitoid, *M. croceipes* (Elzen et al., 1987; Eller et al., 1988). Considering the latter category, host frass has been demonstrated to be an important source of kairomones for several entomophagous insects. The frass kairomones may elicit specific host selection behaviors when a parasitoid contacts the frass (Jones et al., 1971; Nordlund and Sauls, 1981; Nordlund and Lewis, 1985; Takabayashi and Takahashi, 1989), or the volatile components of the frass may serve as long-distance olfactory cues (Hendry et al., 1973; Auger et al., 1989; Eller et al., 1988; Elzen et al., 1987; Lewis and Tumlinson, 1988).

M. demolitor, a solitary braconid that was imported into the United States, parasitizes *Heliothis zea* (Boddie), *H. virescens* (F.), *P. includens*, and *Trichoplusia ni* (Hubner) (Shepard et al., 1983). *M. demolitor* may be considered as a specialist parasitoid because it has this narrow host range, and its host selection behavior was previously shown to be stimulated by the frass of *H. zea* (Nordlund and Lewis, 1985) when the female parasitoid contacts such frass. Our studies demonstrated that the

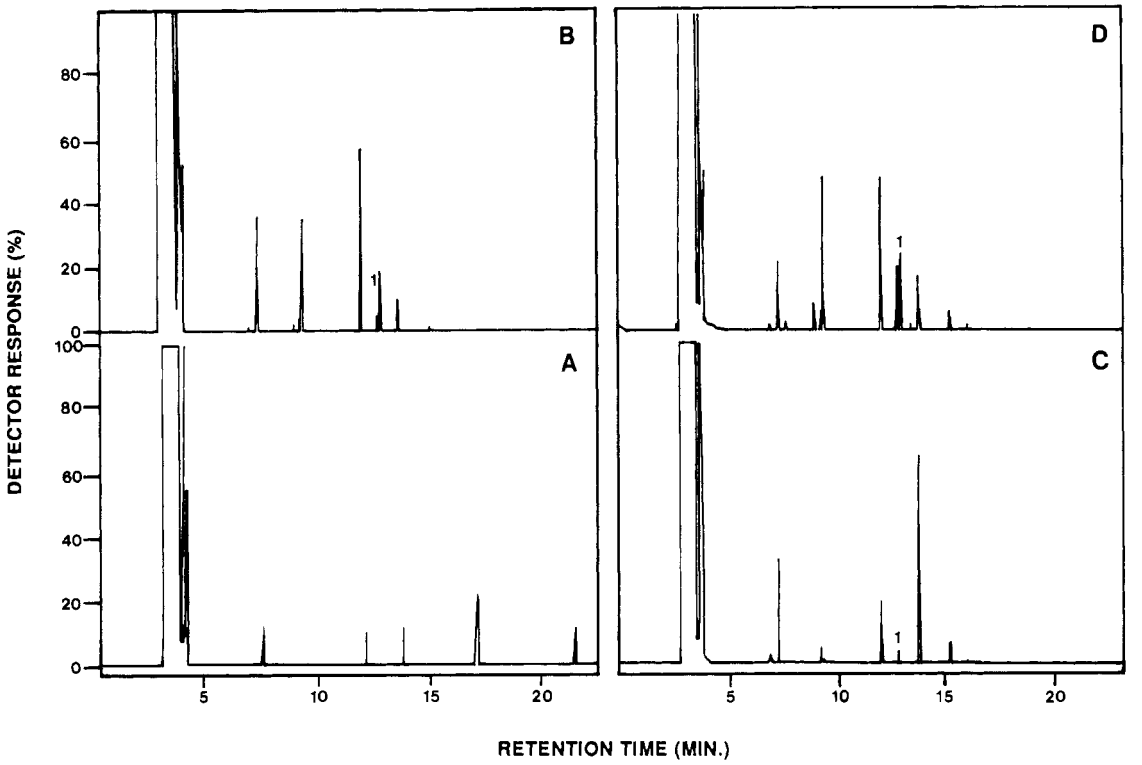

Figure 3. Gas–liquid chromatogram of Tenax-trapped volatiles from (A) intact Davis soybean plants, (B) excised leaves of Davis soybean plants, (C) intact plants of PI 227687, and (D) excised leaves of PI 227687. 1, 3-Octanone.

Table III. Behavioral Responses[a] of Adult Female *M. demolitor* to Authentic Samples of Volatiles Identified in Soybean Looper Frass

chemical and concn	time spent, s ± SEM: treated	control	diff[b]	statist signif
linalool				
10 ng	106 ± 17 (35)	116 ± 21 (39)	11 ± 37	ns
25 ng	87 ± 12 (30)	91 ± 9 (38)	4 ± 15	ns
100 ng	79 ± 14 (27)	125 ± 13 (42)	−46 ± 24	$p < 0.1$
guaiacol				
50 ng	126 ± 13 (52)	59 ± 10 (25)	67 ± 21	$p < 0.01$
100 ng	149 ± 14 (59)	54 ± 8 (22)	94 ± 20	$p < 0.001$
3-octanone				
5 ng	132 ± 17 (44)	63 ± 10 (21)	69 ± 20	$p < 0.01$
10 ng	134 ± 15 (56)	52 ± 11 (21)	81 ± 25	$p < 0.01$
25 ng	156 ± 10 (51)	74 ± 10 (24)	82 ± 19	$p < 0.008$
100 ng	105 ± 27 (37)	116 ± 36 (40)	−11 ± 63	ns
control				
hexane on both sides	98 ± 24 (38)	110 ± 18 (40)	12 ± 14	ns

[a] Observations in the olfactometer were taken for 5 min. Chemical was dissolved in HPLC grade hexane; 5 μL of the solution was dispensed on a 3 cm diameter filter paper (presoaked in white oil) and was tested against a control filter paper treated with 5 μL of hexane. [b] Differences in the time spent in the control vs treated side of the olfactometer were analyzed by the paired *t*-test. Value in parentheses is the percentage of the experimental period spent in treated or control sides.

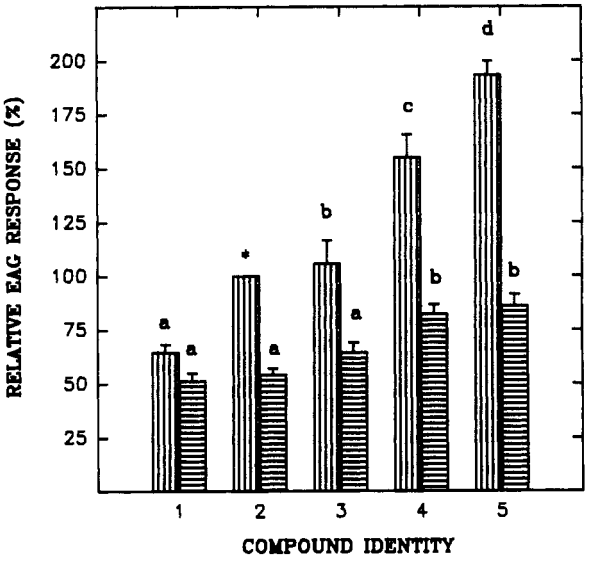

Figure 4. EAG responses (relative to the response elicited by 10 μg of *cis*-3-hexen-1-ol) of female *M. demolitor* to volatile chemicals identified in soybean looper frass. Bars filled with vertical lines represent response to 10-μg dose. Bars filled with horizontal lines represent response to the 1-μg dose. Vertical lines on the bars represent standard error of means. Bars with same letters are not significantly different (Tukey's honestly significant difference, $p < 0.05$). The bar marked with an asterisk was not included in statistical analysis. 1, Hexane; 2, *cis*-3-hexen-1-ol; 3, linalool; 4, guaiacol; 5, 3-octanone.

volatiles from the frass of soybean looper serve also as long-distance olfactory cues for this parasitoid; i.e., the frass volatiles attracted the parasitoids to the source of the stimulus and increased search duration. With a closely related indigenous species, *M. croceipes*, host feces also were reported to be the key odors that attract this wasp to the microhabitat of its hosts (Eller et al., 1988; Elzen et al., 1987). Indeed, investigations of the electroantennogram responses of the parasitoid *M. demolitor* to hydrocarbons of different carbon-chain lengths (Ramachandran and Norris, 1991) indicated that its antennae are tuned to perceive volatiles from sources other than plants, such as larval frass. Our present results confirm that volatiles from host frass are an important source of cues to *M. demolitor*.

Although volatiles in frass have been demonstrated to affect the behavior of several parasitoids, identifications of the specific chemicals involved in these interactions are limited (Hendry et al., 1973; Auger et al., 1989). Analysis and behavioral bioassay of such volatiles and electroan-

tennogram studies with authentic volatiles identified in frass indicate that 3-octanone and guaiacol are two components of frass that elicit attraction in female *M. demolitor*. 3-Octanone has also been reported as a major constituent of the alarm pheromones of several species of ants (Crewe et al., 1972; Crewe and Blum, 1972; Duffield and Blum, 1975). Guaiacol was identified by Aldrich et al. (1976) as the male-specific chemical in the abdominal glands of leaf footed bug, *Leptoglossus phyllopus* (Coreoidea: Hemiptera). Guaiacol was suggested as one of the long-distance male-produced sex pheromones for this species. In three species of millipedes, guaiacol has been isolated from cyanogenic glands and was reported to function as a defense compound (Duffey and Blum, 1977). Another major component of the frass was linalool; however, it only affected the behavior of *M. demolitor* (i.e., was repellent) at the highest dose tested. Linalool has been reported to be an important kairomone for predatory mites, *Amblyseius potentillae* and *Phytoseiulus persimilis* (Dicke et al., 1990). Lima bean plants infested with *Tetranychus urticae* produced large quantities of linalool. Linalool was also suggested to be a component of the spider mite dispersing pheromone. On the basis of our results, we speculate that spider mite frass may also have been an important source of this kairomone in the lima bean–spider mite–predator interaction. Linalool has also been identified in the metathoracic gland secretions of the cotton stainer, *Dysdercus superstitiosus* (F.) (Heteroptera: Pyrrhocoridae), and was hypothesized to be a defense compound (Daroogheh and Olagbemiro, 1982).

On the basis of the observations that many parasitoids do not respond to kairomones in the frass of their hosts fed on artifical diets (Sauls et al., 1979; Nordlund and Sauls, 1981; Elzen et al., 1984; Nordlund and Lewis, 1985) and that they respond differently to frass produced on different plant hosts (Roth et al., 1978; Mohyuddin et al., 1981; Nordlund and Sauls, 1981), it has been suggested that the kairomones discussed above may be of plant origin. Our results in which *M. demolitor* did not respond to volatiles from frass produced on a pinto bean based artificial diet, but was strongly attracted to frass derived from plant substrates, would support this contention. Chemical analysis of volatiles in frass produced on our artificial diet indicated that none of the chemicals identified in the frass volatiles from our plant sources were present. The kairomone of the potato tuber moth parasite, *Orgilus receptor* (Braconidae: Hymenoptera), has been demonstrated to be merely accumulated from the food source and excreted in the frass by potato tuber moth larvae, but the origins of kairomones for other parasitoids have remained unknown (Nordlund et al., 1989; Dicke et al., 1990). In our study and in that of Liu et al. (1989), the soybean looper frass kairomone, 3-octanone, was detected in the foliage volatiles of Davis and PI 227687 soybeans. This chemical was also detected in the volatiles trapped from the foliage of lima beans (Liu et al., 1989). Because the concentration of this kairomone in the frass is ca. 10 times that in the foliage, it would seem to be at least accumulated and concentrated in the frass. It is also interesting to note that several other volatiles which were previously identified from the foliage of these three plants (Liu et al., 1989) were not detected in the frass volatiles.

Guaiacol was not detected in the foliage volatiles of soybeans (Figure 3; Liu et al., 1989) or lima beans (Liu et al., 1989). Linalool, the other major volatile component of the frass with known kairomonal activity to phytosid mites (Dicke et al., 1990), also was not detected in the

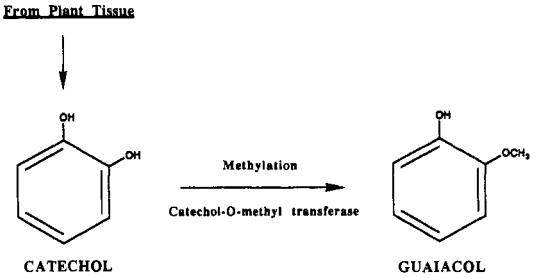

Figure 5. Enzymatic methylation of plant-derived catechol to yield guaiacol in an animal system (Axelrod and Tomchick, 1958).

foliage volatiles of soybeans (Figure 3; Liu et al., 1989) but was present in trace quantities in lima beans (Liu et al., 1989). Although guaiacol is reported to occur in numerous plant species, whether it occurs in the free form is not known (Gibbs, 1974). Three species of millipedes and a coreid bug, *L. phyllopus*, have been shown to biosynthesize guaiacol from tyrosine by tyrosine-phenol lyase (EC 4.1.99.2) (Duffey and Blum, 1977; Duffey et al., 1977). The enzyme seems to be restricted to the glandular tissues where this chemical is synthesized. Although the above studies attempted to preclude the role of bacteria in the biosynthesis, it was concluded that the involvement of a bacterium with high specific culturing requirements, e.g., an anaerobic bacterium, could not be ruled out. Indeed, *Nocardia autotraphica*, a bacterium that transforms organic substances in soil, has been shown to produce guaiacol as a metabolite in the transformation of methoxyphenolic acids (Malarczyk et al., 1987). The possibility that guaiacol was produced by such a saprophytic bacterium after the feces had been excreted (i.e., during the 24-h trapping period) was tentatively ruled out in our studies in which we trapped volatiles from frass treated with antibiotics (0.05% penicillin and streptomycin sulfate). No difference was observed in the quantity of guaiacol detected in antibiotic-treated and in untreated frass samples (unpublished data).

The large, but distinctive, quantities of guaiacol and linalool in the frass volatiles of soybean looper produced on different genotypes of the same species (i.e., Davis and PI 227687), and even on different plant species (*G. max* and *P. vulgaris*), and their absence in volatiles from frass produced on an artificial diet support the interpretation that the quantity of these volatiles in frass is influenced significantly by the herbivore's substrate. The fact that the quantity of guaiacol produced on the herbivore-resistant PI 227687 soybean was much higher than that produced on the more herbivore susceptible Davis soybean foliage especially supports this hypothesis. While such differences would be expected to affect the attractiveness of the frass to the parasitoid, our bioassays were not designed to test for such differences. It is significant that the herbivore resistance in PI 227687 has been demonstrated to be partially due to flavonoids and other phenolic compounds (Chiang et al., 1987; Khan et al., 1986; Norris et al., 1988; Sharma and Norris, 1991), because a herbivorous metabolic route to guaiacol involves the phenolic catechol as the plant-dependent substrate (Figure 5). Prior theoretical considerations regarding tritrophic interactions among plants, herbivores, and natural enemies have led to the suggestion that resistant plants should be bred with both intrinsic (constitutive and induced plant resistance) and extrinsic (e.g., favorability to biological control agents) defenses (Price, 1986). Our results indicating that soybean looper larvae produced higher quantities of guaiacol on resistant PI 227687 than on the susceptible Davis support the interpretation that this soybean genotype would also

significantly favor colonization by *M. demolitor*. Yanes and Boethel (1983) studied the effects of PI 227687 resistance on the development of soybean looper and *M. demolitor* and suggested that such parasitism had an additive effect with plant resistance in reducing leaf consumption by *P. includens*. Thus, it is possible that the relatively wild soybean, PI 227687, has evolved both intrinsic and extrinsic defenses against herbivores and that these defenses have a common phenolic parameter. It is known, however, that part of the herbivore resistance of PI 227687 is attributable to volatile repellants (Norris et al., 1988; Liu et al., 1989); thus, such resistance factors might be found to repel parasitoid colonization of its host on this plant. Whether such combined intrinsic and extrinsic plant defenses significantly deplete both the herbivorous host and the parasitoid populations on PI 227687 is a worthy consideration in efforts to describe the realities in polytrophic interactions among life forms.

Our study, however, has provided the first evidence that volatile kairomones in host frass include not only plant compounds that are concentrated and released in frass (Hendry et al., 1976) but also compounds (e.g., guaiacol) that are strictly larval metabolites of plant precursers. Results of Navasero and Elzen (1989), who found that *M. croceipes* responded to *H. virescens* frass produced on ground cherry (a nonhost of *H. virescens*), although a response to volatiles from the plant was absent, indicate that the kairomones of this parasitoid might also be metabolic products of plant precursors.

The presence of the same one or more chemicals in the frass from a phytophagous host feeding on distinct food sources (which differ markedly in their volatile chemistry; Liu et al., 1989) could provide host-specific cues to a searching parasitoid. It should be evolutionarily advantageous for a host-specific natural enemy, such as *M. demolitor* or *M. croceipes*, to utilize volatile cues associated specifically with the frass of its host in addition to those associated with the host plant of its prey. Our results thus enable a better understanding of the evolutionary significance of the reported differences in the relative importance of volatile cues from plants and host frass for generalist and specialist entomophagous insects (Turlings et al., 1990).

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Registry No. Linalool, 78-70-6; guaiacol, 90-05-1; 3-octanone, 106-68-3.