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ELECTROANTENNOGRAM RESPONSES OF *APANTELES ANGALETI* MUESEBECK, A PARASITOID OF *Pectinophora gossypiella* TO SYNOMONAL COMPOUNDS OF COTTON

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ABSTRACT

Odour responses of males and females of the larval parasitoid Apanteles angaleti were studied by using an Electroantennogram (EAG) to the various synomonal extracts from the leaves, undamaged and damaged buds, and undamaged and damaged bolls of cotton variety LD 327. The hydrocarbon profile of the various extracts analysed by GC indicated the presence of hydrocarbons ranging from C_{10}-C_{30}. Among the different hydrocarbons detected in the various plant parts noncosans, heptacosane, hexacosane and pentacosane were found to be present in higher concentration compared to others,

Maximum amplitude of EAG response for both male and female A. angaleti was recorded for the extract obtained from the Pectinophora gossypiella damaged bolls (147.133 % in female and 124.133% in male), followed by extracts from leaves (134% in female and 93.33% in male), undamaged buds (113% in female and 113.1% in male), damaged buds (113% in female and 93.67% in male) and undamaged bolls (98% in female and 92% in male) respectively. The differential response of this parasitoid to the different plant parts/stages of plant may be due to presence of these saturated hydrocarbons

Key words: *Apanteles angaleti*, Electroantennogram, parasitoid , *Pectinophora gossypiella*, synomonal compounds,

INTRODUCTION

Cotton an important cash crop for farmers in India, occupies nearly 30 per cent of the cropped area and ranks second production wise (13%) in the world (Russell and Kranthi, 2006). A major limiting factor in its cultivation is the damage caused by insect pests, especially the boll worm complex comprising of the American boll worm as the pink boll worm, *Pectinophora gossypiella* (Saunders). It attacks the bud, flower and bolls and also the shoot in early stages and cotton lint and seeds during the later stages thereby reducing not only the yield but also the quality of the lint, with an overall yield loss to the tune of 57-80% (Gupta, 1999). The bollworm being internal feeders are often difficult to control and chemical control has not provided a long term solution for these pests. Parasitoids belonging to the braconid genera, *viz*., *Apanteles* sp has been found to be useful for control of bollworms on cotton.

Insect initially detects and filters information from environment by using peripheral sensory system. The information is then used to produce an environmentally relevant response. Several studies have demonstrated that specific blend of odours produced by herbivore-injured plant are often attractive to certain insect predators and parasitoids (Vet and Dick, 1992). Electroantennogram (EAG) is a slow potential recorded from the antenna, the primary olfactory organ of an insect. It is a bioassay widely used in experimental entomology for the detection of volatiles perceived by the insect (Schneider, 1957). The amplitude of an EAG response increases with increasing concentration of the stimulus until a saturation level is reached. The amplitude is further dependent on the nature of stimulus, the insect species, sex and many less well-defined factors. The EAG has been successfully employed for the pheromone identification over the years and it is now increasingly being used for identifying host and plant-odour volatiles which play an important role in the host searching behavior of parasitoids. Many electroantennographic recording techniques have been developed for insect olfactory sensors to monitor the depolarisation of antennal receptor cells of an olfactory organ when exposed to varying quantities of stimulative chemicals. Pest insects can respond to small quantities of volatile compounds such as ethanol, verbenone, allomones, and kairomones etc. (Weissbecker *et al.,*1990; Salom *et al*., 1992; Raguso and Light, 1998; Zhu et al., 1999; Brockerhoff and Grant, 1999; Han and Cheng, 2002; Soares *et al*., 2003; Yu *et al*., 2004; Malo *et al*., 2005; Olsson *et al*., 2005; Birkett *et al*., 2006; Das *et al*., 2007; Jonsson and Anderson, 2008). Our goal in this study, was to record the subtle differences in the responses of males and females of parasitoids *Apanteles angaleti* to extracts from various plant parts of cotton which mediate its host habitat location.

MATERIALS AND METHODS

Cotton variety, LD 327 was grown in the research farm of the Division of Entomology, Indian Agricultural Research Institute, New Delhi - 110012 during the May - January cropping season of 2005 – 2006. All the recommended agronomic practices for raising a good crop were followed.

The synomones were extracted from the leaves, undamaged buds and bolls and buds and bolls damaged by pink bollworm, *Pectinophora gossypiella* with hexane. 30 g of each plant part were taken at the active vegetative phase and immersed overnight in 300 ml of distilled hexane. The hexane was then filtered through Whatman No. 1 filter paper. Anhydrous sodium sulphate @1g/10g sample was added to the filtrate for dehydration for 2 hrs and then it passed through silica gel (60-120 mesh) column. The hexane extract of different plant parts was eluted through the column and then distilled at 60 - 70^{0}C. The left over residue was collected by rinsing the container with a small quantity of HPLC grade hexane (Merck) and stored in separate vials and kept in fridge till further use for Electroantennogram (EAG) and Gas Liquid Chromatography analysis.

Identification of synomonal compounds was done with Gas Chromatography (Varian 3900 XL) using hydrocarbon standards from Sigma Aldrich, USA. The purified hexane extracts of different plant parts were concentrated and injected into Gas Chromatograph (Varian 3900 XL) fitted with Flame Ionization Detector (FID) in a WCOT fused silica 30 m x 0.32 mm ID, CP-SIL 24 LB/MS (# CP5860) Varian Chrompack capillary column at a temperature range programmed between 100-260^{0}C for 56min. The injector and detector temperatures were maintained at 300^{0}C. Nitrogen was used as carrier gas with a flow rate of 20ml/min. The injection volume was 3µl. The hydrocarbon standards for C_{10}-C_{30} were obtained from Sigma Aldrich, USA. The resultant chromatographs were analyzed with the help of interactive graphics (Varian Star Chromatography workstation, Version 6.0) software. For calculating the quantity of unknown saturated hydrocarbon the following formula was used:

$$\text{Concentration of Unknown saturated Hydrocarbon} = \frac{\text{Area of unknown saturated Hydrocarbon}}{\text{Area of standard saturated Hydrocarbon}} \times \text{Conc. of standard saturated Hydrocarbon}$$

The mass culturre of the larval parasitoid, *A. angaleti* was initiated from the *A. angaleti* culture maintained continuously on the laboratory host *Corcyra cephalonica* in the Biological Control Laboratory, Division of Entomology, Indian Agricultural Research Institute, New Delhi - 110012. Male and female adults were kept in glass cages of 20x20x20 cm size and provided with 1-2 day old larvae of *C. cephalonica* for parasitization for 24-36 hrs. The adult parasitoids were fed with opened resin. The parasitized larvae of *C. cephalonica* were transferred to glass jars filled with broken maize grains till the formation of parasitoid pupae in silk cocoons and emergence of parasitoid adults.

The electrophysiological response of both male and female parasitoids to the various synomonal extracts were evaluated through electroantennogram. Antennae of ♂ and ♀ of *A. angaleti* were cut out off the head at the base by using a micro scissor and clipped off under 0.1M electrolyte solution and then used for EAG recording. The amplitudes of EAG response were measured and presented in a graph. 10% honey solution was used as reference. A voltage of 1mV was used. To compensate for the decline in antennal activity during recording session, the value of maximum EAG responses was expressed relative to the responses to the references. In this normalization the responses to the references was defined as to be 100%.

The stimulus from synomonal extracts was applied by using a stimulus air controller (Type CS-05, Syntech, Netherlands) designed to deliver controlled air flow towards the antenna. The continuous air flow was maintained at 4-5 m/s with a complementary air supply @ 4 m/s connected to the stimulus applicator and mixing tube assembly using a Pasteur pipette/disposable micro pipette tip having the stimulus applied on Whatman No.1 filter paper strip (2.5 x 0.5 cm).

The stimulus was delivered at various different doses of extracts @ 10, 15, 20, 25, 30, 50, and 100 µl per filter paper strip individually to find the maximum effective biologically active dose. The dose of 30µl which was able to elicit better EAG response for *A. angaleti* and therefore selected as an optimum dose for all EAG recordings. The antennal preparation was exposed to a series of different stimuli i.e. synomonal extract with intermittent application of reference stimuli (10% honey solution) in recording session with an interval of 40 seconds for recovery. The recording was repeated with at least 5 individual adult parasitoids with 3 replications of stimulus puff through stimulus applicator. The EAG responses for males and females of *A. angaleti* were recorded separately.

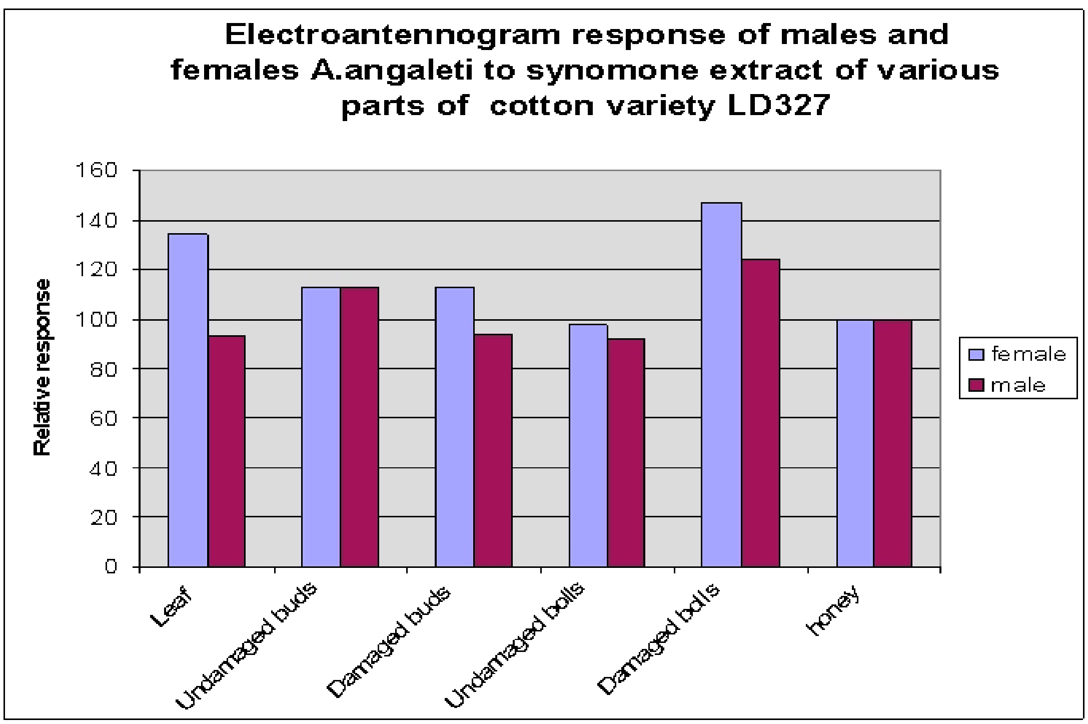
RESULTS AND DISCUSSION

Gas liquid chromatography analysis of synomone extracts indicated that out of the five synomonal extracts from cotton variety LD 327, damaged bud extract was found to possess the maximum number of hydrocarbons (14 hydrocarbons)) compared to damaged boll (13 hydrocarbons), leaf (12 hydrocarbons), undamaged bud (10 hydrocarbon) and undamaged boll extract (9 hydrocarbon). The group of long chain hydrocarbon ranging from C_{25} to C_{30} was detected in all these extracts except for heptacosane which was not present in the undamaged boll extract. In contrast, the whole group of hydrocarbons from C_{10} to C_{13} were absent. Tetradecane and pentadecane were not detected in both leaf and undamaged boll extracts. Hexadecane was found in all extracts but heptadecane was found only in leaf and damaged boll extracts. Octadecane and tetracosane was not detected in any of the extracts of this variety while nonadecane was found only in the damaged bud extract. Among all the long chain saturated hydrocarbon ranging from C_{25}-C_{30} hexacosane was present with highest concentration in damaged bud extract followed by heptacosane in undamaged boll extract, However, pentacosane was not detect in undamaged boll but present in other extracts, nonacosane in damaged boll extract and undamaged boll extract. The quantity of triacotane was highest in damaged boll extract followed by undamaged boll, leaf, damaged bud and undamaged bud extracts [Table 1]

Table 1. Hydrocarbon profile of synomonal extracts of cotton variety LD 327

Carbon No.	Hydrocarbon	Leaf	Undamaged bud	Damaged bud	Undamaged boll	Damaged boll
C_{10}	Decane	ND	ND	ND	ND	ND
C_{11}	Undecane	ND	ND	ND	ND	ND
C_{12}	Dodecane	ND	ND	ND	ND	ND
C_{13}	Tridecane	ND	ND	ND	ND	ND
C_{14}	Tetradecane	ND	331.99	1122.40	ND	998.33
C_{15}	Pentadecane	ND	174.34	730.47	ND	ND
C_{16}	Hexadecane	6257.89	1022.81	4955.26	9771.93	2077.19
C_{17}	Heptadecane	1448.55	ND	ND	ND	1010.15
C_{18}	Octadecane	ND	ND	ND	ND	ND
C_{19}	Nonadecane	ND	ND	2780.26	ND	ND
C_{20}	Eicosane	5381.54	ND	2587.40	11549.25	1558.12
C_{21}	Heniecosane	1921.55	73.49	1116.19	9395.23	2783.88
C_{22}	Docosane	1288.55	ND	715.42	3607.71	1171.77
C_{23}	Tricosane	915.16	ND	2273.13	ND	868.37
C_{24}	Tetracosane	ND	ND	ND	ND	ND
C_{25}	Pentacosane	825.91	192.01	1596.52	ND	3932.92
C_{26}	Hexacosane	6003.18	1866.45	124756.76	5784.58	5458.66
C_{27}	Heptacosane	3318.74	683.16	5444.34	77336.82	15670.79
C_{28}	Octacosane	3383.87	286.90	3252.37	1575.69	4267.27
C_{29}	Nonacosane	2453.80	8227.27	3169.90	31265.28	37666.92
C_{30}	Triacotane	5919.29	236.59	4656.16	7319.51	10760.09

All the values indicate concentration in ppm; ND = Not detected



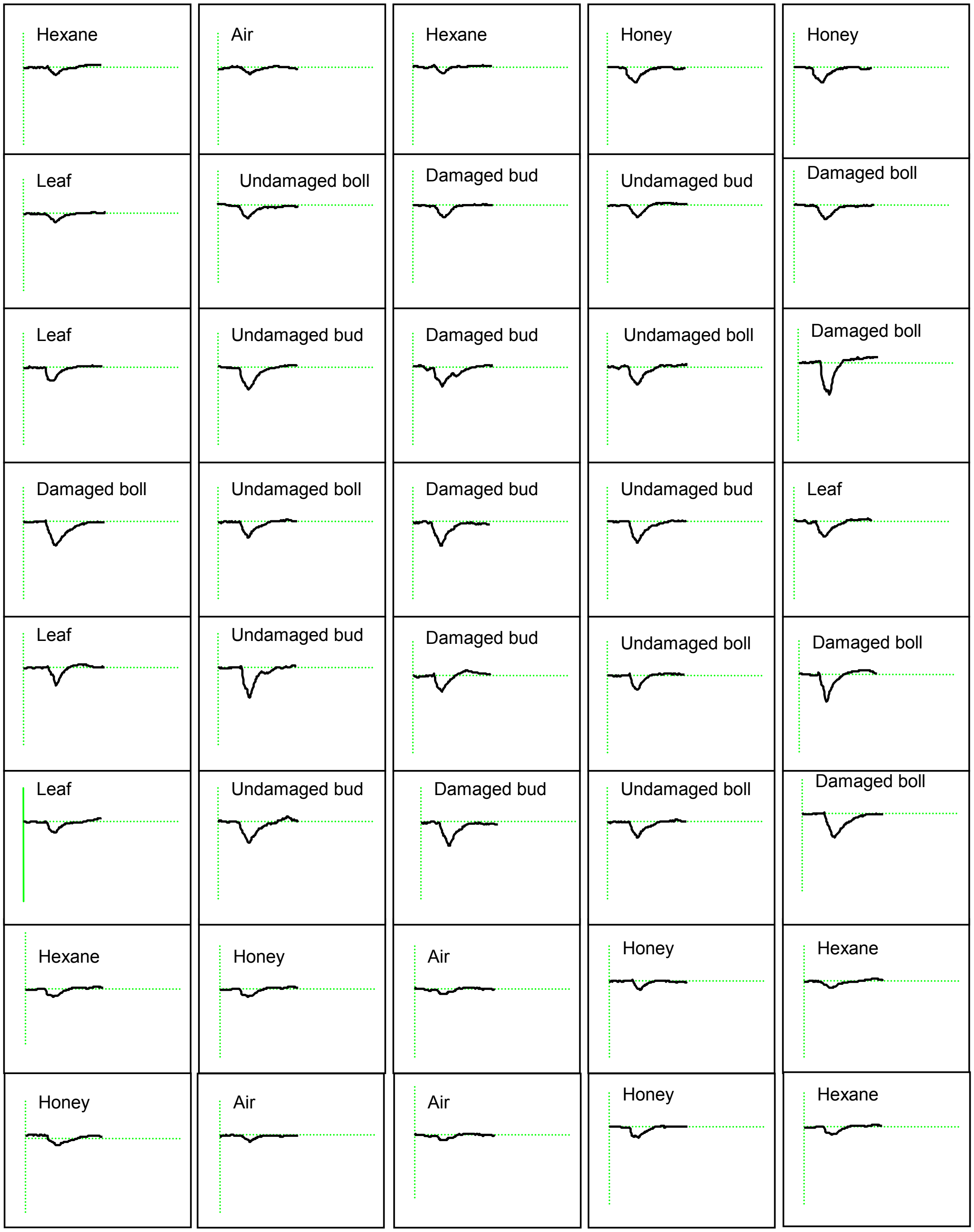
Different synomonal extracts of variety LD 327 were used to record the EAG responses of male and female parasitoids. Maximum mean relative EAG responses of 124.1% and 147.1% respectively were seen in male and female wasps respectively for the damaged boll extract while the lowest response was seen for both males and females for undamaged boll extract (Fig 1., Plate 1, 2). The results showed that all of the compounds in the induced odours were perceived by the parasitoids yet there were some differences in the intensity of response to particular compounds, indicating potential differential sensitivity of the parasitoids to the various volatiles. The EAG response of *A. angaleti* showed significant difference between males and female to different plant part extracts. This is similar to the results of Rojas (1999) who observed responses of females *M. brassicae* was higher to all other green leaf volatiles except 1-hexanol and Z-(3)-hexynyl acetate. In contrast to the current results, Jyothi *et al.* (2002) reported that the EAG response of males and females were not significantly different but they found that the EAG response of female parasitoids increased with increase in length of carbon atom in response to stimulation with alchohol, aldehydes and terpenoids

Long-chain hydrocarbons are commonly encountered in both the plant and animal kingdom. In fact *n*-alkanes are among the commonest constituents of all plant waxes (Baker, 1982; Jeffree, 1986). They play a role in several bitrophic herbivore–plant interactions: as attractants for oviposition, or as attractant or deterrent for feeding (Bernays and Chapman, 1994). They also play a role in parasitoid–herbivore (Rutledge, 1996) and predator–herbivore (Yasuda, 1997) interactions, as well as in insect chemical mimicry (Liepert and Dettner, 1996). The main factor with respect to plant waxes that is thought to determine the foraging success of predators or parasitoids is their ability to attach to the plant surface.

Hydrocarbons with n-C_{27} (nonacosane) and n-C_{31} (hentriacontane) were reported as the most abundant in plants (Hellmann and Stoesser, 1992; Dutton, 2002) which has been further confirmed by our results. These compounds are known to constitute the texture of the epicuticular leaf surface (Baker, 1982). The amount and composition of alkanes in apple leaves change depending on the season, developmental age of the leaves, and on apple tree varieties (Hellmann and Stoesser, 1992). This increase might result in a different plant texture, which may contribute to the observed response of the female parasitoids. As evidenced by gas chromatography (GC), the hydrocarbon mixture usually contains up to 100 different hydrocarbons (Nelson *et al*., 1981). The length of hydrocarbon chains usually varies from 23 to 47 carbon atoms (Blomquist and Dillwith, 1985). The gas-liquid chromatography analysis of different plant parts from cotton variety LD 327 were mainly targeted to identify the saturated straight chain hydrocarbons and it revealed a wide variation in number of hydrocarbon and their concentration in the various synomonal extracts. The chain length of the hydrocarbon of synomonal extracts ranged from C_{14} to C_{30}. Jones *et al* (1973) found tricosane as the most active compound to elicit a high response in the egg parasitoid *Trichogramma evanescences*. In addition, the quantity of these compounds increases as a result of leaf miner herbivory. This increase might result in a different plant texture, which may contribute to the observed response of the female parasitoids. Indeed, texture has been shown to be an important physical cue for host location by several parasitoid species (Schmidt, 1991; Vinson, 1985).

Plate 1. EAG responses of females of *A. angaleti* to synomonal extracts of cotton variety LD 327 (Repeated with 5 individual wasps)

Plate 2. EAG responses of males of *A. angaleti* to synomonal extracts of variety LD 327 (Repeated with 5 individual wasps)



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Phản ứng EAG của *Apanteles angaleti*, ong ký sinh của *Pectinophora gossypiella* đối với hợp chất Synomone của cây bông

Sử dụng điện sinh lý trên râu đầu (electroantennogram) cả hai giới tính của loài ong ký sinh *A. angaleti* đối với synomone (hydrocarbon) chiết trích từ bề mặt của lá, nụ hoa và trái (bị gây hại /không gây hai bởi *Pectinophora gossypyella*) của giống bông vải LD 327 để nghiên cứu tập tính tìm kiếm và định vị ký chủ của loài ong ký sinh này. Kết quả EAG cho thấy phản ứng của con đực và con cái *A. angaleti* đối với chất trích từ trái bị gây hại bởi *P. gossypiella* có biên độ cực đại (147.133 % đối với con cái và 124.133% đối với con đực) kế đến là chất trích từ lá (134% đối với con cái và 93.33% đối với con đực), nụ hoa không gây hại bởi *P. gossypiella* (113% đối với con cái và 113.1% đối với con đực), nụ hoa gây hại bởi

P. gossypiella (113% đối với con cái và 93.67% đối với con đực) và trái không gây hại bỏi *P. gossipiella* (98% đối với con cái và 92% đối với con đực). Kết quả phân tích các hydrocarbon bằng phương pháp sử dụng sắc ký khí (GC) cho thấy số lượng hydrocarbon phát hiện từ C_{10}-C_{30}. Trong số các hydrocarbon hiện diện noncosans, heptacosane, hexacosane and pentacosane có nồng độ cao hơn so với các hydrocarbon khác. Nghiên cứu cho thấy con cái *A. angaleti* bị hấp dẫn bởi một số hydrocarbon có phân tử lượng cao (C_{25}-C_{30}) so với con đực. Nồng độ và tỷ lệ của các hydrocarbon có trong thành phần hóa học của cây ký chủ cũng góp phần trong việc giúp con cái phân biệt để tìm kiếm và đinh vị ký chủ của nó. Mục đích sử dụng EAG trong nghiên cứu semiochemical là để tìm ra một số hợp chất hấp dẫn các loài ong ký sinh trong thiên nhiên từ đó có thể tạo ra một số semiochemical nhân tạo giúp đạt được hiệu quả cao trong việc sử dụng thiên địch trong phòng trừ sâu hại.