

## ISOLATION AND IDENTIFICATION OF ALLELOCHEMICALS THAT ATTRACT THE LARVAL PARASITOID, *Cotesia marginiventris* (CRESSON), TO THE MICROHABITAT OF ONE OF ITS HOSTS

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**Abstract**—Volatiles released from corn seedlings on which beet armyworm larvae were feeding were attractive to females of the parasitoid, *Cotesia marginiventris* (Cresson), in flight tunnel bioassays. Analyses of the collected volatiles revealed the consistent presence of 11 compounds in significant amounts. They were: (*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexen-1-yl acetate, linalool, (*3E*)-4,8-dimethyl-1,3,7-nonatriene, indole,  $\alpha$ -*trans*-bergamotene, (*E*)- $\beta$ -farnesene, (*E*)-nerolidol, and (*3E,7E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. A synthetic blend of all 11 compounds was slightly less attractive to parasitoid females than an equivalent natural blend. However, preflight experience with the synthetic blend instead of experience with a regular plant-host complex significantly improved the response to the synthetic blend. Our results suggest that *C. marginiventris* females, in their search for hosts, use a blend of airborne semiochemicals emitted by plants on which their hosts feed. The response to a particular odor blend dramatically increases after a parasitoid experiences it in association with contacting host by-products.

**Key Words**—Hymenoptera, Braconidae, *Cotesia marginiventris*, corn, parasitoid, host searching, semiochemicals, synomones.

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## INTRODUCTION

Although many studies have demonstrated the attraction of parasitoids to volatile semiochemicals, only a few of these chemicals have been isolated and identified (Weseloh, 1981; Vinson, 1981; Eller, 1990). Recent findings that show that responses to semiochemicals by parasitoids are often flexible and can be modified by experience (e.g., Alphen and Vet, 1986; Lewis and Tumlinson, 1988; Vet and Groenewold, 1990) have led to increased interest in the subject from the biological control perspective. It appears possible to condition parasitoid females with specific chemicals so that their subsequent responses to these chemicals are intensified significantly (Lewis and Tumlinson, 1988). This may have positive consequences for mass release programs that so far have suffered from low searching efficiency and high departure frequencies from target areas by released parasitoids (Lewis and Nordlund, 1985). Prerelease experience with volatiles that will guide the wasps to larval pests in a target area is likely to increase the rate of parasitization and, thereby, the effectiveness of the wasps as control agents. To make this possible, identification and formulation of the essential semiochemicals will be necessary.

*Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), a larval endoparasitoid, attacks many economically important Lepidopterous species such as *Spodoptera frugiperda*, *S. exigua*, *Pseudoplusia includens*, and *Heliothis* spp. (Lepidoptera: Noctuidae). The parasitoid frequently causes high mortality among these pests (e.g., Tingle et al., 1978; Pair et al., 1982; McCutcheon and Turnipseed, 1981), but might be much more effective after well-timed augmentative releases. Like other parasitoids, *C. marginiventris* increases its responses to volatile semiochemicals emitted by a plant-host complex after experiencing certain contact kairomones in association with these volatiles (Dmoch et al., 1985; Turlings et al., 1989, 1990a). Several host-derived cues stimulate close-range searching behavior in *C. marginiventris* (Loke et al., 1983; Loke and Ashley, 1984a-c). However, plants damaged by the host larvae are the main source of the volatiles (synomones) that attract the wasp in the vicinity of hosts (Turlings et al., 1991). Here we present the results of a study in which these synomones were collected, tested for their attractiveness to the parasitoid, and eventually identified. The activity of the identified compounds was confirmed by testing synthetic versions in flight-tunnel bioassays.

## METHODS AND MATERIALS

*The Insects.* *Cotesia marginiventris* were reared and held as described by Turlings et al. (1989). All flight-tunnel tests were conducted with 3- to 5-day-old mated females 4-8 hr into the photophase. In all cases, late second- or early

third-instar beet armyworm (BAW), *Spodoptera exigua* (Hübner), larvae were used as hosts in the experiments. They were reared according to the procedure described by King and Leppla (1984).

*Mass Collections of Volatiles for Bioassays and Identification.* Volatiles released by BAW larvae feeding on corn seedlings were collected using an all glass push-pull odor collection system. Before entering the system, air was humidified in a gas dispersion tube and purified with in-line activated charcoal filters. The air then entered the first of three Pyrex glass tubes connected in series. The first tube contained a glass frit, which ensured that a laminar air flow would enter the second tube, which contained the odor source. The first tube ended in a 110- to 115-mm male ground-glass joint that was connected to a female counterpart of the second joint. The second tube was ~36 cm long (including joints) with an outside diameter of 11 cm. Its outlet was a 50- to 55-mm male ground-glass joint that fitted the inlet of the third tube, which ended in three collector ports. To optimize the collection, two ports were used simultaneously by attaching stainless-steel tubes (0.64 cm OD  $\times$  0.5 cm ID) to each port. The third port was not used and was sealed with a glass stopper. A collection trap was connected to the upwind end of each steel tube, while the downwind end was connected with Tygon tubing to Alborg flowmeters, which were connected to house vacuum.

Collection traps consisted of 3.7-mm-ID  $\times$  4-cm-long glass tubes with a 325-mesh stainless-steel frit sealed across the diameter of each tube about 4 mm from the upwind end. Approximately 50 mg of Super Q adsorbent (80-100 mesh) (Alltech, Deerfield, Illinois) was placed on top of the frit and held in place with a small plug of glass wool. Before each collection, the traps were rinsed with methylene chloride.

In the collection chamber, we placed 100 early third-instar BAW larvae on 40 10-day-old greenhouse-grown corn seedlings that were cut just before collection, and their stems were wrapped in wetted cotton. With the odor source and traps in place, a collection was started by balancing house air and house vacuum so that approximately 300 ml/min passed through each trap and the pressure was only slightly higher inside the system than outside. The pressure could be read with a user-designed glass pressure gauge that was connected to a side arm at the downwind part of the collection chamber.

Each collection was run for 24 hr. After 10-12 hr, traps were removed and extracted with 300  $\mu$ l of pure methylene chloride, then connected back on to the system and extracted once more after another 12-14 hr. The amount of volatiles collected is expressed in collection minute equivalents (CME). Each collection contains 1440 CME (= 24 hr).

*Collections for Quantifications.* For more exact quantification of the volatiles released by a complete plant-host complex, a smaller system was used. The glass collection chambers consisted of two parts. Purified air entered the

first part through a 2-cm-long  $\frac{1}{4}$ -in.-OD inlet, which widened into a section (6 cm long; 3 cm ID) containing a glass frit. The second part, also 3 cm in diam, was 15 cm long with a 2-cm-long  $\frac{1}{4}$ -in. outlet. The second part contained the odor source. Both parts had fitting glass ball joints that were clamped together. One collection trap with 25 mg Super Q adsorbent was connected to the  $\frac{1}{4}$ -in. outlet with a brass Swagelock fitting containing Teflon ferules. Three corn seedlings that had been fed upon overnight were placed with 15 BAW in the chambers. Volatiles were collected for 1 hr (300 ml/min). Each trap was rinsed with 200  $\mu$ l methylene chloride, and internal standards were added (1  $\mu$ g each of octane and nonyl-acetate in 50  $\mu$ l methylene chloride). Of each collection sample (total volume 250  $\mu$ l), 1–2  $\mu$ l was analyzed using capillary gas chromatography (GC) (see below). The collection was repeated on six different days with new plant and herbivore material. The same procedure was followed to collect volatiles from undamaged seedlings. Collections with only wetted cotton wool inside the chamber were performed to determine system impurities.

*Collections of Different Components of Complete Plant-Host Complex.* The above procedure also was used to collect volatiles of the three main components of a complete plant-host complex. A complex of three overnight damaged corn seedlings was divided into larvae, frass, and damaged leaves as described by Turlings et al. (1991). The larvae were starved for 2 hr and then washed with water. The frass was wiped off the leaves with two pieces of cotton wool (one wet and one dry), and the damaged leaves were washed with water to remove any remaining larval by-products. Volatiles of the three components and of a complete plant-host complex were collected simultaneously for 3 hr. Collections were repeated five times, and GC analyses were used to determine the volatiles present in each of the samples.

*Chemical Analyses.* Analyses were conducted on a Varian model 3700 GC and a Hewlett-Packard model 5890 GC, both equipped with split-splitless capillary injector systems and flame ionization detectors. Data collection, storage, and subsequent analysis was performed on a Perkin Elmer chromatographic data system. Helium at a linear flow velocity of 19 cm/sec was used as a carrier gas. Most of the initial analyses of the volatile collections were performed on two fused silica capillary columns. They were 50 m  $\times$  0.25 mm ID with a 0.25- $\mu$ m-thick film of bonded methyl silicone (007) and 50 m  $\times$  0.25 mm ID with a 0.25- $\mu$ m-thick film of bonded cyanopropyl methyl silicone (CPS-1). Both columns were obtained from Quadrex Corporation (New Haven, Connecticut) and were run at an initial temperature 50°C for 3 min, then temperature programmed at 5°C/min to 180°C. All injections of 1–3  $\mu$ l were made in the splitless mode.

Samples were also analyzed by GC-mass spectroscopy (GC-MS) with a Nermag model R1010 mass spectrometer in both the electron impact and the

chemical ionization modes. The methyl silicone and CPS-1 columns used in the previous analyses were used in the GC-MS analyses with helium as carrier gas. Methane and isobutane were used as reagent gases for chemical ionization.

Vapor-phase infrared spectra were obtained from a Nicolet 20SXC GC-FTIR spectrometer interfaced to a Hewlett-Packard model 5890 GC. Samples were introduced to the FTIR via the methyl silicone column described above.

Several compounds that required [ $^1\text{H}$ ]NMR analysis for full identification were analyzed with a Nicolet 300 MHz Fourier transform NMR spectrometer interfaced to a Nicolet model 1280 data system. The natural compounds as well as synthetic standards were purified by micropreparative GLC on a 30-m  $\times$  0.53-mm-ID SPB-1 (1.5- $\mu\text{m}$  film thickness) column (Supelco) in a Hewlett-Packard model 5890 gas chromatograph. To allow injection of large volumes (up to 100  $\mu\text{l}$ ), a deactivated column (30 m  $\times$  0.53 mm, Quadrex Corporation) preceded the SPB-1 column [see Grob (1982) for details on this technique]. Just before entering the detector, the effluent from the column was split, one part going to the FID detector, the other exiting into a collector as originally designed by Brownlee and Silverstein (1968). The split ratio was manipulated so that  $>95\%$  of the sample entered a glass capillary collection tube (35 cm long, 1.2 mm ID) in the collector. Dry Ice in acetone was used to cool the part of the capillary tube furthest away from the GC. Tubes were inserted into the collector just prior to elution of the compound to be collected and removed immediately after elution. For collection of the most volatile compounds, approximately 8 mg of Super Q adsorbent was packed between two glass wool plugs at the cold end of a collection tube. Temperature programs varied for the collection of different compounds. The collected material was transferred into an NMR tube by rinsing the glass capillary with approximately 25  $\mu\text{l}$  of benzene- $\text{D}_6$ . The NMR tubes were 5 mm (OD) at the top, with a 50  $\times$  2-mm (OD) coaxial extension at the bottom (Wilma Glass Company, Buena, New Jersey). Data points were collected with a 6- $\mu\text{sec}$  pulse (90° tip angle). Where necessary, proton decoupling was accomplished by standard decoupling techniques (decoupler power ca. 0.5 W).

All spectra of the natural products were compared with those of candidate synthetic compounds. (3*E*)-4,8-Dimethyl-1,3,7-nonatriene and (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene were synthesized by the Wittig reaction of geranial and farnesal, respectively, with methylenetriphenyl phosphorane (analogous to Maurer et al., 1986).  $\alpha$ -*trans*-Bergamotene was provided by Douglas B. McIlwaine at Brown University, Providence, Rhode Island. All other synthetic standards used in this study were obtained from commercial sources.

*Flight-Tunnel Bioassays.* The Plexiglas flight tunnel described by Turlings et al. (1991) was used to test the attractiveness of specific samples to females

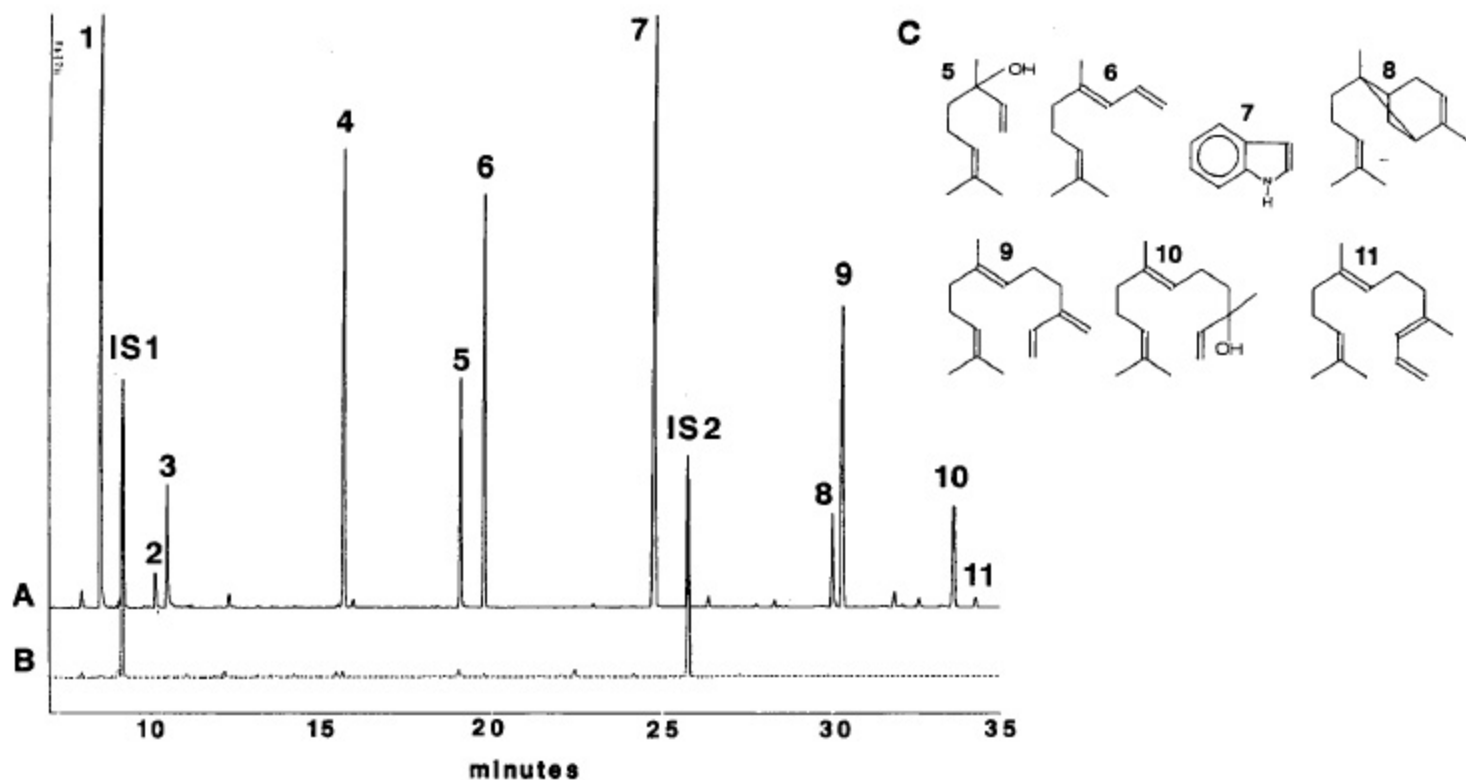


FIG. 2. (A) Chromatogram of volatiles released by a complex of corn seedlings damaged by BAW larvae. The 11 major compounds were identified as 1, (*Z*)-3-hexenal; 2, (*E*)-2-hexenal; 3, (*Z*)-3-hexen-1-ol; 4, (*Z*)-3-hexen-1-yl acetate; 5, linalool; 6, (*3E*)-4,8-dimethyl-1,3,7-nonatriene; 7, indole; 8,  $\alpha$ -*trans*-bergamotene; 9, (*E*)- $\beta$ -farnesene; 10, (*E*)-nerolidol; 11, (*3E,7E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. (B) Chromatogram of volatiles released by undamaged corn seedlings. (C) Structures of the last seven compounds with corresponding peak numbers. Chromatograms were obtained by analysis of the volatiles on a 50-m 007 methyl silicone column (0.25 mm ID, 0.25  $\mu$ m film thickness), after a 2-hr collection. IS1 and IS2 are the internal standards *n*-octane and *n*-nonyl-acetate.

TABLE 1. COMPOUNDS IDENTIFIED IN VOLATILES COLLECTED FROM CORN SEEDLINGS FED UPON BY BAW LARVAE

Peak <sup>a</sup>	Compound	Kovats <sup>b</sup>	Amount (ng/hr <sup>c</sup> )	Relative %
1	(Z)-3-hexenal	775	2015 (785)	5-21
2	(E)-2-hexenal	834	310 (108)	1-4
3	(Z)-3-hexen-1-ol	845	502 (118)	2-5
4	(Z)-3-hexen-1-yl acetate	991	2058 (286)	12-20
5	Linalool	1089	427 (40)	2-4
6	(3E)-4,8-dimethyl-1,3,7-nonatriene	1110	676 (140)	3-7
7	Indole	1266	2333 (617)	9-26
8	$\alpha$ -trans-bergamotene	1441	834 (503)	2-9
9	(E)- $\beta$ -farnesene	1451	3153(1867)	8-35
10	(E)-nerolidol	1551	947 (329)	6-10
11	(3E,7E)-4,8,12-trimethyl-1,3,7,11-tri-decatetraene	1569	61 (16)	0-1

<sup>a</sup>Correspond with peak numbers in Figure 2.

<sup>b</sup>Kovats GLC index (Kovats, 1965) for the 48-m methyl silicone capillary column.

<sup>c</sup>Average amounts released by three corn seedlings that had been fed upon overnight. During the 1-hr collections, 15 BAW larvae fed on the seedlings ( $n = 6$ ). Standard deviations are shown in parentheses.

(Visser et al., 1979; Stenhagen et al., 1974). They were (Z)-3-hexenal, (E)-2-hexenal, (Z)-3-hexen-1-ol, (Z)-3-hexen-1-yl acetate, linalool, and indole. All are commonly found in plants (e.g., Visser et al., 1979; Tollsten and Bergström, 1988). Compounds 6 and 8-11 are less common but have all been reported several times as plant-produced chemicals (see Discussion). Synthetic candidates for these compounds, when analyzed by GC, had the same retention times on the two different columns as their natural versions. Moreover, MS, IR and [<sup>1</sup>H]NMR spectra were identical to the natural products. The [<sup>1</sup>H]NMR spectra matched with the spectra reported by others: (3E)-4,8-dimethyl-1,3,7-nonatriene and (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (Maurer et al., 1986; Dicke et al., 1990a),  $\alpha$ -trans-bergamotene (Corey et al., 1971), (E)- $\beta$ -farnesene (Bowers et al., 1972), and (E)-nerolidol (Dorskotch et al., 1980).

*Sources of Collected Volatiles.* The damaged plants released, by far, most of the volatiles (Figure 3). All compounds were collected from the damaged leaves in significant amounts, except for compounds 1-3. Preliminary data, however, indicated that when starved larvae are allowed to feed on the leaves, the three most volatile compounds are released in large amounts. Clearly, the highly volatile compounds are only released in the observed amounts as a result of active plant damage. No detectable amounts of the 11 volatiles were released

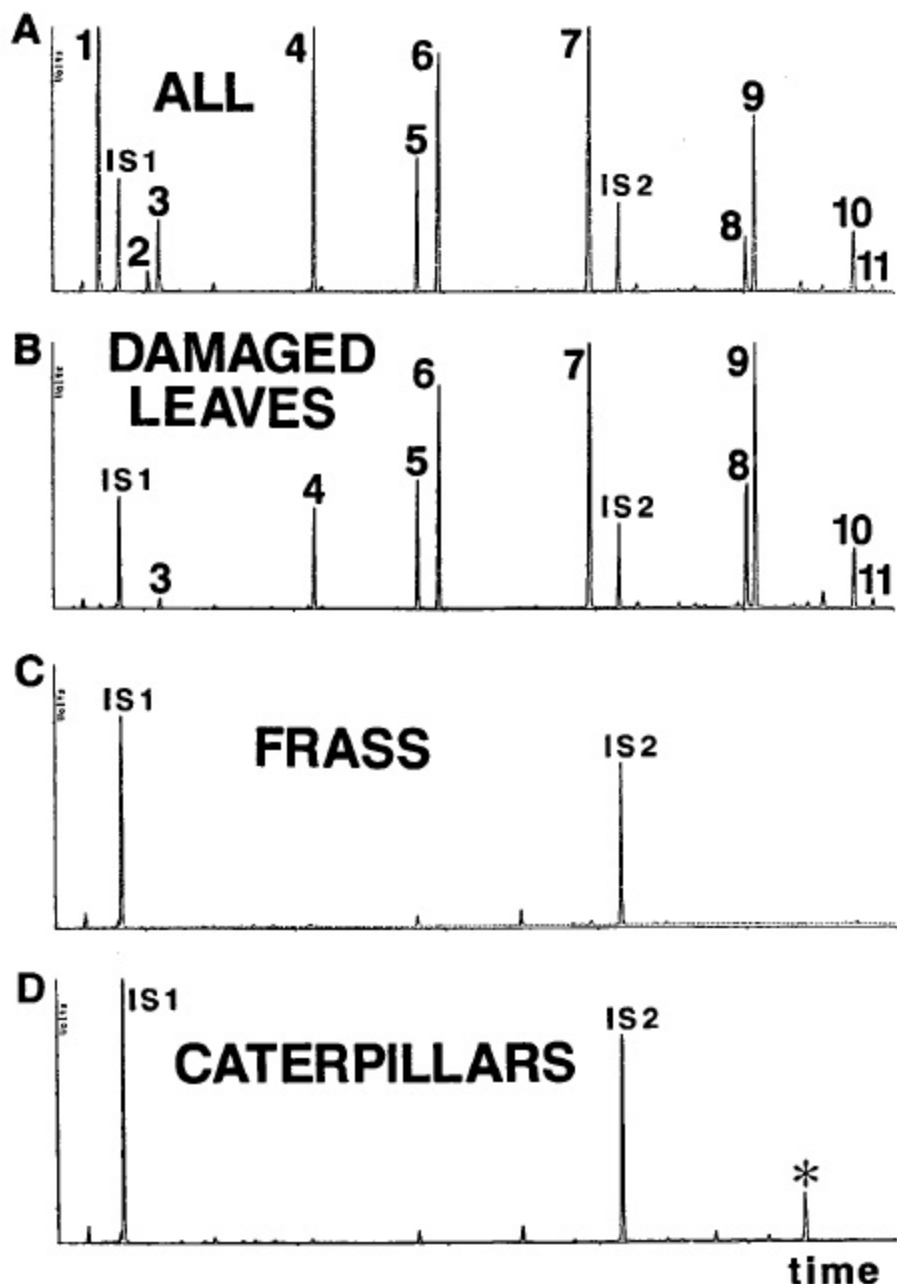


FIG. 3. Volatiles released by different components of a complete plant-host complex: (A) complete complex of BAW larvae feeding on corn seedlings; (B) water-washed corn seedlings that were damaged by BAW larvae; (C) BAW frass wiped from the damaged corn seedlings; and (D) starved water-washed BAW larvae. Peak numbers correspond with those in Figure 1. The asterisk marks pentadecane, a volatile emitted by the oral secretions from BAW larvae. Details on chromatography and internal standards are given with Figure 1.

by frass or larvae (Figure 3). In the collections of the starved larvae, however, an additional peak showed up. This compound was emitted by oral secretions that the larvae regurgitated while fighting among themselves. This was confirmed when collections from hexane extracted filter paper saturated with the oral secretion resulted in the same peak. The compound was identified as pentadecane by GC-MS.

*Responses to Synthetic Odor Blend.* A collected natural blend and a synthetic mimic (Table 2) were used in further bioassays. Collection procedures were different for Table 1 and Table 2. Volatiles in Table 1 were collected for only 2 hr the day after the larvae had started feeding on the corn seedlings. In contrast, the collections for Table 2 started with larvae on fresh leaves and were continued over a 24-hr period. Several compounds are not released when the larvae just start feeding (Turlings et al., 1990b). Hence, the large differences obtained for some of the major compounds.

A majority of the insects would fly to the extracts of volatiles collected from BAW feeding on corn seedlings (Table 3). Females that were experienced on the synthetic blend and those that had a natural experience responded equally well to the naturally derived volatiles. However, experience did make a difference for the females that were tested to a synthetic blend of the identified compounds. Significantly fewer wasps that had received a natural experience flew

TABLE 2. QUANTITATIVE COMPARISON OF NATURAL AND SYNTHETIC BLENDS USED FOR BIOASSAYS

Peak <sup>a</sup>	Compound	Relative % <sup>b</sup>	
		Natural	Synthetic
1	(Z)-3-hexenal	49.3	48.5
2	(E)-2-hexenal	1.7	1.8
3	(Z)-3-hexen-1-ol	10.4	10.3
4	(Z)-3-hexen-1-yl acetate	15.3	14.9
5	Linalool	3.2	2.8
6	(3E)-4,8-dimethyl-1,3,7-nonatriene	2.5	2.3
7	Indole	2.3	2.2
8	$\alpha$ -trans-bergamotene	3.2	3.4
9	(E)- $\beta$ -farnesene	10.0	11.7
10	(E)-nerolidol	1.6	1.6
11	(3E,7E)-4,8,12-trimethyl-1,3,7,11-tri-decatetraene	0.5	0.5

<sup>a</sup>Peak numbers in Figure 2.

<sup>b</sup>Percentage of total amount in blend (for each test sample 1% = 205 ng).

TABLE 3. FLIGHT-TUNNEL RESPONSES OF *C. marginiventris* FEMALES WITH DIFFERENT EXPERIENCES TO METHYLENE CHLORIDE EXTRACTS OF SUPER Q-TRAPPED VOLATILES COLLECTED FROM BAW LARVAE FEEDING ON CORN SEEDLINGS (NATURAL), SYNTHETIC MIMIC OF SAME VOLATILES CONTAINING 11 MAJOR COMPOUNDS IN METHYLENE CHLORIDE SOLUTION (SYNTHETIC), AND METHYLENE CHLORIDE ONLY (SOLVENT)

Odor source <sup>a</sup>	Experienced odor	n	Number of flights	Average % of flights <sup>b</sup>
Natural	NATURAL	36	24	66.7 (16.7) A
	SYNTHETIC	36	21	58.3 (16.0) A
Synthetic	NATURAL	36	11	30.6 (11.5) B
	SYNTHETIC	36	19	52.8 (15.0) A
Solvent	NATURAL	36	0	0.0 C

<sup>a</sup>Blends from Table 2 were used in amounts equivalent to 200–300 CME (1% = 25 ng).

<sup>b</sup>On six different days six females were tested for each treatment. Standard deviations in parenthesis. Analysis of variance was performed on the daily percentages after arcsin transformation, followed by Duncan's multiple-range test. Different letters indicate significant differences between treatments ( $P < 0.05$ )

to the synthetic blend than did females that had received a synthetic experience. The responsiveness exhibited by females with a synthetic experience to a synthetic odor blend did not differ from that of females that were tested to the natural extract. None of the tested females flew to the solvent alone.

#### DISCUSSION

*C. marginiventris* females responded in a dose-related manner to the collected volatiles (Figure 1). The reduced response at the highest dose tested indicated that for optimal bioassay results release rates should be balanced carefully. By applying the extracts to filter paper, the obtained release rates were obviously far from natural. Before the importance of each individual compound in the attraction of *C. marginiventris* can be determined, exact formulations resulting in release rates similar to a natural situation (Table 1) must be obtained.

The technique of collecting plant volatiles directly from an airstream passed over the odor source has some major advantages over the often applied harsh methods of extraction or steam distillation. The latter two techniques give no indication of how much of each identified compound is actually released into the environment and may result in destruction or isomerization of essential chemicals. This may account for our finding that (Z)-3-hexenal was one of the major components, whereas elsewhere it is seldom reported as a plant volatile. Due to its high volatility, (Z)-3-hexenal is much harder to collect than the other

green leaf volatiles, including (*E*)-2-hexenal. This is illustrated by our preliminary attempts to re-collect the compounds from the GC as described earlier. Initially, a (U-) tube immersed in liquid nitrogen was used; collection efficiency was extremely poor for (*Z*)-3-hexenal, while it was high for all other volatiles. Only when the collection tubes were used that contained a small amount of Super Q adsorbent was it possible to collect (*Z*)-3-hexenal effectively. High amounts of (*Z*)-3-hexenal are not only characteristic for BAW damage or corn. Preliminary results showed that artificial damage of corn and damage of other plants (i.e., cotton, tomato, and cowpea) resulted in the release of similar relative amounts of (*Z*)-3-hexenal (unpublished data). Buttery et al. (1987) reported on the fast isomerization of (*Z*)-3-hexenal [probably into (*E*)-2-hexenal] in crushed tomato leaves. Our respective results suggest that damaged green leaves release much more (*Z*)-3-hexenal than previously reported.

When Buttery and Ling (1984) collected the volatiles of corn plants that were cut at the stem, they also found (*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexen-1-yl acetate, linalool, and (*E*)- $\beta$ -farnesene. Thompson et al. (1974) extracted the essential oil from corn and identified 59 compounds, including indole and nerolidol. No previous reports on corn volatiles mention  $\alpha$ -*trans*-bergamotene, (3*E*)-4,8-dimethyl-1,3,7-nonatriene, or (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. The latter two apparently related methylene terpenoids were recently reported by Maurer et al. (1986), who found them in the oil of *Elettaria cardamomum* (cardamom oil). Kaiser (1987) (as referenced by Dicke et al., 1990a) reported these compounds from night-scented flowers of different plant species that are pollinated by moths. These compounds are released by Lima bean leaves (Dicke et al., 1990a) and cucumber leaves (Dicke et al., 1990b) that have been subjected to spider mite infestation (see below).

The terpenoid  $\alpha$ -*trans*-bergamotene appears to be rare. It has been reported from cotton (Minyard et al., 1966) and from buck's horn (staghorn sumac) (*Rhus typhina*) (Bestmann et al., 1988). (*E*)- $\beta$ -Farnesene was the most predominant terpenoid in the collections. It has been identified previously from corn leaves by Buttery and Ling (1984), but they found it in much smaller amounts relative to the other compounds. (*E*)- $\beta$ -Farnesene attracts a chalcid wasp (Kamm and Buttery, 1983) and serves as an alarm pheromone for aphids (Bowers et al., 1972; Edwards et al., 1973; Wohlers, 1981). The *E* isomer of nerolidol is present in the essential oil of several *Melaleuca* tree species (Jones and Harvey, 1936; Naves, 1960; Doskotch et al., 1980). Picker et al. (1976) found it also in the bark of the Australian tree *Flindersia laevis*. Doskotch et al., (1980), who isolated (*E,S*)-nerolidol from *Melaleuca leucadendron* leaves, showed that it functions as a feeding deterrent for gypsy moth larvae.

We still need to establish which optical isomers of linalool,  $\alpha$ -*trans*-bergamotene, and (*E*)-nerolidol are released by corn seedlings. We used racemic mixtures of the latter three in our synthetic blend, and this may explain why

females with a natural experience did not respond well to the synthetic blend (Table 3). An experience with the blend that contained the racemic mixtures, however, increased the responses to a level comparable to the responses to a natural blend. These results again illustrate the significant effects of experience on the responses to airborne semiochemicals in parasitoids. If, in the future, synthetic blends are used to condition parasitoids to perform more effectively when released in host-infested areas, the best results are likely to be obtained with the closest mimics of naturally released odors.

The damaged plants are clearly the main source of the identified compounds (Figure 3). This agrees with the responses of the parasitoids to the different components of a complete plant-host complex (Turlings et al., 1991). The wasps are significantly more attracted to the damaged plants than to frass or to larvae. Undamaged plants are far less attractive (Turlings et al., 1991), and here it is shown that undamaged plants release only minute amounts of some of the compounds (Figure 2). That undamaged plants are relatively odorless may be a trait that makes them inconspicuous to herbivores. When under attack by herbivores, however, a dramatic change occurs in the number and amounts of volatiles plants release. Results similar to ours were obtained by Dicke and coworkers, who found that spider mite-infested plants initiate the release of terpenoids (Dicke and Sabelis, 1988; Dicke et al., 1990a,b). The compounds were not released by undamaged plants, nor were they detected from artificially damaged leaves. Of the compounds that we identified as being released by BAW-damaged corn, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexen-1-yl acetate, linalool, and the two methylene terpenoids also were released by spider mite-infested Lima bean (Dicke et al., 1990a). At least one of the methylene terpenoids, (*3E*)-4,8-dimethyl-1,3,7-nonatriene, is involved in the attraction of predatory mites that feed on spider mites (Dicke and Sabelis, 1988; Dicke et al., 1990a,b). Preliminary results suggest that these methylene terpenoids are released by several green plants that have been under attack by herbivores (unpublished data), and they may elicit behavioral responses in a variety of insects. These plant responses may be direct defenses against their attackers, as high terpenoid contents inhibit insect feeding in several cases (e.g., Doskotch et al., 1980; Mihaliak et al., 1987; Gunasena et al., 1988; Turlings and Tumlinson, 1991). In addition, however, the volatiles released by the injured plants may serve as signals to attract natural enemies of herbivores (Dicke and Sabelis, 1988; Dicke et al., 1990a,b; Turlings et al., 1990b; Turlings and Tumlinson, 1991).

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