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ELECTROANTENNOGRAM RESPONSES OF *Campoletis sonorensis* (HYMENOPTERA: ICHNEUMONIDAE) TO CHEMICALS IN COTTON (*Gossypium hirsutum* L.)

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Abstract—Combined gas chromatography-electroantennogram (GC-EAG) recording of *Campoletis sonorensis* (Cameron) responses to cotton plant volatile chemicals was performed. *C. sonorensis* antennal olfactory receptors respond differentially to green leaf, mono-, and sesquiterpene chemicals that have been identified previously in cotton. EAG depolarizations to green leaf chemicals were greater than to terpenes.

Key Words—*Campoletis sonorensis*, parasitoid, Hymenoptera, Ichneumonidae, cotton, *Gossypium hirsutum*, host habitat location, green leaf chemical, monoterpene, sesquiterpene, electroantennogram, olfaction, volatile.

INTRODUCTION

Parasitoid host-finding behavior includes the steps of habitat preference, potential host community location, and host location (Vinson, 1984). While proceeding through this hierarchy of behavioral events, parasitoid sensory systems are bombarded by environmental stimuli, some of which are more important in eliciting behavioral responses than others.

Plants and their chemicals are important in parasitoid habitat location (Vinson, 1981). *Diaeretiella rapae* responds to allyl isothiocyanate as a cue to locate its host on crucifers (Read et al., 1970). Similarly, *Heydenia unica* uses α-pinene as a cue to locate its host on pine trees (Camors and Payne, 1972). Elzen et al. (1983, 1984) described short-range orientation and attraction of *Campoletis sonorensis* to host-free cotton and to some volatile cotton chemicals

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and subsequently (1986) demonstrated the long-range orientation and attraction of *C. sonorensis* to host-free cotton in a wind tunnel bioassay.

Parasitoid host habitat location in these cases involves perception of volatile chemicals. The sensory cells that detect volatile, low molecular weight organic molecules are olfactory receptor neurons usually located on the antennae (Schneider, 1964). The electroantennogram (EAG) (Schneider, 1957) records cumulative antennal olfactory receptor responses to volatile chemicals. Visser (1983) suggested that herbivores show differential olfactory sensitivities depending on the volatile chemistry of their host plant. It would be adaptively advantageous for parasitoids to evolve similar receptors for plant chemicals and utilize them in host habitat location. While Visser (1983) stressed the importance of green leaf chemicals (short-chain alcohols and aldehydes that smell like grass; Visser et al., 1979) in habitat location by herbivores, he realized that there are many other volatile plant chemicals that influence leaf odor and perhaps insect behavior. A similar approach must be taken to decipher host habitat location by insect parasitoids. The objective of this study was to use combined gas chromatography–electroantennogram (GC-EAG) studies (Arn et al., 1975) to record cumulative olfactory receptor responses of *C. sonorensis* to volatile chemicals that have been previously identified in cotton plants.

METHODS AND MATERIALS

Insects. C. sonorensis were reared on a culture of *Heliothis virescens* larvae, which were fed an artificial diet described by Vanderzant et al. (1962). Adult parasitoids were mated and maintained in 300-ml screened cages and fed honey–distilled water (1:2) on cotton pads. Cages were held in an environmental chamber at 26 $\pm$ 2°C with a photoperiod of 16:8 hr (light–dark).

Chemical Stimuli. Chemicals used for olfactory stimuli are listed in Table 1. These chemicals were chosen based on their presence in cotton essential oil (Minyard et al., 1967; Hedin et al., 1971; Elzen et al., 1984), their volatility as defined by Amoore (1982), their attraction to insects of various species (Camors and Payne, 1972; Visser and Ave, 1978), and their behavioral effects described for *C. sonorensis* (Elzen et al., 1984). All chemicals were diluted to desired concentration with hexane (pesticide grade, Fisher Scientific).

Combined Gas Chromatography–Electroantennogram Methodology. The GC-EAG technique used was similar to that previously described by Struble and Arn (1984). Chemicals were injected in 1.0-μl aliquots via an on-column injector (Scientific Glass Engineering, Inc.) to a 0.22-mm ID $\times$ 25-m vitreous silica BP1 capillary column in a Varian 3700 GC equipped with a flame ionization detector (FID). Upstream from the FID, makeup gas was added at 20 ml/min and effluent was then split 5:1 (EAG–FID) by a fixed outlet splitter

TABLE 1. SOURCE AND PURITY OF ODOROUS STIMULI USED IN EAG STUDIES.

Compound	Chemical purity (%)	Source
Green Leaf		
Hexanal	99	Aldrich
cis-3-Hexen-1-ol	98	Aldrich
trans-2-Hexen-1-ol	97	Aldrich
trans-2-Hexenal	99	Aldrich
Heptanal	95	Aldrich
Monoterpenes		
α-Pinene	>99	Aldrich
β-Pinene	>98	Aldrich
Myrcene	85	Aldrich
Limonene	97	Aldrich
Sesquiterpenes		
β-Caryophellene	>94	International Flavors and Fragrances
α-Humulene	>96	Fluka
β-Caryophellene oxide	>98	Givaudan
Gossonorol	>98	Synthesized

system (Williams and Vinson, 1980). EAG routed effluent passed through a 200°C exit port into a modified water cooled (24°C) condenser (1 cm ID). The distance between effluent introduction into the condenser and antenna preparation was 20 cm. Purified air, humidified by bubbling through distilled water at 300 ml/min, swept the effluent over the antenna preparation.

EAGs were recorded using Ag–AgCl electrodes encased in glass capillaries filled with 0.75% NaCl. Heads of mated 6-day postemergent female *C. sonorensis* were excised, and the antenna tip was inserted into the recording electrode. The indifferent electrode was inserted into the mandibles. EAG signal was amplified 100-fold by a DC preamplifier (George Johnson Electronics) and viewed on a Tektronix 561B oscilloscope. EAG and FID signals were simultaneously recorded for later analysis on Hewlett Packard 3390A integrators.

Dilutions of heptanal (10^{1}–10^{-2} μg) were administered to a series of antenna preparations. All EAG responses were measured relative to a 1.0-μg heptanal standard. Each presentation of stimuli was performed as an individual GC injection and was followed by the application of a standard. Green leaf chemicals were presented in 1.0-μg doses while the mono- and sesquiterpenes were tested at 1.0- and 10.0-μg doses. Test chemicals were applied in a sequence within each of the three chemical groups with each sequence being applied to a different antenna preparation. Prior to testing data for significance, a regression of mean and variance was performed to determine if a pattern in the data was present that required a data transformation (Ott, 1984). The mean percent response relative to the standard was tested for all chemicals at all doses using Duncan's multiple range test ($P < 0.05$) (Duncan, 1955).

RESULTS AND DISCUSSION

A typical GC-EAG response is shown in Figure 1. The mean response of female *C. sonorensis* to the 1.0-μg heptanal standard was −1.02 mV (SE ±0.14, $N = 10$). Precision of flame ionization and antenna detection time is evident. The reliability of the EAG was tested by dose response (Figure 2). EAG responses to each dose of heptanal were significantly different by Duncan's multiple range test ($P < 0.05$).

Response to Green Leaf Chemicals. Green leaf volatile chemicals elicited larger *C. sonorensis* EAG responses than the mono- and sesquiterpenes tested, with heptanal producing the strongest response (Figure 3A). Six-carbon alcohols and aldehydes elicited EAG responses that were significantly smaller than heptanal.

Differing olfactory responses to green leaf volatile chemicals are not surprising. Similar results have been shown for several orders of insect (Dickens, 1984; Dickens and Boldt, 1985; Dickens et al., 1986; Guerin and Visser, 1980;

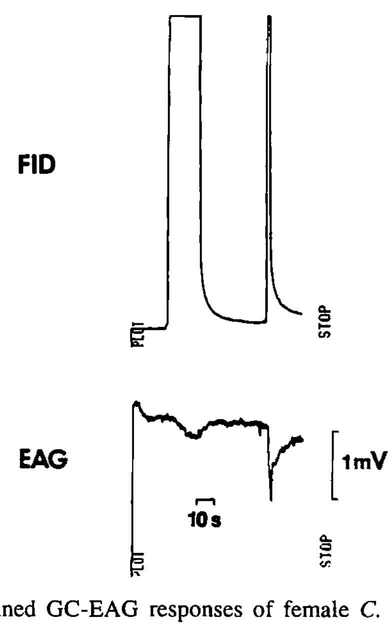

FIG. 1. Typical combined GC-EAG responses of female *C. sonorensis* to 1.0 μg of heptanal.

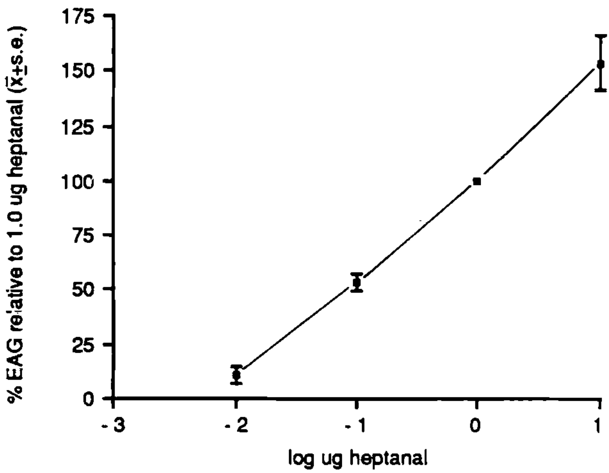

FIG. 2. Mean EAG response of female *C. sonorensis* to dilutions of heptanal.

Van der Pers, 1981; Visser, 1979). Although Dickens et al. (1986) have demonstrated this phenomenon in Hymenoptera, and Lecomte and Pouzat (1985) have recorded parasitic Hymenoptera EAG responses to plant chemicals, this is the first suggestion of different olfactory response to similar green leaf chemicals in parasitic Hymenoptera.

Response to Monoterpenes. Monoterpenes elicited significantly smaller EAG responses than most green leaf chemicals and smaller, but not significantly smaller, responses than sesquiterpenes. α-Pinene was the only monoterpene that stimulated *C. sonorensis* at a 1.0-μg dose (Figure 3B). *C. sonorensis* responded electrophysiologically to 10.0 μg of α-pinene, myrcene, and limonene, while β-pinene never elicited any response at either dose. Responses to 10.0 μg of α-pinene and limonene were significantly greater than to the other monoterpenes tested. These data suggest that *C. sonorensis* is capable of discriminating differences not only in green leaf molecular structure but also in monoterpene structure, based on differences in EAG response to α-pinene and β-pinene.

Response to Sesquiterpenes. C. sonorensis EAG responses to sesquiterpenes were similar to responses elicited by monoterpenes in that doses of 10.0 μg were needed to approach parity of responses elicited by 1.0 μg of green leaf chemicals (Figure 3C). All the sesquiterpenes tested except gossonorol produced small EAG responses at a 1.0-μg dose. *C. sonorensis* responded to all the sesquiterpenes tested at 10.0 μg. β-Caryophellene oxide elicited the strongest responses at both 1.0 and 10.0 μg.

While it is important that EAG data and data from behavioral studies are compared, it must be stressed that EAG responses do not necessarily reflect

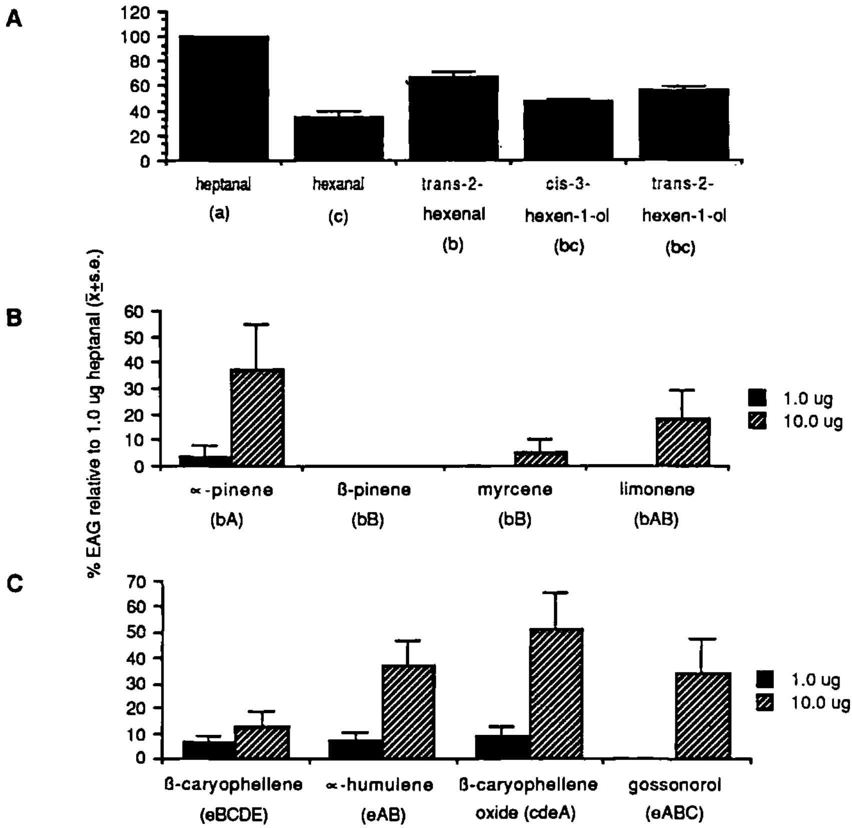

FIG. 3. Mean EAG response of female *C. sonorensis* to 1.0-μg doses of green leaf chemicals (A), 1.0- and 10.0-μg doses of monoterpenes (B), and 1.0- and 10.0-μg doses of sesquiterpenes (C). Chemicals followed by the same letter are not significantly different with capital letters representing 10.0-μg doses, Duncan's multiple range test ($P < 0.05$, $N = 10$).

central integration of peripheral stimuli and therefore do not indicate the resulting behavioral response. When electrophysiology data described here are compared to *C. sonorensis* behavioral Y-tube bioassay data of Elzen et al. (1984), minor differences are evident. The strongest EAG response to sesquiterpenes was toward β-caryophellene oxide, while behavioral data indicate a greater attraction to gossonorol. Behaviorally *C. sonorensis* did not respond to β-caryophellene, which was the least responsive sesquiterpene tested electrophysiologically. Behavior and EAG data appear to agree well with respect to α-humulene and β-caryophellene oxide responses.

CONCLUSION

Little research has been conducted on insect parasitoid attraction to specific plant chemicals and chemical blends other than those previously mentioned of Camors and Payne (1972), Elzen et al. (1984), and Read et al. (1970). While electrophysiological techniques have been utilized as tools in insect herbivore olfactory-mediated behavior studies, to date few electrophysiological studies have been conducted on parasitoids. Such research may elucidate the mechanisms involved in perception of behavioral stimuli by organisms used for biological control of insects.

The most striking similarity between our data and those of EAG studies with insect herbivores (Guerin and Visser, 1980; Kozlowski and Visser, 1981; Visser, 1979) is that substantially larger doses of terpenes than of green leaf chemicals are required to elicit responses near parity. While such data may be a function of volatility or chemical plating in the odor delivery tube, it may also suggest that molecules with smaller dose olfactory sensitivities (green leaf chemicals) could be more important in longer distance orientation to an odor source while chemicals with larger dose olfactory sensitivities (terpenes) may be more important at shorter ranges. Evidence supporting this supposition has been provided by studies comparing glanded and glandless cotton varieties, which differ mainly in production of terpenes (Elzen et al., 1985). *C. sonorensis* preferentially landed on glanded (high terpene) cotton if a choice were allowed, but landed on glandless (low terpene) cotton in no-choice wind tunnel studies (Elzen et al., 1986). Future behavioral studies may clarify such questions.

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