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OLFACTORY BEHAVIOR AND ELECTROANTENNOGRAPHIC RESPONSES OF THE COCOA BEETLE, *STEIRASTOMA BREVE* (COLEOPTERA: CERAMBYCIDAE)

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ABSTRACT

With the aim of studying the olfactory behavior of one of the main pests in neotropical cocoa plantations, the cocoa beetle *Steirastoma breve* (Sulzer) (Coleoptera: Cerambycidae), we studied behavioral and antennal responses towards different odor sources in a two-choice olfactometer and an electroantennographic system, respectively. Odor sources tested as stimuli in olfactometric experiments were chopped pieces of cocoa branches, adult males, adult females of *S. breve*, and combinations of these. Extracts of female and male body parts in *n*-hexane were tested in electroantennographic experiments. Statistically significant attraction responses in the olfactometer were observed only when *S. breve* individuals were stimulated with odors from pieces of cocoa branches. Both sexes showed active EAG responses to odors of cocoa branches, and females showed active EAG responses to adult male odors. These results suggest that olfactory behavior of *S. breve* is mediated by volatiles derived from cocoa trees and from adult male insects.

Key Words: behavior, cocoa, *Theobroma cacao*, pheromone, kairomone, ethological control, electroantennography

RESUMEN

Una de las principales plagas del cultivo de cacao en el neotrópico, es la comúnmente conocida “Gota del Cacao”, *Steirastoma breve* (Sulzer) (Coleoptera: Cerambycidae). A fin de conocer el comportamiento olfativo de *S. breve*, se evaluó la respuesta del insecto hacia diferentes fuentes de aroma. Para ello se usó un dispositivo olfatómetrico de dos vías de selección y se evaluaron las respuestas de antenas de machos y hembras en un electroantenógrafo. En el olfatómetro se evaluaron tres fuentes de aroma: trozos de ramas cacao, machos adultos, hembras adultas y sus combinaciones; y en el electroantenógrafo adicionalmente se midieron las respuestas de las antenas hacia extractos de partes del cuerpo de machos y hembras. Se observó que las únicas fuentes de aroma que demostraron un efecto significativo de atracción en el comportamiento olfativo y respuestas electroantenográficas de *S. breve* fueron: los trozos de ramas de cacao que resultaron atractivos para hembras y machos en el olfatómetro, y que además estimularon la antena de individuos de ambos sexos; y los volátiles producidos por el macho resultaron ser atractivos para las hembras y produjeron una fuerte respuesta electroantenográfica en la antena de la hembra. Los resultados sugieren que el comportamiento olfativo de *S. breve* se encuentra modulado por kairomonas provenientes de plantas de cacao y por una feromona secretada por el macho de este insecto en su estado adulto.

Translation provided by the authors.

Various species of Coleoptera such as Scolytidae, Curculionidae, Dermestidae, Nitidulidae, Scarabeidae, Dynastidae, and Cerambycidae have been reported as pests (Rochat et al. 1991, 2000a,b; Finnegan & Chambers 1993; Jaffe et al. 1993; Fettöther et al. 1995; Malosse et al. 1995;

Cosse & Bartelt 2000). *Steirastoma breve* (Sulzer) (Coleoptera: Cerambycidae), known as the “cocoa beetle”, is one of the main pests in cocoa (*Theobroma cacao*, Sterculiaceae) plantations in many countries, and its presence has been reported in South America from Argentina to Venezuela, in

addition to some Caribbean islands such as Trinidad, Grenada, Martinique, Puerto Rico, and Jamaica among others (Entwistle 1972; Sánchez & Capriles 1979). According to the literature on *S. breve*, the egg phase lasts an average of 4.2 days, the larval phase 54.9 days, the pupal phase 10.9 days, and the adult phase 35 days for males and 69 days for females (Mendes & García 1984).

Adults feed on epidermal tissues of the main stem and branches of young plants, producing a characteristic gnawed area, which produces damage to the floral clusters due to the fact that flowers grow in tight clusters on the stem and branches. Moreover, female adults lay eggs inside slits cut into the bark with their mandibles. Usually, both sexes attack young trees aged from six months to four years (Entwistle 1972; Sánchez & Capriles 1979).

Larvae cause severe damage in stem and branches. After eclosion, larvae bore into the bark where they feed on the cambial tissues and the bark itself. First, larvae make a round chamber, progressively enlarging and elongating it until it forms a tunnel or irregular spiral-like galleries. This results in a ringed stem or ringed branch. Depending on the age and location of the damage, these events can kill the apical area. If the main stem is attacked, they can quickly kill the entire plant (Entwistle 1972; Sánchez & Capriles 1979).

Because the use of insecticides has progressively decreased, it is very important to search for alternative pest control methods that are safe for the environment and highly efficient in the management of *S. breve* populations. In most cases, ethological control has been applied successfully (Nakamuta et al. 1997; Howse et al. 1998; Seybold et al. 2000). This entails previous study of olfactory behavior, as well as the identification and evaluation of the chemical compounds involved in insect communication (Hernández et al. 1992; Howse et al. 1998). An olfactometer has been used to study the olfactory behavior of many coleopteran pests (Rochat et al. 1991; Jaffe et al. 1993; Cerda et al. 1996, 1999). Electroantennography (EAG) is a technique that has been used for the analysis of biologically active compounds (Marion-Poll & Thiéry 1996). In Coleoptera, EAG and gas chromatography coupled with electroantennographic detection (GC-EAD) have been used to identify the chemical structure and stereochemistry of the sex pheromone of another cerambycid *Anaglyptus subfaciatus* Nakamuta et al. (1994). Since nothing is known about the olfactory behavior of *S. breve* towards odors derived from its host plant or from odors emitted by adult insects, the objectives of this study are evaluation of olfactory behavior and electroantennographic responses of *S. breve* when stimulated by odors derived from cocoa plant tissues and by volatiles produced by males, females, and *n*-hexane extracts of insect body parts.

MATERIALS AND METHODS

Collection of Insects and Cocoa Plant Branches

Insects and cocoa plant branches (CPB) were collected at experimental cocoa plantations of the Instituto Nacional de Investigaciones Agrícolas (INIA), located in Campo Central and Padrón, in Municipio Acevedo, Miranda State, Venezuela. Adult insects used in all experiments were collected directly from the field and then placed individually in plastic containers (7 cm high \times 11 cm \varnothing), transported to the laboratory in a thermal container, and kept under controlled laboratory conditions. Insects were fed with pieces of CPB for 12 to 16 h before olfactometric and electroantennographic experiments were performed. The pieces of CPB were collected from 2- to 4-month old EEM-003 and Ocumare 61 cocoa plant cultivars.

Olfactometric Experiments

Olfactometric bioassays were carried out at Laboratory of Entomology INIA-Miranda, Cagua, in a two-choice olfactometer (Cerda et al. 1996) with some modifications. Males and females were evaluated alternately with a minimum of 25 individuals of each sex. Odor sources used to stimulate the insects were adult females, adult males, apical chopped pieces of cocoa branches (2 to 4 months old) from EEM-003 selection, and combinations of the above totaling seven different odor sources. These were: (1) males, (2) females, (3) cocoa plant branches (CPB), (4) females + males, (5) females + CPB, (6) males + CPB, and (7) air. Each individual was placed in a chamber located in the olfactometer for 3 min before the evaluation of insect responses, in order to get them used to the system. After that period, the chamber door was opened and a small fan with a 200-ml/min flow located at the back of the olfactometer chamber was immediately switched on in order to disperse odors inside the system. Insect behavior was observed for 15 min with (1) quantification of individuals reaching either odor source, and (2) quantification of inactive or undecided individuals recorded. Each tested individual was considered as a replicate. At the end of each experiment, all remaining cue odors were removed by applying 70% v/v ethanol, and then circulating a hot air stream with a hair dryer through the device for approximately 10 min. All bioassays were performed from 8:00 to 12:00 and 13:00 to 15:00, and mean values of temperature and relative humidity were recorded during each experiment. Experimental data were analyzed by the binomial test (Wiedenhöfer 1993) after comparison of the frequencies of individuals selecting one odor source vs. the other.

Insect Extracts

Extracts of body parts from 25 males and 25 females of *S. breve* were prepared by placing each individual for 5 min in a refrigerator at 5°C in order to diminish insect activity. Then, each was dissected in a Petri dish without any fluid into three parts: head, prothorax, and pterothorax + abdomen. Each group of body parts was immediately placed in a 5-ml clean glass vial, containing 3 ml of *n*-hexane (HPLC grade), and extracted during a 48-h period. Then, the supernatant was removed with a Pasteur pipette and placed in a 4-ml clean glass vial. The extracts were concentrated to approximately 50 µl by a gentle nitrogen stream, and kept at -5°C until electroantennographic experiments were conducted.

Electroantennographic Experiments

EAG experiments were performed in the Laboratorio de Comportamiento, Universidad Simón Bolívar. EAG responses of *S. breve* males and females were evaluated while being stimulated by (1) pieces of CPB of Ocumare 61 clone (four pieces 5 cm long × 1 cm Ø), (2) males (4 individuals), (3) females (4 individuals), (4) 1 µl of *n*-hexane extract of body parts (head, prothorax, pterothorax + abdomen), or (5) *n*-hexane. In experiments 1-3, the odor source was introduced into a glass chamber where the stimulus source was placed; a system to produce a wet air current, calibrated to 300 ml/min flow, and a stimulus controller (Syntech® model CS-05, Hilversum, The Netherlands), was adjusted to a time pulse of 0.5 s and 500 ml/min flow. In experiments 4 and 5 (*n*-hexane extracts and control), stimuli were released from Pasteur pipettes containing a piece of filter paper previously impregnated with 1 µl of each extract or solvent after the solvent had been allowed to evaporate. The puff was delivered into the continuous air stream, after placing the pipette tip into the hole of the tube carrying the air stream. The antennal responses were amplified and recorded with a Syntech data acquisition controller and software.

Male or female antennae were excised and fixed between silver-gold electrodes with two droplets of an electrically conductive gel (Spectra 360® electrode gel, Parker, Orange, NJ) applied to the electrodes. Each stimulus was tested in 10 replicate experiments in which the antenna received three stimulus pulses at 3-min intervals. For quantification of EAG amplitudes (mV) we only considered the first pulse applied in each replicate. Antennal responses (mV) from males and females were compared by means of the Mann-Whitney *U*-Test.

RESULTS

The responses of *S. breve* in olfactometric bioassays when stimulated with various odor

sources are shown in Table 1. Only three sets of experiments showed statistically significant differences. For example, when CPB vs. male volatiles were tested as odor sources, 55% of the insects showed a statistically significant orientation towards CPB while 29% responded to male odors, with 16% remaining undecided or inactive. There was a clear preference of *S. breve* towards CPB (67%, $P < 0.05$ Binomial test) versus a clean air stimulus.

When results from orientation of both sexes towards female vs. male odors are compared, we observed that 67% of the evaluated females oriented toward male odors (Binomial test, $P < 0.05$). Male to male, male to female, and female to female orientations were not statistically different. The treatments with other odor source combinations (♀ + CPB, ♂ + CPB, ♂ + ♀) did not show significant differences (Binomial test, $P > 0.05$) (Table 1).

Table 2 shows the results of EAG experiments when male and female antennae were stimulated with female odors, male odors, CPB, and clean wet air. Female antennae produced significantly higher amplitudes (Mann-Whitney *U*-test, $P < 0.05$) than males, and gave stronger responses when stimulated with male odors. Male and female electric potential average responses were similar when exposed to CPB odors. Table 2 shows that male and female antennae stimulated with clean wet air showed slight variations in electrical potentials (mV). EAG responses of female antennae towards male + CPB odor produced a larger antennal depolarization that was significant compared to male antennae (Mann-Whitney *U*-test, $P < 0.05$) (Table 2).

Additional EAG results obtained from stimulating male and female antennae with *n*-hexane extracts of body parts are shown in Table 3. Female antennal responses were stronger than males, especially when male prothorax extract was applied as stimulus. Female antennal responses were higher (Mann-Whitney *U*-test, $P < 0.05$) when they were exposed to male extracts. On the other hand, these results also show that female head extract produced a stronger signal from male antennae, even though they are both of low intensity, and that prothorax extract generated a significant, slightly higher electroantennographic response from female antennae.

DISCUSSION

The results from olfactometric and EAG experiments suggest that *S. breve* olfactory behavior is highly influenced by odors emitted by cocoa plants (kairomones) and also quite possibly by a sex pheromone. The fact that during olfactometric tests *S. breve* female and male individuals were attracted by CPB volatiles, and that additionally these plant odors produced a significant electrical potential deflection (mV) in antennae of

TABLE 1. RESPONSES OF *S. BREVE* ADULTS IN OLFACTOMETRIC BIOASSAYS STIMULATED BY VARIOUS ODOR SIGNALS.

Odor sources		Frequencies		
		♂	♀	Total
Air vs. Air	Air	9	6	15
	Air	8	5	13
	Not decided	5	4	9
	Total	22	15	37
CPB vs. Air	CPB	17	13	30*
	Air	6	5	11
	Not decided	2	1	3
	Total	25	20	45
CPB vs. ♂	CPB	17	15	32*
	♂	10	7	17
	Not decided	3	6	9
	Total	30	28	58
CPB vs. ♀	CPB	10	13	23
	♀	16	4	20
	Not decided	6	11	17
	Total	32	28	60
♂ vs. ♀	♂	20	20*	40
	♀	15	6	21
	Not decided	6	4	10
	Total	41	30	71
♂ vs. ♂ + ♀	♂	12	9	21
	♂ + ♀	14	9	23
	Not decided	6	10	16
	Total	32	28	60
♂ + ♀ vs. ♀	♂ + ♀	7	12	19
	♀	9	12	21
	Not decided	9	1	10
	Total	25	25	50

*Statistically significant differences, Binomial test, $P < 0.05$.

both sexes of *S. breve*, suggests that volatile compounds emitted by cocoa plant tissues are used by the insects as cues to locate cocoa plants as their

hosts. After the insect arrives, the host could be a site for feeding, mating, and oviposition, as has been reported for other coleopteran pests of the

TABLE 2. ELECTROANTENNOGRAPHIC RESPONSES OF MALES AND FEMALES OF *S. BREVE*, WHEN STIMULATED WITH AIR, VOLATILE COMPOUNDS EMITTED BY INSECTS, AND BY COCOA PLANT BRANCHES (N = 10 REPLICATES).

Treatments Volatile compounds emitted by:	EAG responses from males (mV)	EAG responses from females (mV)
	Average ± SD	Average ± SD
♀	0.7 ± 0.1	1.2 ± 0.3*
♂	0.2 ± 0.1	7.5 ± 0.4*
CPB	1.4 ± 0.1	1.6 ± 0.3
♀ + CPB	0.6 ± 0.1	0.5 ± 0.1
♂ + CPB	1.5 ± 0.2	3.4 ± 0.3
♂ + ♀	1.3 ± 0.2	1.3 ± 0.2
Air	0.1 ± 0.0	0.1 ± 0.0

*Statistically significant differences (Mann-Whitney U-Test, $P < 0.05$) when column values are compared.

TABLE 3. ELECTROANTENNOGRAPHIC RESPONSES FROM MALE AND FEMALE ANTENNAE OF *S. BREVE* WHEN STIMULATED WITH *N*-HEXANE EXTRACTS OF BODY PARTS FROM *S. BREVE* (*N* = 10 REPLICATES).

Treatments <i>n</i> -hexane extracts of:	EAG response from males (mV)	EAG response from females (mV)
	Average \pm SD	Average \pm SD
Head ♂	0.4 \pm 0.1	6.0 \pm 0.8*
Prothorax ♂	0.5 \pm 0.2	15.5 \pm 3.0*
Pterothorax + abdomen of ♂	1.3 \pm 0.3	6.5 \pm 1.0*
Head ♀	1.0 \pm 0.2	0.5 \pm 0.1*
Prothorax ♀	1.2 \pm 0.3	2.1 \pm 0.3*
Pterothorax + abdomen of ♀	0.6 \pm 0.1	0.8 \pm 0.5
<i>n</i> -hexane	0.2 \pm 0.3	0.1 \pm 0.0

*Statistically significant differences (Mann-Whitney *U*-test, *P* < 0.05) when the columns are compared.

Curculionidae and Cerambycidae families (Jaffe et al. 1993; Hanks 1999). The presence of a characteristic superficially gnawed area over the attacked cocoa plant cortex is evidence that *S. breve* use plants as a substrate for feeding.

In another Cerambycidae, *Anaglyptus subfasciatus*, the existence of a male-produced sex pheromone was first suspected by the fact that females were attracted to males in wind tunnel bioassays (Nakamura et al. 1994). This was later confirmed by Leal et al. (1995), who identified the pheromone components, but Nakamura et al. (1997) showed that a blend of host plant volatiles and the male sex pheromone used as baits in yellow water traps were more attractive than sex pheromone or host attractant alone. In some other Cerambycidae species, the existence of sex pheromones in males and also in females is confirmed, along with their host plant relationships (Schröder et al. 1994; Fetzko et al. 1995; Bento et al. 1993; Fukaya et al. 1996).

The presence of a male sex pheromone in *S. breve* is suggested by olfactometric test results which indicate that females are significantly more attracted to males. In addition, female antennae showed a very strong deflection in EAG experiments only when stimulated by male odors. Female antennae generated strong amplitude signals with all *n*-hexane male extracts. Male prothorax extract was the source that caused the strongest response.

Sources for pheromone production in Coleoptera vary according to the kind of tissues involved. In *Carpophilus freemani* (Nitidulidae) the pheromone gland has been located in the abdomen (Dowd & Bartelt 1993). However, in other coleopterans the gland is located in the prothorax. In the case of *Anthonomus grandis* Boheman (Curculionidae), the aggregation pheromone is produced in the fat bodies associated with the digestive tract (Wiygul et al. 1982), and in *Rhynchophorus palmarum* L. (Curculionidae), the pheromone glands are located in the male prothorax (Sánchez et al. 1996). In the cerambycid

Hylotrupes bajulus the pheromone is produced in a gland situated in the male prothorax (Fetzko et al. 1995). According to the strong evidence provided by the olfactometric and electroantennographic experiments of the present study, it is highly probable that the *S. breve* pheromone production system is also located in the prothorax. That each of the *n*-hexane body extracts elicited EAG responses implies that the *S. breve* digestive system could be associated with pheromone dispersion, as has been reported for other coleopteran insects. Therefore, once male insects arrive at a plant having been attracted by volatile host compounds, it is very probable that males start releasing a pheromone in order to enhance female searching behavior for mating.

In conclusion, the results of the present study when combined with the findings reported in the literature indicate that the chemical communication system and olfactory behavior of *S. breve* is probably similar to that described for the cerambycid *A. subfasciatus*. However, it is necessary to continue research, currently in progress, in order to identify the chemical compounds involved in the *S. breve* communication system. This may enable their use as safe tools for the control of this important neotropical pest.

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