

# Parasitic wasps orient to green leaf volatiles\*

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## Summary

Undamaged plants emit low levels of green leaf volatiles (GLVs), while caterpillar-damaged and artificially damaged plants emit relatively higher levels of certain GLVs. Female braconid parasitoids, *Microplitis croceipes*, oriented to both damaged plants and to individual GLVs in no-choice tests in a wind tunnel, but seldom oriented to undamaged plants. Female ichneumonid parasitoids, *Netelia heroica*, also oriented to individual GLVs in a wind tunnel. Males of both wasp species failed to orient to the GLVs. These data show that leaf-feeding caterpillars can cause the release of GLVs, and that parasitic wasps can respond to these odors by flying upwind (chemoanemotactic response), which brings the wasps to their caterpillar

hosts. This supports the hypothesis that plants communicate with members of the third trophic level, *i.e.*, plants under herbivore attack emit chemical signals that guide natural enemies of herbivores to sites of plant damage. In this interaction, the GLVs serve as tritrophic plant-to-parasitoid synomones. That parasitoids from two different wasp families oriented to GLVs suggests that the response may be widespread among the Hymenoptera.

## Key words

green leaf volatile, semiochemical, synomone, volatile attractant, tritrophic, host location, parasitoid behavior, Hymenoptera, Braconidae, *Microplitis*, Ichneumonidae, *Netelia*, Lepidoptera, Noctuidae, *Heliothis*

## Introduction

Plant chemicals are important in host finding by wasp parasitoids of herbivorous insects (Eller *et al.* 1988a; Sugimoto *et al.* 1988; Whitman 1988a; Ding *et al.* 1989; Navasero & Elzen 1989; Sheehan & Shelton 1989; Martin *et al.* 1990). However, few of these attractive plant odors have been identified (Whitman 1988a). In this paper we show that green leaf volatiles (GLVs) are released from plants as caterpillars feed, and that some GLVs are attractive to parasitic wasps in a wind tunnel. We speculate that in nature parasitic wasps use GLVs to orient to sites where caterpillars are damaging leaves. These GLVs would thus serve as plant-to-parasitoid synomones.

The GLVs, also known as green leaf odors, are six carbon alcohols, aldehydes and derivative esters commonly found in green plants (Visser *et al.* 1979; Hamilton-Kemp *et al.* 1988, 1989; Hernandez *et al.* 1989), including *Heliothis zea* food plants (Thompson *et al.* 1971; Hedin *et al.* 1973, 1975; Liu *et al.* 1988; Lwande *et al.* 1989; Dicke *et al.* 1990). The GLVs are released or produced when plant tissue is cut (Saijo & Takeo 1975; Wallbank & Wheatley 1976; Buttery *et al.* 1985; Tollsten & Bergström 1988) and are responsible in part for the odor of damaged leaves (*e.g.*, the odor of fresh-mowed grass). The adaptive significance of GLVs is unknown.

The two wasp species used in this study seek out and oviposit into plant-feeding caterpillars. *Microplitis*

*croceipes* (Cresson) is a braconid parasitoid of the corn earworm caterpillar *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae). Its host seeking behavior and biological control potential for *H. zea* have been well studied (Drost *et al.* 1986, 1988; Eller *et al.* 1988a, b; Powell *et al.* 1989). *Netelia heroica* Townes is a nocturnal ichneumonid parasitoid of various species, including *Heliothis* spp. (Townes 1939). The three plant species used in our experiments (cotton, cowpea, and hyacinth bean) serve as *H. zea* food plants (Johnson *et al.* 1986).

## Materials and methods

### Insects

Larval *M. croceipes* were reared on *Heliothis zea* at the IBPMR Laboratory at Tifton, Georgia. The *Heliothis* were cultured on artificial diet (Burton 1969). Adult parasitoids were kept communally and allowed to mate in Plexiglas cages (30 × 30 × 17 cm) at 28 °C, at 50–70% relative humidity, with a 16:8 light-dark photoperiod, and they were provided daily with fresh water and honey (Lewis & Burton 1970).

*N. heroica* were collected in the field from Tifton, Georgia and thereafter cultured at the IBPMR Laboratory on *Heliothis virescens* (Fabricius). Adult *N. heroica*

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were kept communally and allowed to mate in  $45 \times 38 \times 38$  cm screen cages under similar environmental conditions as described for *M. croceipes*.

### Plants

Cotton (*Gossypium hirsutum* L.) and purple-hull cowpea (*Vigna unguiculata* L.) seedlings were grown in a greenhouse. Hyacinth bean (*Dolichos lablab* L.) leaves were obtained from mature field-grown plants.

### Collection and analysis of volatiles

Plant volatiles were collected using the volatile collection system described by Eller *et al.* (1988a) with Super-Q (80/100 mesh; Alltech, Deerfield, IL) as the adsorbent and extracted in methylene chloride. Volatiles were collected from undamaged, artificially damaged and caterpillar-damaged cowpea seedlings. Volatiles from undamaged cowpea were collected from three cowpea seedlings with their roots removed and the cut end wrapped with wet cotton. Artificially damaged cowpeas were treated as described for undamaged cowpeas except each seedling had two 9-mm diameter holes punched in its leaves. Caterpillar-damaged cowpea odor was collected from cowpeas on which third and fourth instar *Heliothis* larvae were feeding. Three collections were made for each of the three treatments.

Collected volatiles and synthetic compounds were analyzed by gas-liquid chromatography (GLC) to determine the relative amounts of GLVs present in the various treatments. GLC analyses were conducted on Varian model 3700 and Hewlett-Packard model 5890 gas chromatographs, equipped with flame ionization detectors. A Perkin Elmer Chromatographics 3 data system was used for data collection, storage, and analysis. Injections were made in the splitless mode and after 30 seconds the injector was changed to the split mode. Fused silica capillary columns, OV-101 (0.25  $\mu\text{m}$ , 50 m  $\times$  0.25 mm ID) and CPS-1 (0.25  $\mu\text{m}$ , 50 m  $\times$  0.25 mm ID), were used in the Varian and Hewlett-Packard gas chromatographs, respectively. Helium was used as the carrier gas (linear flow velocity of 18.0 cm/s). The temperature program was: initial temperature of 40°C for 1 min, then increased 5°C/min to 200°C. The injector and detector temperatures were 220°C and 260°C, respectively. The internal standards used were n-octane and nonyl acetate.

GLC-mass spectral analyses were conducted with a Nermag model R 10-10 mass spectrometer in the electron impact (EI) mode using an ionization energy of 70 eV. The mass spectrometer was interfaced to a Hewlett Packard model 5890 gas chromatograph equipped with a Hewlett Packard on-column injection system. A fused silica SPB-1 capillary column (0.25  $\mu\text{m}$ , 30 m  $\times$  0.25 mm ID) was used. Helium was used as the carrier gas (linear flow velocity of 18.0 cm/s). The temperature program was: 60°C for 1 min, then increased 20°C/min to 230°C.

### Flight tunnel

We tested the ability of wasps to fly to plant volatiles in a  $75 \times 75 \times 200$  cm laminar-flow Plexiglas wind tunnel (wind speed: 31 cm/s; temperature: 26–27°C; relative

humidity: 40–80% RH; chamber light provided by four overhead 80 W fluorescent bulbs). An electric fan, placed outside the building, pulled air through the tunnel and vented it outside to avoid contamination from recycled air.

### Chemicals

The green leaf volatiles used in this study (*E*-2-hexenal, hexanal, *Z*-3-hexen-1-ol, *E*-2-hexen-1-ol, *Z*-3-hexenyl acetate, *E*-2-hexenyl acetate, *Z*-3-hexenyl propionate, *Z*-3-hexenyl butyrate) were obtained from Sigma Chemical Co. and Aldrich Chemical Co. Following the tests, chemicals were analyzed by GLCMS; purity ranged from 93–99%.

### Preparation of water extract of *H. zea* frass

Ten grams of fresh moist *H. zea* frass (collected from caterpillars feeding on cowpea seedlings) was thoroughly blended with 100 ml of distilled water. The resultant slurry was filtered through Whatman No. 1 filter paper, producing a green-colored filtrate. This filtrate was kept frozen and served as the stock extract throughout the tests. To produce the dried extract, approximately 1 ml of stock extract was unfrozen and shaken. Then, 30  $\mu\text{l}$  of extract was collected into a capillary tube and spotted onto the center of a 110 cm disk of Whatman No. 1 filter paper. After the 1 cm diameter moist area dried (~7 min), the filter paper was used to stimulate wasps.

### Bioassays

*General methods* – Insects were tested individually; all trials were single choice tests. Treatments were balanced for sex, day, and time of day. The odor source was placed in the flight chamber 13 cm above the chamber floor, along the longitudinal midline of the chamber, and 20 cm downwind from the upwind end of the chamber.

In tests using *M. croceipes*, wasps were 3–7 days old and were taken from stock cultures never exposed to plant or host materials as adults. Hence, they were naive. Prior to testing, each wasp was assigned to one of two groups: (1) "Naive" wasps were transferred directly from their communal stock cage in a clean 4 ml shell vial, which was immediately placed in a vertical position, directly downwind from the odor source. (2) "Stimulated" wasps were treated in a similar manner except that prior to placement in the wind tunnel, they were allowed to contact the dried water extract of *H. zea* frass for 30 s. When female wasps contacted the dried extract they usually performed an intense and stereotypic antennation behavior (Lewis & Jones 1971; Lewis & Tumlinson 1988). The small percentage (9%) of females that did not perform antennation behavior was discarded. Male *M. croceipes* showed little interest in the extract; for males, pre-flight stimulation consisted of allowing the insect to walk on or near the dried extract for a total of 30 s. When placed in the wind tunnel, wasps usually walked upwards to the opening of the shell vial, encounter the lower portion of the odor plume, faced into the wind, and after 5 s to 5 min took flight. Wasps that did not take flight in 5 min or wasps incapable of normal flight were discarded. If a wasp flew toward and landed on the odor

source or a 1 cm<sup>2</sup> paper target placed near the odor source, a positive response was recorded and the trial was ended. If the wasp took flight, but failed to land on the odor source or adjacent target, a negative response was recorded. Each wasp was given two successive chances to fly to the odor source, and then was discarded.

In contrast to the *M. croceipes*, none of the *Netelia heroica* were naive; all had experienced host or host-plant odors, and most of the females had previously oviposited into a *Heliothis* caterpillar. Immediately prior to testing, each *N. heroica* was either "stimulated" or "not-stimulated" (see below).

**Attraction of wasps to damaged versus undamaged plants** – To investigate the ability of *M. croceipes* to orient to plants, individual female wasps were exposed to one of seven possible odor sources (no odor and damaged or undamaged cotton, cowpea, or hyacinth bean). Whole cotton or cowpea seedlings (~2 weeks old with ~9 cm<sup>2</sup> leaf surface) were used. Roots were placed in an 8 ml vial containing water, with cotton, wrapped around the stems, as a plug for the vial. For hyacinth bean, a leaf of ~9 cm<sup>2</sup> area from a mature flower-bearing plant was placed with a cotton plug in a water-filled shell vial. Plants were either placed directly into the wind tunnel (undamaged) or were damaged first by making three 1 cm long cuts in leaves with scissors. Old plants were replaced with new ones every half-hour. A water-filled vial with a cotton plug and a 9 cm<sup>2</sup> filter paper target served as a control. Wasps were released 30 cm downwind from the odor source. Thirty naive females were tested to each treatment.

**Release and calibration of green leaf volatiles** – Sustained evaporative release of GLVs was obtained from 0.5 µl capacity microcapillary tubes (Drummond Co). filled with 10 mm of a selected green leaf volatile substance. Each capillary tube was attached to a vertically positioned disposable glass pipette so that it was suspended crosswise in the wind tunnel, but at a 45° angle from vertical. This arrangement assured that the lower meniscus of test liquid always remained just at the lower orifice of the tube, producing a uniform evaporative surface. If the tube was placed vertically or horizontally, uniform evaporation was not achieved; with a vertical tube, a droplet of test liquid sometimes emerged from the tube, increasing volatilization. With a horizontal tube, capillary action sometimes pulled the liquid into the center of the tube, decreasing volatilization. A 1 cm<sup>2</sup> disk of white filter paper held vertical and crosswise to the wind direction served as a target. It was attached to the capillary tube so that the lower end of the disk was 3 mm from the lower end of the tube. Wasps orienting to volatiles emerging from the capillary tube usually landed on the paper target. Release rates were calculated from volumetric changes in the internal liquid when the tubes were kept in the wind tunnel for 24 h. Mean release rates (N = 5) for the eight GLVs tested ranged from 7.4 to 52 µg/h.

**Attraction of *Microplitis croceipes* wasps to individual green leaf volatiles** – Naive and stimulated male and female *M. croceipes* wasps were tested for their ability to orient to eight different GLVs (*E*-2-hexenal, hexanal, *Z*-3-hexen-1-ol, *E*-2-hexen-1-ol, *Z*-3-hexenyl acetate, *E*-2-hexenyl acetate, *Z*-3-hexenyl propionate, *Z*-3-hexenyl butyrate) and two controls. A blank control consisted of a 1 cm<sup>2</sup> paper disk.

A hexane control consisted of a 1 cm<sup>2</sup> paper disk with hexane (released from a 0.5 µl capillary tube) as an odor source. Wasps were released 100 cm downwind from the odor source.

**Attraction of *Netelia heroica* wasps to individual green leaf volatiles** – Non-stimulated and stimulated male and female *N. heroica* wasps were tested for their ability to orient to six different GLVs (*E*-2-hexenal, hexanal, *Z*-3-hexen-1-ol, *E*-2-hexen-1-ol, *E*-2-hexenyl acetate, *Z*-3-hexenyl butyrate) in the wind tunnel. Wasps were also tested with the same blank and hexane controls as described for *M. croceipes*. All tests were conducted using the same colony of 45 individual *N. heroica*. Each day, 10 females and 10 males were removed from the communal colony, individually tested, and returned to the colony. "Non-stimulated" wasps were removed from the communal cage in a 40 ml vial and placed directly into the wind tunnel, 100 cm downwind from the odor source. "Stimulated" wasps were treated in a similar manner, except that they were allowed to contact fresh *Heliothis* frass for 10 s prior to testing. Female *N. heroica* always reacted strongly to the caterpillar frass by antennating it. Male *N. heroica* showed little interest in the frass. Each wasp was given two successive chances to orient and land on the odor source or paper target. Wasps that did not fly after 5 min were discarded.

## Results

### Attraction of *Microplitis croceipes* wasps to damaged versus undamaged plants

When naive female *M. croceipes* wasps were exposed in the wind tunnel to undamaged and artificially damaged leaves, a greater percentage of wasps successfully oriented to the damaged leaves (Table 1). This suggests that female *M. croceipes* can orient to plant volatiles, and that damaged leaves are more attractive than undamaged leaves.

### Collection and analysis of volatiles

Gas chromatograms for the three volatile collections and the synthetic GLVs are shown in Figure 1. Undamaged leaves produced only trace amounts of volatiles, while the artificially damaged and caterpillar-damaged leaves produced a greater variety and quantity of volatiles including two compounds identified as *Z*-3-hexen-1-ol and *Z*-3-hexenyl acetate. The mean ( $\pm$  SEM) collected amounts of these two compounds are shown in Table 2.

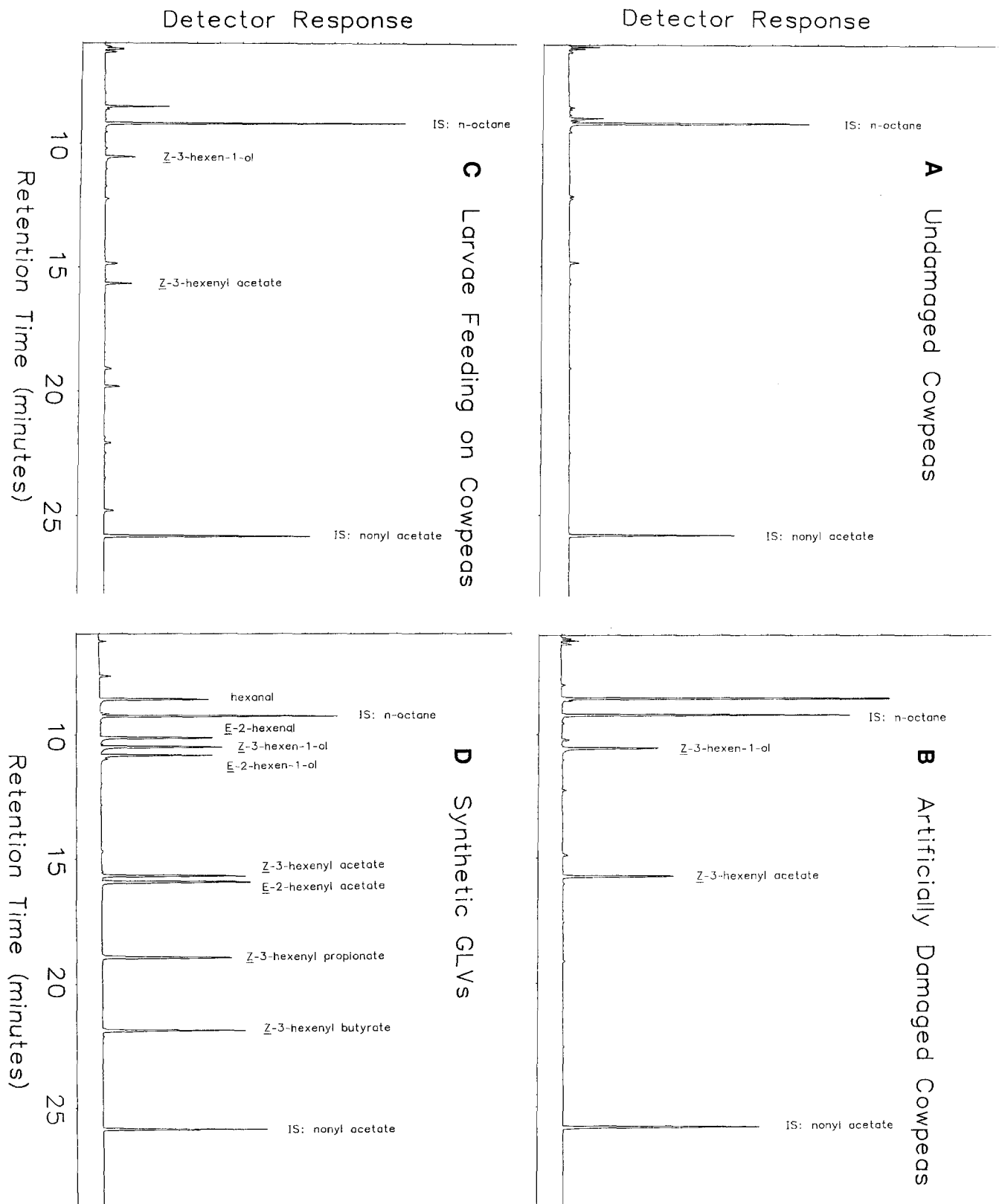
**Table 1** Response of naive female *Microplitis croceipes* wasps to undamaged and artificially damaged leaves in a wind tunnel<sup>a</sup> (N = 30/ treatment)

Plant	% Successful orientation		P
	Undamaged plant	Damaged plant	
Control (No plant)	0		
Cotton	0	13	0.057 <sup>b</sup>
Cowpea	0	23	0.0053 <sup>b</sup>
Hyacinth bean	10	43	<0.025 <sup>c</sup>

<sup>a</sup> See Methods section for experimental conditions

<sup>b</sup> Fisher exact test

<sup>c</sup> Chi-square test



**Fig. 1** Gas chromatogram (OV-101 column) of volatiles from (A) undamaged cowpea plant, (B) artificially damaged cowpea plant, (C) cowpea plant with a feeding *Heliothis zea* caterpillar, and (D) various synthetic green leaf volatiles. Internal standards n-octane and nonyl acetate are shown

**Table 2** Green leaf volatiles produced by undamaged, artificially damaged, and caterpillar-damaged cowpea seedlings

Treatment	Mean $\pm$ SEM amount produced (ng/plant h)	
	Z-3-hexen-1-ol	Z-3-hexenyl acetate
Undamaged cowpeas	trace	trace
Artificially damaged cowpeas	169 $\pm$ 21	236 $\pm$ 52
Caterpillar-damaged cowpeas	131 $\pm$ 30	93 $\pm$ 29

#### Attraction of *Microplitis croceipes* to green leaf volatiles

When tested in the wind tunnel, neither naive nor stimulated male *M. croceipes* wasps oriented to individual GLVs (Table 3); instead, males usually flew about randomly. In contrast, many of the GLVs were moderately or highly attractive to both naive and stimulated female *M. croceipes* wasps (Table 3). Stimulated females generally demonstrated a greater ability to orient than naive wasps. No wasps oriented to controls.

Wasps that made successful flights to odor sources almost always performed a stereotypic sequence of behaviors that has been previously interpreted (Drost *et al.* 1986, 1988; Eller *et al.* 1988b) as functioning in chemoanemotactic orientation. Such wasps stood on the lip of the vial, faced into the wind with their antennae spread at a 45° angle, took flight, cast back and forth into and out of the odor plume until they locked onto the center of the plume, and then flew directly upwind to the odor source. Wasps that exited the odor plume or flew beyond (upwind of) the odor source, re-initiated casting, either immediately or after returning downwind. As wasps neared the odor source, they often decelerated and hovered 5–10 cm directly downwind from the source before landing. In contrast, wasps that did not orient to the odor source seldom performed these behaviors, and instead, flew randomly about the wind tunnel and landed on the walls or ceiling. These results demonstrate that certain GLVs are highly attractive to female *M. croceipes*, that the wasps per-

form stereotypic chemoanemotactic behaviors in response to their presence, and that orientation to these volatiles is improved following preflight stimulation.

#### Attraction of *Netelia heroica* to green leaf volatiles

When tested in the wind tunnel, neither stimulated nor non-stimulated male *N. heroica* oriented to any of the GLVs (Table 4). In contrast, some GLVs were highly attractive to female *N. heroica* wasps. GLVs attractive to female *N. heroica* were not necessarily the same as those attractive to *M. croceipes*. For example, 50% of stimulated *N. heroica* females oriented to *E*-2-hexenal, while only 13% of *M. croceipes* females oriented to this compound. Conversely, no *N. heroica* oriented to Z-3-hexenyl butyrate, yet this compound elicited a response of 72% from stimulated *M. croceipes* females (Tables 3 and 4). As with *M. croceipes*, *N. heroica* females given preflight stimulation oriented more successfully in the wind tunnel. When orienting to the odor source, *N. heroica* generally did not cast back and forth, but instead flew slowly (almost hovering), directly toward the target, with their 25–30 mm long antennae spread at a 75 degree angle. Wasps never oriented to controls. These results suggest that female *N. heroica* respond to certain GLVs with chemoanemotactic behaviors, and that this response improves following preflight stimulation.

#### Discussion

Our results showed that undamaged plants released small amounts of GLVs, whereas larger amounts of GLVs were released when caterpillars fed on leaves. Female parasitic wasps oriented to both damaged plants and individual green leaf volatile substances in a wind tunnel. Based on these results, we suggest that in nature caterpillars feeding on leaves cause the release of GLVs, and that female wasps respond to the odors of these GLVs by flying upwind (chemoanemotaxis), which brings them to sites where their caterpillar hosts are feeding. Thus, GLVs can guide parasitoids to their hosts. In this interaction, the GLVs function as synomones; they benefit the plant through the reduction or elimination of herbivores, and benefit the wasps by facilitating host location.

**Table 3** Response of naive and stimulated *Microplitis croceipes* wasps to individual green leaf volatiles tested in a wind tunnel<sup>a</sup>

Odor Source	% Successful orientation			
	Males Naive N = 20	Stimulated N = 20	Females Naive N $\geq$ 30	Stimulated N $\geq$ 30
Blank control	0	0	0	0
Hexane control	0	0	0	0
<i>E</i> -2-hexenal	0	0	0	13
Hexanal	0	0	0	20 <sup>bc</sup>
Z-3-hexen-1-ol	0	0	0	50 <sup>bc</sup>
<i>E</i> -2-hexen-1-ol	0	0	10	43 <sup>bc</sup>
Z-3-hexenyl acetate	0	0	17 <sup>b</sup>	46 <sup>bc</sup>
<i>E</i> -2-hexenyl acetate	0	0	26 <sup>b</sup>	38 <sup>bc</sup>
Z-3-hexenyl propionate	0	0	33 <sup>b</sup>	67 <sup>bc</sup>
Z-3-hexenyl butyrate	0	0	56 <sup>b</sup>	72 <sup>b</sup>

<sup>a</sup> See Methods section for experimental conditions

<sup>b</sup> Significantly different from 0 at  $P < 0.05$ ; Fisher exact test

<sup>c</sup> Significantly different from naive at  $P < 0.05$ ; Fisher exact test or Chi-square test

**Table 4** Response of non-stimulated and stimulated *Netelia heroica* wasps to individual green leaf volatiles<sup>a</sup> (N = 10)

Odor Source	% Successful orientation			
	Males		Females	
	Non-stimulated	Stimulated	Non-Stimulated	Stimulated
Blank control	0	0	0	0
Hexane control	0	0	0	0
<i>E</i> -2-hexenal	0	0	20	50
Hexanal	0	0	30	50
<i>Z</i> -3-hexen-1-ol	0	0	10	30
<i>E</i> -2-hexen-1-ol	0	0	0	30
<i>E</i> -2-hexenyl acetate	0	0	40	70
<i>Z</i> -3-hexenyl butyrate	0	0	0	0

<sup>a</sup> See Methods section for experimental conditions

These findings are important because they suggest that herbivore-damaged plants can communicate with members of the third trophic level.

We believe our interpretation of the results is valid for a number of reasons. In our experiments, wasps had free access to food and water immediately prior to testing, suggesting that orientation to the damaged plants or GLVs was not for reasons of obtaining food or moisture (nectar, honeydew, etc.). Indeed, attraction to the GLV was female-specific implying a female-specific need (*i.e.*, host location). The fact that both a braconid and an ichneumonid wasp oriented to GLVs suggests that the phenomenon may be widespread among the parasitic Hymenoptera. Many ichneumonids, such as *Netelia*, are nocturnal and probably rely heavily on semiochemicals to locate hosts.

Parasitic wasps use a variety of host-produced kairomones (frass, saliva, silk, exuviae, etc.) and plant-produced synomones such as secondary plant compounds when host-searching (Vinson 1984; Nordlund *et al.* 1988; Whitman 1988a, b). Because caterpillars presumably ingest GLVs or their precursors as they feed, it is possible that GLVs emanate from caterpillar feces as well as from caterpillar-damaged leaves.

Our results show that wasps were extremely sensitive to the GLVs; *M. croceipes* females responded to release rates as low as 7.4 µg/h. We also demonstrated that naive (*i.e.*, without exposure to plant or host cues as adults) *M. croceipes* oriented to certain GLVs the first time they encountered the odors. These results imply that wasps have a highly refined, innate, and genetically based proclivity to respond to these substances and suggests a long evolutionary association between wasps and GLVs. One can speculate how such a plant-parasitoid synomonal relationship could have arisen. The parasitic Hymenoptera evolved from phytophagous Hymenoptera (Gauld 1988) that presumably had the ability to respond to host plant chemicals. Indeed, various phytophagous and carnivorous Hymenoptera respond to various GLVs or related compounds (Crewe *et al.* 1972; Blum 1976; Kamm & Buttery 1983; Veith 1984; Aldrich *et al.* 1985, 1986; Baehrecke *et al.* 1989; Thiery *et al.* 1990). During their evolution to a carnivorous life style, parasitic wasps probably retained and refined their sensitivity to plant substances. Over time, wasps that retained an ability to orient to GLVs found more hosts and left more offspring, and perhaps plants that released these volatiles at sites of herbivory suffered less herbivory.

Our results also demonstrate that the ability of wasps to orient to GLVs significantly improves following a preflight exposure. Whether this increased response is due to sensitization or learning is unknown at this point. Pre-test exposure to host-associated stimuli often improves the host-finding response of wasp parasitoids (Vet & van Opzeeland 1985; Drost *et al.* 1986, 1988; Eller *et al.* 1988a; Hérard 1988a, b; Lewis & Tumlinson 1988; Cardé & Lee 1989; Sheehan & Shelton 1989).

The two wasp species that we tested differed in their response to individual GLVs. Substances attractive to *M. croceipes* were not necessarily attractive to *N. heroica* and *vice versa*. Plant species vary quantitatively and qualitatively in GLV composition, which may allow wasps to specialize on specific plant taxa. A better understanding of the interplay between plant GLV variability and the learned and innate responses of parasitoids to GLVs could enhance biological control of crop pests.

#### The function of green leaf volatiles in plants

Our results engender a broader and perhaps more interesting question: Have green leaf and other plant volatiles evolved to serve tritrophic communicative roles for plants? In other words, do plants scream "Help!" when attacked by herbivores? Some authors have suggested this (Whitman 1988b; Dicke & Sabelis 1989; Dicke *et al.* 1990) and some indirect evidence supports such a hypothesis. First, many studies show that insect predators and parasitoids are much more attracted to damaged than undamaged plants (Sato 1979; Elzen *et al.* 1983; Loke *et al.* 1983; Odell & Godwin 1984; Nadel & van Alphen 1987; Dicke & Sabelis 1988; Whitman 1988b). Plant volatiles such as the GLVs are often released from plants only following tissue damage (Wallbank & Wheatley 1976; Tollsten & Bergström 1988; Dicke *et al.* 1990), such as when a caterpillar feeds upon a leaf. Thus, these volatiles are released at specific, relevant sites. Plants may in effect reserve the use of these chemical communication substances until they are needed. For example, some plants appear to lack high concentrations of GLVs, but quickly produce them through enzymatic reactions following leaf tissue damage (Saijo & Takeo 1975; Buttery *et al.* 1985). Phytophagous insects also orient to green leaf odors (Visser & Avé 1978; Visser 1983, 1986; Liu *et al.* 1988; Mitchell 1988; Dickens 1989; Dickens *et al.* 1990); however, once a plant is under herbivore attack, the advantage of attracting natural enemies may outweigh the disadvantage of attracting additional herbivores.

If plants use volatile synomones to communicate with natural enemies such as parasitoids, then insect herbivores should have evolved measures to counter this communication. This may be the case. Some caterpillars feed in short bouts, then move to other plant areas. Others actively fling away their fecal pellets (which may contain green leaf and other plant volatiles), or sever partially eaten leaves from the plant (Heinrich 1979; Heinrich & Collins 1983; Edwards & Wanjura 1989; Lederhouse 1990). These behaviors serve to separate herbivores from the chemical beacons that might attract parasitoids.

Alternative hypotheses for the evolutionary function of GLVs exist: 1) GLVs may defend plants against herbivores or microorganisms; 2) they may be allelopathic substances; 3) they may be plant excretory products; 4) they may serve unknown metabolic functions; 5) they may be plant pheromones; 6) they may have no purpose, but occur from incidental chemical reactions following tissue damage. These hypotheses all suffer from certain logical flaws and a paucity of supportive data. We hope to address these various hypotheses in future publications.

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### References

- Aldrich JR, Kochansky JP, Sexton JD (1985) Chemical attraction of the eastern yellowjacket, *Vespa maculifrons* (Hymenoptera: Vespidae). *Experientia* 41:420-422
- Aldrich JR, Lusby WR, Kochansky JP (1986) Identification of a new pre-daceous stink bug pheromone and its attractiveness to the eastern yellowjacket. *Experientia* 42:583-585
- Baehrecke EH, Williams HJ, Vinson SB (1989) Electroantennogram responses of *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) to chemicals in cotton. *J Chem Ecol* 15:37-45
- Blum MS (1976) Pheromonal communication in social and semisocial insects. Pp 49-60 in Kono T & Ishii S (eds) *Proceedings of a Symposium on Insect Pheromones and their Applications*. Kasumigaseki, Japan: Ministry Agric Forestry
- Burton RL (1969) Mass rearing the corn earworm in the laboratory. U.S. Dept Agric Tech Bull ARS Ser 33-134:1-8
- Buttery RG, Xu C-J, Ling LC (1985) Volatile components of wheat leaves (and stems): possible insect attractants. *J Agric Food Chem* 33:115-117
- Cardé RT, Lee H (1989) Effect of experience on the responses of the parasitoid *Brachymeria intermedia* (Hymenoptera: Chalcididae) to its host, *Lymantria dispar* (Lepidoptera: Lymantriidae), and to kairomone. *Ann Entomol Soc Am* 82:653-657
- Crewe RM, Blum MS, Collingwood CA (1972) Comparative analysis of alarm pheromones in the ant genus *Crematogaster*. *Comp Biochem Physiol* 43B:703-716
- Dicke M, Sabelis MW (1988) How plants obtain predatory mites as bodyguards. *Netherlands J Zool* 38:148-165
- Dicke M, Sabelis MW (1989) Does it pay plants to advertise for bodyguards? Toward a cost-benefit analysis of induced synomone production. Pp 341-358 in Lambers H, Cambridge ML, Konings H & Pons TL (eds) *Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants*. The Hague: SPB Academic Publishing
- Dicke M, Van Beek TA, Posthumus MA, Ben Dom N, Van Bokhoven H, De Groot AE (1990) Isolation and identification of volatile kairomone that affects acarine predator-prey interactions involvement of host plant in its production. *J Chem Ecol* 16:381-396
- Dickens JC (1989) Green leaf volatiles enhance aggregation pheromone of boll weevil, *Anthonomus grandis*. *Entomol exp appl* 52:191-203
- Dickens JC, Jang EB, Light DM, Alford AR (1990) Enhancement of insect pheromone response by green leaf volatiles. *Naturwissenschaften* 77:29-31
- Ding D, Swedenborg PD, Jones RL (1989) Chemical stimuli in host-seeking behavior of *Macrocentrus grandii* (Hymenoptera: Braconidae). *Ann Entomol Soc Am* 82:232-236
- Drost YC, Lewis WJ, Zanen PO, Keller MA (1986) Beneficial Arthropod behavior mediated by airborne semiochemicals. I. Flight behavior and influence of preflight handling of *Microplitis croceipes* (Cresson). *J Chem Ecol* 12:1247-1262
- Drost YC, Lewis WJ, Tumlinson JH (1988) Beneficial arthropod behavior mediated by airborne semiochemicals. V. Influence of rearing method, host plant, and adult experience on host-searching behavior of *Microplitis croceipes* (Cresson), a larval parasitoid of *Heliothis*. *J Chem Ecol* 14:1607-1616
- Edwards PB, Wanjura WJ (1989) Eucalypt-feeding insects bite off more than they can chew: sabotage of induced defences? *Oikos* 54:246-248
- Eller FJ, Tumlinson JH, Lewis WJ (1988a) Beneficial arthropod behavior mediated by airborne semiochemicals. II. Olfactometric studies of host location by the parasitoid *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae). *J Chem Ecol* 14:425-434
- Eller FJ, Tumlinson JH, Lewis WJ (1988b) Beneficial arthropod behavior mediated by airborne semiochemicals: source of volatiles mediating the host-location flight behavior of *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a parasitoid of *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae). *Environ Entomol* 17:745-753
- Elzen GW, Williams HJ, Vinson SB (1983) Response by the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) to chemicals (synomones) in plants: implications for host habitat location. *Environ Entomol* 12:1872-1876
- Gauld ID (1988) Evolutionary patterns of host utilization by ichneumonoid parasitoids (Hymenoptera: Ichneumonidae and Braconidae). *Biol J Linn Soc* 35:351-377
- Hamilton-Kemp TR, Andersen RA, Rodriguez JG, Loughrin JH, Patterson CG (1988) Strawberry foliage headspace vapor components at periods of susceptibility and resistance to *Tetranychus urticae* Koch. *J Chem Ecol* 14:789-796
- Hamilton-Kemp TR, Rodriguez JG, Archbold DD, Andersen RA, Loughrin JH, Patterson CG, Lowry SR (1989) Strawberry resistance to *Tetranychus urticae* Koch: effects of flower, fruit, and foliage removal-comparisons of air- vs nitrogen-entrained volatile compounds. *J Chem Ecol* 15:1465-1473
- Hedin PA, Thompson AC, Gueldner RC (1973) The boll weevil-cotton plant complex. *Toxicol Environ Chem Rev* 1:291-351
- Hedin PA, Thompson AC, Gueldner RC (1975) Survey of air space volatiles of the cotton plant. *Phytochemistry* 14:2088-2090
- Heinrich B (1979) Foraging strategies of caterpillars leaf damage and possible predator avoidance strategies. *Oecologia* 42:325-337
- Heinrich B, Collins SL (1983) Caterpillar leaf damage, and the game of hide-and-seek with birds. *Ecology* 64:592-602
- Hérard F, Keller MA, Lewis WJ, Tumlinson JH (1988a) Beneficial arthropod behavior mediated by airborne semiochemicals. III. Influence of age and experience on flight chamber responses of *Microplitis demolitor* Wilkinson. *J Chem Ecol* 14:1583-1596
- Hérard F, Keller MA, Lewis WJ, Tumlinson JH (1988b) Beneficial arthropod behavior mediated by airborne semiochemicals. IV. Influence of host diet on host-oriented flight chamber responses of *Microplitis demolitor* Wilkinson. *J Chem Ecol* 15:1597-1606
- Hernandez HP, Hsieh TC-Y, Smith CM, Fischer NH (1989) Foliage volatiles of two rice cultivars. *Phytochemistry* 28:2959-2962
- Johnson SJ, King EG, Bradley JR Jr (eds) (1986) *Theory and tactics of Heliothis population management: I. Cultural and biological control*. Southern Coop Ser Bull 316
- Kamm JA, Buttery RG (1983) Response of the alfalfa seed chalcid, *Bruchophagus roddi*, to alfalfa volatiles. *Entomol exp appl* 33:129-134
- Lederhouse RC (1990) Avoiding the hunt: primary defenses of lepidopteran caterpillars. Pp 175-189 in Evans D & Schmidt J (eds) *Insect Defenses*. Albany, New York: State University of New York Press
- Lewis WJ, Burton RL (1970) Rearing *Microplitis croceipes* in the laboratory with *Heliothis zea* as hosts. *J Econ Entomol* 63:656-658
- Lewis WJ, Jones RL (1971) Substance that stimulates host-seeking by *Microplitis croceipes* (Hymenoptera: Braconidae), a parasite of *Heliothis* species. *Ann Entomol Soc Am* 64:471-473
- Lewis WJ, Tumlinson JH (1988) Host detection by chemically mediated associative learning in a parasitic wasp. *Nature* 331:257-259
- Liu S-H, Norris DM, Marti E (1988) Behavioral responses of female adult *Trichoplusia ni* to volatiles from soybeans versus a preferred host, lima bean. *Entomol exp appl* 49:99-109
- Loke WH, Ashley TR, Sailer RI (1983) Influence of fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), larvae and corn plant damage on host finding in *Apanteles marginiventris* (Hymenoptera: Braconidae). *Environ Entomol* 12:911-915

- Lwande W, McDowell PG, Amiani H, Amoke P (1989) Analysis of airborne volatiles of cowpea. *Phytochemistry* 28:421-423
- Martin WR Jr, Nordlund DA, Nettles WC Jr (1990) Response of parasitoid *Eucelatoria bryani* to selected plant material in an olfactometer. *J Chem Ecol* 16:499-508
- Mitchell BK (1988) Adult leaf beetles as models for exploring the chemical basis of host-plant recognition. *J Insect Physiol* 34:213-225
- Nadel H, van Alphen JJM (1987) The role of host- and host-plant odours in the attraction of a parasitoid, *Epidinocarsis lopezi*, to the habitat of its host, the cassava mealybug, *Phenacoccus manihoti*. *Entomol exp appl* 45:181-186
- Navasero RC, Elzen GW (1989) Responses of *Microplitis croceipes* to host and nonhost plants of *Heliothis virescens* in a wind tunnel. *Entomol exp appl* 53:57-63
- Nordlund DA, Lewis WJ, Altieri MA (1988) Influences of plant-produced allelochemicals on the host/prey selection behavior of entomophagous insects. Pp 65-90 in Barbosa P & Letourneau D (eds) *Novel Aspects of Insect-Plant Interactions*. New York: John Wiley
- Odell TM, Godwin PA (1984) Host selection by *Blepharipa pratensis* (Meigen), a tachinid parasite of the gypsy moth, *Lymantria dispar* L. *J Chem Ecol* 10:311-320
- Powell JE, Bull DL, King EG (1989) Biological control of *Heliothis* spp. by *Microplitis croceipes*. *Southwestern Entomol Suppl* 12:1-115
- Saijo R, Takeo T (1975) Increase of *cis*-3-hexen-1-ol content in tea leaves following mechanical injury. *Phytochemistry* 14:181-182
- Sato Y (1979) Experimental studies on parasitization by *Apanteles glomeratus*. IV. Factors leading a female to the host. *Physiol Entomol* 4:63-70
- Sheehan W, Shelton AM (1989) The role experience in plant foraging by the aphid parasitoid *Diaeretiella rapae* (Hymenoptera: Aphididae). *J Insect Behav* 2:743-759
- Sugimoto T, Kameoka H, Kusatani S, Invi O, Otsuka K (1988) Foraging for patchily-distributed leaf-miners by the parasitoid, *Dapsilarthra rufiventris* (Hymenoptera: Braconidae) V. Plant odour as a cue to long range patch-location. *Appl Entomol Zool* 23:135-143
- Thiery D, Bluet JM, Pham-Delègue M-H, Etiévant P, Masson C (1990) Sunflower aroma detection by the honeybee: study by coupling gas chromatography and electroantennography. *J Chem Ecol* 16:701-711
- Thompson AC, Baker DN, Gueldner RC, Hedin PA (1971) Identification and quantitative analysis of the volatile substances emitted by maturing cotton in the field. *Plant Physiol* 48:50-52
- Tollsten L, Bergström G (1988) Headspace volatiles of whole plants and macerated plant parts of *Brassica sinapis*. *Phytochemistry* 27:4013-4018
- Townes HK (1939) The Nearctic species of *Netelia* (*Paniscus* of Authors) and a revision of the genera of Neteliini (Hymenoptera, Ichneumonidae) *Lloydia* 1:168-231
- Veith HJ (1984) 2-Methyl-3-butene-2-ol, a major component of the alarm pheromone of the hornet *Vespa crabro*. *Naturwissenschaften* 71:328
- Vet LEM, van Opzeeland K (1985) Olfactory microhabitat selection in *Leptopilina heterotoma* (Thomson) (Hym.: Eucoilidae), a parasitoid of drosophilidae. *Neth J Zool* 35:497-504
- Vinson SB (1984) Parasite-host relationships. Pp 205-233 in Bell WJ & Cardé RT (eds) *Chemical Ecology of Insects*. London: Chapman and Hall
- Visser JH (1983) Differential sensory perceptions of plant compounds by insects. Pp 215-230 in Hedin PA (ed) *Plant Resistance to Insects*. ASC Symposium Series 208. Washington, D.C.: American Chemical Society
- Visser JH (1986) Host odor perception in phytophagous insects. *Annu Rev Entomol* 31:121-144
- Visser JH, Avé DA (1978) General green leaf volatiles in the olfactory orientation of the Colorado beetle, *Leptinotarsa decemlineata*. *Entomol exp appl* 24:538-549
- Visser JH, Van Straten S, Maarse H (1979) Isolation and identification of volatiles in the foliage of potato, *Solanum tuberosum*, a host plant of the Colorado beetle, *Leptinotarsa decemlineata*. *J Chem Ecol* 5:13-25
- Wallbank BE, Wheatley GA (1976) Volatile constituents from cauliflower and other crucifers. *Phytochemistry* 15:763-766
- Whitman DW (1988a) Plant natural products as parasitoid cuing agents. Pp 386-396 in Cuttler HG (ed) *Biologically Active Natural Products Potential use in Agriculture*. ASC Symp Ser 380. Washington D.C.: American Chemical Society
- Whitman DW (1988b) Allelochemical interactions among plants, herbivores, and their predators. Pp 11-64 in Barbosa P & Letourneau D (eds) *Novel Aspects of Insect-Plant Interactions*. New York: John Wiley