SHORT COMMUNICATIONS

EAG Responses of Parasitoids, *Ascogaster reticulatus* WATANABE (Hymenoptera: Braconidae), to the Female Sex Pheromone¹

Osamu HIDOH, Toshie KAWASHIMA,²
Jun-Ichi FUKAMI and Yooichi KAINOH³

Laboratory of General Biology, Meiji College of Pharmacy,
Nozawa, Setagaya-ku, Tokyo 154, Japan

³Institute of Agriculture and Forestry, University of
Tsukuba, Tsukuba-shi, Ibaraki 305, Japan

(Received November 14, 1991)

(Accepted July 23, 1992)

Key words: EAG, sex pheromone, (Z)-9-hexadecenal, parasitoid, *Ascogaster reticulatus*

The braconid parasitoid, *Ascogaster reticulatus* WATANABE (Hymenoptera: Braconidae), is an egg-larval parasitoid of the smaller tea tortrix, *Adoxophyes* sp. (Lepidoptera: Tortricidae). KAMANO et al. (1989) observed the mating behavior of *A. reticulatus* and found pheromonal activity in a hexane extract of the female bodies. The male located the extract on a Petri dish with antennal contact. This observation suggests that the perception of a pheromone is mediated by the contact chemoreception. Recently, however, the active principal was identified as (Z)-9-hexadecenal (Z9-16:Ald) (KAINOH et al., 1991), which was a common component in sex attractant-pheromones in some moth species (ARN et al., 1986). An odorous role of this chemical is supposed in the parasitoids. In the present study, we employed a conventional electroantennogram (EAG) technique to confirm the olfactory sensitivity of the antennae to a pheromonal odor.

MATERIALS AND METHODS

The parasitoids used were from our stock culture based on the rearing method by KAINOH and TAMAKI (1982) and KAINOH (1986). Emerged adults were sexed and 10 to 20 insects of each sex were kept in a transparent plastic container (15 cm dia., 12 cm ht.) in which honey and wet cotton

were provided. Unmated adults 3- or 4-d-old were used for the experiments. Insect rearing and experiments were conducted at $25\pm 1^\circ\text{C}$ and $50\pm 20\%$ RH with 16L-8D photoperiod.

An insect's head with antennae cut off was mounted on cotton soaked with saline composed of 120 mM NaCl, 10 mM KCl, 4 mM CaCl_2 , 8 mM MgCl_2 , and 5 mM HEPES. The pH of this solution was adjusted to 7.0 with NaOH. Two or 3 distal segments of an antenna were cut off. For EAG recording, a fine glass capillary electrode was filled with the above saline and connected to the amplifier with Ag-AgCl wire inserted into the electrode. The recording electrode was manipulated over the antennal tip. An indifferent electrode made of Ag-AgCl wire was embedded into the cotton. Electrical signals, passed through a high cut-off filter (at 1,000 Hz) were amplified and recorded.

A hexane solution of an authentic pheromone compound, Z9-16:Ald, or its geometrical isomer, (E)-9-hexadecenal (E9-16:Ald) (both of a purity of >99%) was applied onto a filter-paper disc (5 mm dia.). The disc was inserted into a glass pipette (4.5 mm i.d.) after evaporation of hexane. A 5-ml puff of air was directed onto the antenna through the glass pipette from a distance of 2 cm by means of a glass syringe connected with silicon tube.

RESULTS AND DISCUSSION

A negative-deflection EAG was obtained from stimulation with Z9-16:Ald. Since larger EAGs were obtained by the stimulation (10 $\mu\text{g}/\text{disc}$) on the ventral side of an antenna than on the dorsal side of the same antenna (Table 1), Z9- and E9-16:Ald were applied to the ventral side of an antenna in the following experiments. A male often searches for the sex pheromone deposited on a substrate by keeping the ventral side of the antennae in contact with it (KAMANO et al., 1989). This antennal searching corresponds to the higher sensitivity of the ventral side. However, it remains unclear whether the higher sensitivity is caused by the number of receptors and/or by the type of receptors.

The responses obtained from female antennae are also shown in Table 1. There was no response in female antennae to 10 $\mu\text{g}/\text{disc}$ of Z9-16:Ald, a

¹ Appl. Entomol. Zool. **27** (4): 587-589 (1992)

² Present address: Ciba-Geigy Co., Ltd., Hamamatsu-cho, Minato-ku, Tokyo 105, Japan

level at which the male antennae responded well. The female EAG response at 0 $\mu\text{g}/\text{disc}$, which was considered to be a mechanical response to the air puff, was smaller than that in a male antenna. Female antenna showed low sensitivity to the pheromonal odor and mechanical stimulation.

The amplitude of EAG obtained from male responses to Z9-16:Ald increased in a dose-dependent manner (Fig. 1). Under our experimental conditions, 20 $\mu\text{g}/\text{disc}$ of Z9-16:Ald caused maximum EAG, whereas the same dose, or a higher dose of E9-16:Ald, elicited smaller EAGs. Male parasitoids did not show any behavioral response to E9-16:Ald in the Petri dish bioassay at any dose between 5×10^{-4} to 5 ng/dish, which was an effective dose range for Z9-16:Ald (KAINOH et al., 1991).

The present EAG data correlate well with the observation. Because male antennae respond to an air puff (0 $\mu\text{g}/\text{disc}$ in Table 1), an EAG amplitude larger than 0.69 mV would contribute to the real response of both chemicals. Dose-response relationships suggested that about a 100-fold dose was required for E9-16:Ald to attain the same response as that of Z9-16:Ald. In EAG response of lepidopterous insects, the geometrical isomer of a real pheromone component usually produces 3 to 10 times lower activity (ROELOFS, 1984). In contrast, the low sensitivity to E9-16:Ald in the present study suggests that antennal specificity to the pheromone component, Z9-16:Ald, is more rigid in this parasitoid.

In our preliminary study, male parasitoids did

Table 1. EAG responses of antennae in male and female *Ascogaster reticulatus* to Z9-16:Ald.

	Dose ($\mu\text{g}/\text{disc}$)	Side ^a	No.	Amplitude ^b (mV)
Male	0	ventral	12	$0.69 \pm 0.05\text{A}$
	10	ventral	12	$1.43 \pm 0.07\text{B}$
	10	dorsal	7	$0.96 \pm 0.11\text{A}$
Female	0	ventral	6	$0.27 \pm 0.07\text{C}$
	10	ventral	6	$0.32 \pm 0.08\text{C}$

^a Z-9-16:Ald puff directed onto the ventral side of an antenna (ventral) or onto the dorsal side (dorsal).

^b Mean $\pm$ S.E. of EAG amplitude. Means followed by the same letter were not significantly different at $p=0.01$ by DUNCAN's multiple range test.

Fig. 1. Dose-response relationship of EAG amplitude elicited by Z9- and E9-16:Ald in male *Ascogaster reticulatus*. Filled and open circles indicate application of Z9-16:Ald and E9-16:Ald, respectively. The horizontal dotted line indicates the control response to an air puff as determined from Table 1. Each point is a mean of 6-12 EAGs with $\pm$ S.E. represented by the vertical bar.

not show any positive behavioral response to the female odor in an olfactometer. From this behavioral observation and male antennal searching behavior, we had assumed that a pheromone Z9-16:Ald acts as a contact chemical. However, we confirmed that the male antennae responded to the pheromone as a volatile in this study. In the contact chemoreceptive sensillum, a mechanoreceptor cell is often associated with the chemoreceptor cells (ZACHARUK, 1980), and STÄDLER (1984) proposed that close-range olfaction is included in contact chemoreception. Present observation supports the notion that olfaction is associated with contact chemoreception. It will be necessary to elucidate the interaction between mechanosensory and chemosensory mechanisms in perception of the pheromone.

ACKNOWLEDGMENTS

Thanks are due to Dr. H. SUGIE of the National Institute of Agro-Environmental Sciences for his kindly providing the Z9- and E9-16:Ald and also to Dr. S. TATSUKI of the University of Tokyo for

his valuable suggestions and critical reading of an earlier draft.

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Ecdysteroids in Developing Eggs of *Antheraea mylitta* (Lepidoptera: Saturniidae)¹

Kiyoshi ASAKA,² Corinne ROYER³
and Bernard L. MAUCHAMP³

²National Institute of Sericultural and Entomological
Science, Ohwashi 1-2, Tsukuba, Ibaraki 305, Japan

³Institut National de la Recherche Agronomique,
78000 Versailles, France

(Received November 21, 1991)

(Accepted July 15, 1992)

Key words: ecdysteroids, eggs, enzyme immunoassay, *Antheraea mylitta*

Antheraea mylitta is a fairly large insect species commercially reared in India for silk production. Since the size of an egg is approximately 3 mm in diameter, it is advantageous to use it for the investigation of hormonal aspects for embryonic development.

The occurrence of ecdysteroids has been reported in the eggs of various insect species. Several kinds of free and conjugated ecdysteroids have been found in insect eggs and distinct changes of the ecdysteroid levels have been observed during embryonic development (for review see HOFFMAN and LAGUEUX, 1985). We investigated free ecdysteroids in the eggs of *A. mylitta* and compared the results with some other insect species previously reported.

MATERIALS AND METHODS

Silkmoth larvae were reared on an artificial diet recently developed by AKAI et al. (1991). Eggs were incubated at 25°C and 80% R.H. Ten eggs were sampled daily, weighed, and stored in the freezer (-40°C).

The samples were homogenized in a mixture of 2 ml hexane, 700 µl methanol and 300 µl water. The homogenate was centrifuged to separate the hexane and methanol-water phases at 3,000 g. After eliminating the hexane phase with Pasteur pipette, 10 ml water was added to the ecdysteroid-

¹ *Appl. Entomol. Zool.* **27** (4): 589-592 (1992)