ELECTROANTENNOGRAM RESPONSES OF CIHELONUS BLACKBURNI CAMERON, EGG-LARVAL PARASITOID OF SPOTTED BOLL WORM *Earias vitella* TO INFOCHEMICALS ON COTTON ECOSYSTEM

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ABSTRACT

EAG response was recorded from parasitoid wasp Chelonus blackburni Cameron (Hymenoptera, Braconidae) to a broad range of volatile plant compounds of cotton variety Pusa 8-6. The response profile was restricted to small numbers of volatile that evoke substantial EAG. Large EAG responses was particularly found among green leaf extract (151.4%) followed by Earias vitella damaged bud (144.3%), damaged boll (137.7%), undamaged bud (130.3%) and undamaged boll (12.1%) respectively. The values of the maximum EAG responses are expressed relative to the response to the references defined to be 100%. The straight chain saturated hydrocarbon in different extract was analyzed by gas-liquid chromatography and the hydrocarbon present in these extracts was found to be high quantity in Heptacosan, Hexacosane, Nonnacosane and Triacotane compared to other compounds. These of hydrocarbon were reported that to be favorable hydrocarbon for activity of parasitoids.

Keywords: *Chelonus blackburni, Earias vitella EAG, hydrocarbon, parasitoid.*

INTRODUCTION

Insect parasitoids have ability to use variety of cues to locate, identify and acceptance their potential hosts (Vinson, 1976; 1977, Lewis *et al.,* 1976). Generally, parasitoid use infochemical for host selection depend on their reliability and detectability (Vet and Dicke, 1992). Synomone (host plant stimuli) have low reliability but high detectability while kairomone (stimuli from host and host by-products) have low detectability and high reliability (Vet and Dicke, 1992). To solve this reliability and detectability problem female parasitoid use a hierachic search in which the more detectable stimuli such as synomone are use for host habitat location and more reliable stimuli such as host kairomones are use for host location and host acceptance. (Vinson, 1976). The chemical elicited by plant in response to herbivore damaged are often used most successful in host location process (Zwolfer and Kraus, 1957, Vet and Dick, 1992) it is consider as long cues distance whereas chemical elicited by herbivore or its by-products is short cue distance (Weseloh, 1981; Vinson, 1985).

Electroantennogram (EAG) assay is a widely used method for detection of behaviorally active compounds, especially in pheromone research. By stimulating an antenna with a volatile compound the olfactory receptor potential, as a result of receptor membrane depolarization, can be measured. Compounds eliciting a potential larger than the spontaneous antennal activity, are considered to be electrophysiologically active. The EAG method is conclusive in the sense that a compound not eliciting an electrophysiological response can be excluded as an olfactory cue, provided that olfaction is mediated by receptors on the antenna only (Roelofs and Comeau, 1971).

Spotted boll worm, *Earias vitella* (Fab.) is known to be serious pest attack on cotton and lady finger in India, *C. blacknurni* Cameron (Hym: Braconidae) is solitary endoparasitoid of *E. vitella* and is one of the major mortality agent in biocontrol of cotton boll worm including spotted boll worm. The

measurement response the olfactory sensitivity of *C. blackburni* antennae to various infochemicals in tritrophic level by EAG technique is necessary information in order to enhance high efficiency of this parasitoid in pest management of *E. vitella* in agrocotton crops.

MATERIAL AND METHODS

Rearing of host insects Pink bollworm Earias vitella

E. vitella was collected from cotton field and reared under laboratory condition at 27 ± 2^{0}C and 70% RH on artificial diet. The adults were fed on 10% glucose solution for egg laying. The eggs were collected everyday for extracting kairomone.

Rearing of the parasitoid C. blackburni

The parasitoids were reared on the unsterilized egg of *Corcyra cephalonica* under similar condition. Adults were fed on 100% honey and provided *C. cephalonica* egg card for parasitized. Adults were used for EAG studies.

Preparation of synomones and kairomones.

Extraction of synomone

Leaves, undamaged buds, damaged bud, undamaged bolls and damaged bolls by *P. gossypiella* of different plant parts of cotton varieties Pusa 8-6, at the flowering stage were collected for extraction of synomones. Thirty grams of each plant part were taken at the active vegetative phases and immersed overnight in 300ml of distilled hexane. The hexane was then filtered through Whatman No. 1 filter paper. Anhydrous sodium sulphate @1 g/10g sample was added to the filtrate for dehydration for 2 hrs and passed through silica gel (60-120 mesh) column. The hexane extract of different plant parts 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 for further study

Extraction of kairomone

0.5g of *E. vitella* eggs, ten gram of egg of *C. cephalonica*, 1g of moth scale and 5g of frass of *E. vitella* were immersed overnight in 10 ml, 100 ml, 10 ml and 50 ml distilled hexane respectively and filtered through Whatman No.1 filter paper and the procedure was the similar to synomonal extracts.

Gas-liquid chromatograpgy

The purified hexane extracts of different parts of host plants, egg of *C. cephalonica*, frass and eggs of *E. vitella* 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 56minutes. 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 C10-C30 were obtained from Sigma Aldrich, USA. GLC of the synomonal and kairomonal extracts were carried out to detect the presence of these saturated hydrocarbons. The resultant chromatographs were analyzed with the help of interactive graphics (Varian Star Chromatography workstation, Version 6.0) software.

EAG recording apparatus.

The EAG apparatus was used in the present studies is the assortment of EAG module IDAC-232, stimus air controller CS-05, Micro manipulator MP-15, developed by Syntech. The EAG was recorded from excised heads of parasitoids with antennae. The tip of antenna was mounted into the capillary electrode positioned at the different electrode holder that was connected with the probe and data acquisition interface. The stimulus from synomonal and kairomonal extracts were applied by using a stimulus air controller 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 @ 4m/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). All the stimulus sources synomone and kairomone were dissolved in HPLC grade hexane to supply source of stimuli.

RESULTS AND DISSCUSSION

Electroantennogram response of C. blackburni to synomonal extracts

The mean relative EAG response of *C. blackburni* varied significantly for the extracts of the various plant parts of cotton variety Pusa 8-6. The extract of the leaf elicited maximum mean relative EAG responses (151.4%) followed by damaged bud (144.3%) and damaged boll extract (137.7%), undamaged bud extract (130.3%). The mean relative EAG response was least for undamaged boll extract (121.1%) (table 3, fig1 and plate 1).

Electroantennogram response of C. blackburni to kairomonal extracts

The amplitude of EAG responses increased with increasing stimulus i.e with concentration of the odour until a saturation level is reached. Mean relative EAG responses were recorded for different concentrations of each kairomonal extract to define the optimum concentration giving the maximum mean relative EAG response of *C. blackburni*. Mean relative EAG response of parasitoids to egg wash extract of *E. vitella* (153.5%) and *C. cephalonica* (145.2%) were found maximum at C3 (100ppm) concentration. While the mean relative EAG response increased with increasing concentration of adult scale extract of *E. vitella*, the parasitoid's response was maximum (142.8%) at C5 concentration. EAG response was highest in C3 concentration for *E. vitella* frass extract (149.8%) but it decreased at C5 (135.2%). (table 4, fig 2& 3 and plate 2).

The EAG response of *C. blackburni* to kairomonal extracts was higher than synomonal extracts. In the present studies, the olfactory perception of *C. blackburni* exposed to different kairomonal extracts with different concentrations was compared, all the concentrations proved to be significantly different from the controls. However, significantly higher responses were observed only at C3 (100ppm) concentration in egg extract from *C. cephalonica* and *E. vitella* and at C5 (10^4ppm) concentration in scale extract from adult *E. vitella* and at C4 (10^3ppm) concentration in frass extract of *E. vitella*. Many studies have indicated that there is a variation in the EAG responses between different individuals (Park and Hardie, 1998) and the normalization of the EAG responses has been widely used to exclude this individual variation for evaluating the EAG responses (Park and Hardie, 1998).

Gas liquid chromatography analysis of infochemicals

Hydrocarbon profile of synomonal extracts

The gas-liquid chromatography analysis of saturated hydrocarbons present in the waxy layer of leaves, buds and bolls of variety Pusa 8-6 revealed that udamaged bud extract contained maximum number (13) of hydrocarbons ranging from C14 to C30 followed by leaf extract (12) and damaged boll extract (12). Undamaged boll extract showed the presence of only 8 hydrocarbons compared to damaged boll extract (13) (table 1)

Decane, undecane, dodecane and tridecane were absent in all synomonal extracts. Tetradecane was not founded in both damaged bud and undamaged boll extracts and pentadecane was detected only in damaged bud extract. Heptadecane was found in all extracts except in undamaged boll extract. Octadecane was detected only in leaf and damaged boll extracts but not in undamaged bud, damaged bud and undamaged boll extracts. All the extracts were devoid of nonadecane. Long chain saturated hydrocarbons like hexacosane, heptacosane, octacosane, nonacosane and triacotane were found in extracts of all the plant part of this variety. Among them heptacosane was present in the highest concentration followed by damaged boll extract, nonacosane was also found to be at a higher concentration in damaged bud extract and damaged boll extract. Pentacosane was present only in damaged bud and undamaged boll extracts. Triacotane was found to be in higher

concentration in leaf and undamaged bud extracts than in other extracts.

Hydrocarbon profile of kairomonal extracts

Out of the four kairomonal extracts from different sources analyzed by gas-liquid chromatography, egg, adult scale and frass extract of from *E. vitella* contained highest number of hydrocarbons compared to egg extract from *C. cephalonica*. Almost all the hydrocarbons were found in these extracts except decane which was absent in all the extracts. Undecane was detected in both *C. cephalonica* egg and *E. vitella* adult scale extracts but not in the rest of the extracts. Tridecane (C13), a short chain hydrocarbon was found in all extracts except in frass of *E. vitella*. The hydrocarbons ranging from C14 to C18 were detected in all extracts but C15 (pentadecane) was absent in adult scale extract of *E. vitella.* Nonadecane was found in egg and frass extracts of *E. vitella*. Eicosane, heniecosane, docosane, tricosane tetracosane were detected in all extracts except tricosane was absent in egg extract of *E. vitella*. Eicosane and heniecosane were found in highest concentration in adult scale extract of *E. vitella*. Similarly egg extract of *C. cephalonica* indicated the presence of high concentration of heniecosane. Long chain hydrocarbon ranging from C25 to C30 were found in all the of kairononal extracts except heptacosane which was absent in egg extract of *C. cephalonica*. Almost all the favorable hydrocarbons were present in the kairomonal extracts in high concentration (table 2)

Cuticular hydrocarbons are also known to serve as kairomones in many herbivore - parasitoid associations (Borges *et al*., 2003; Peri *et al*., 2006). In parasitoid - herbivore interactions long-chain hydrocarbons are used for both habitat and host location. However, it has been observed that these compounds originate from the host or its by-products and not from the plants. In the present studies some of these compounds were detected from the different parts of Pusa 8-6 cotton variety; egg extracts from *C. cepalonica* and *E. vitella* and frass extract of *E. vitella*. The hydrocarbon profiles of semiochemicals present in each extract were different both in quality and quantity. Plant waxes are known to influence the foraging success of predators or parasitoids in their ability to attach to the plant surface. Also, the wax layer can modulate the detectability of host kairomones (Rostas *et al*., 2008). Dutton (2002) reported that n-C27 (nonacosane) and n-C31 (hentriacontane) are the most commonly found hydrocarbons, they are also reported as most abundant in the apple leaves (Hellmann and Stoesser, 1992). This is also confirmed by our results. Indeed, texture has been shown to be an important physical cue for host location by several parasitoid species (Schmidt, 1991; Vinson, 1985). Hydrocarbons represent universal constituents of the insect cuticle (Wigglesworth, 1965; Blomquist and Dillwith, 1985; Hadley, 1985., Chapman, 1998). Cuticular hydrocarbons (CHCs) are considered to be stable end products of genetically controlled metabolic pathways (Ross *et al*., 1987; Grunshawn *et al*., 1990); insects are known to synthesize most hydrocarbons de novo by elongation–decarboxylation pathway (Lockey, 1985, 1988; Howard and Blomquist, 2005). The common function of CHCs is to protect insects against desiccation. Species living in dry conditions generally contain longer hydrocarbon chains in comparison to their relatives living in wet conditions (Lockey, 1988). Insect species usually possess complex mixtures of hydrocarbons including n-alkanes, branched mono-, di-, or trimethylalkanes, and others (Jackson and Blomquist, 1976). Hydrocarbon profiles may serve as fingerprints defining particular species. The composition of CHCs has been extensively studied in social insects, and in termites it is often used in taxonomic discrimination (Watson *et al*., 1989; Kaib *et al*., 1991; Bagine *et al*., 1994; Haverty *et al*., 1996; Takematsu and Yamaoka, 1999; Haverty and Nelson, 1997) and sibling species recognition (Haverty and Nelson, 1997).

The gas-liquid chromatography analysis of different plant parts from three cotton varieties and kairomones from different hosts were mainly targeted to identify the saturated

straight chain hydrocarbons. The GC analysis revealed a wide variation in number and concentration of hydrocarbons in both synomonal and kairomonal extracts. Mostly, kairomonal extracts showed greater number of hydrocarbons compared to synomonal extracts and ranged from C11 to C30 whereas the number of hydrocarbons of synomonal extracts ranged from C14 to C30. Jones *et al*., (1973) found tricosane as the most active compound and elicited high response in *T. evanescences.* This agrees well with our results, as tricosane was seen to be present in all the kairomonal extracts except egg extract from *E. vitella* but not in the synomonal extracts. That can account for parasitoid *C. blackburni* responding more to kairomonal extracts than synomonal extracts due to the presence of favorable hydrocarbons like docosane, tricosane, tetracosane and pentacosane (Jones *et al.,* 1973).

CONCLUSION

Plant waxes are known to influence the foraging success of predators or parasitoids in their ability to attach to the plant surface. Also, the wax layer can modulate the detectability of host kairomones. The hydrocarbon profiles of semiochemicals present in each extract were different both in quality and quantity. Almost all the favorable hydrocarbons were present in the kairomonal extracts in high concentration. These compounds have been found to be of importance in several bitrophic herbivore - plant interactions: as attractant for oviposition, or as attractant or deterrent for feeding. The mean relative EAG response of *C. blackburni* varied significantly for the extracts of the various plant parts of Pusa 8-6 cotton variety. The EAG response of *C. blackburni* to kairomonal extracts was higher than synomonal extracts. The present study demonstrates that different odour compounds can be distinguished by comparing the relative EAG responses of the antennae from different insect species using the multiple antennae recording technique. In parasitoid - herbivore interactions long-chain hydrocarbons are used for both habitat and host location.

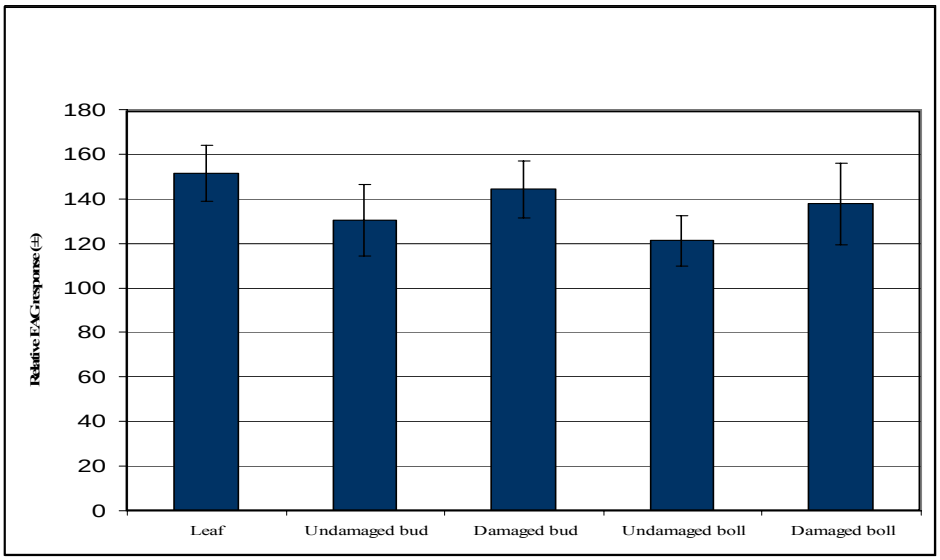


Figure 1. Electroantennogram responses of *Chelonus blackburni* to different part extracts of Pusa 8-6 cotton variety

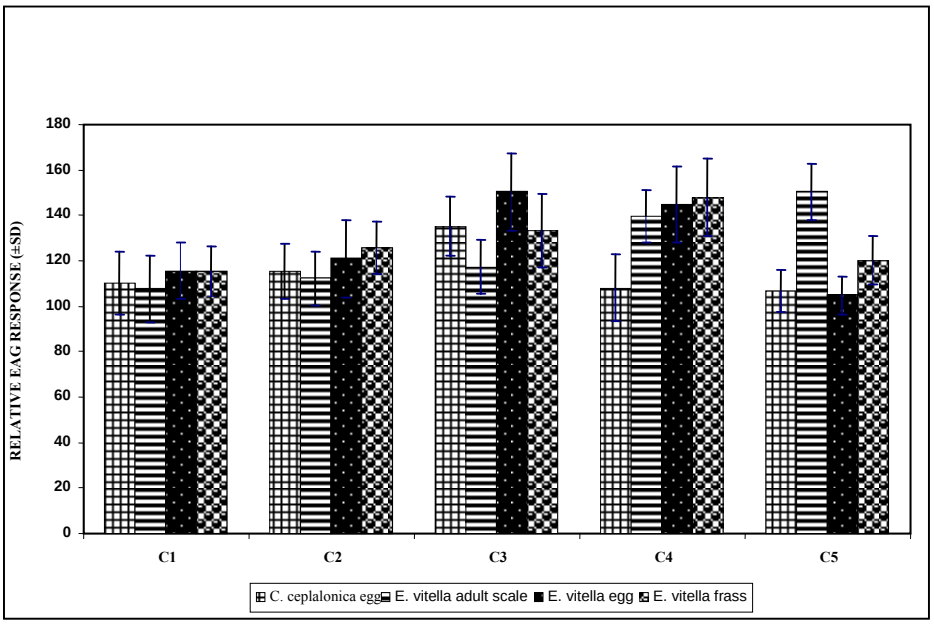


Figure 2. EAG response of ***C. blackburni*** to different concentrations of different kairomonal extracts

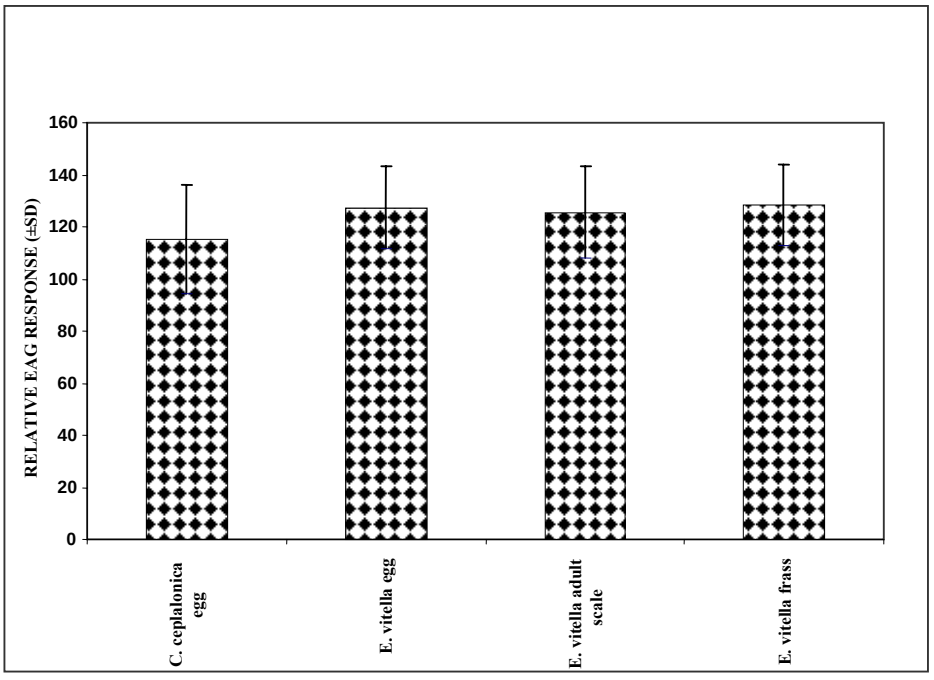


Figure 3. EAG responses of ***C. blackburni*** to different kairomonal extracts

Table 1. Hydrocarbon profile of synomonal extracts of cotton variety Pusa 8-6 (quantity in ppm)

Carbon No.	Hydrocarbon	Leaf	Undamaged bud	Damaged bud	Undamaged boll	Damaged boll
C10	Decane	ND	ND	ND	ND	ND
C11	Undecane	ND	ND	ND	ND	ND
C12	Dodecane	ND	ND	ND	ND	ND
C13	Tridecane	ND	ND	ND	ND	ND
C14	Tetradecane	1672.77	2462.95	ND	ND	1469.61
C15	Pentadecane	ND	ND	389.03	ND	ND
C16	Hexadecane	7051.75	7767.54	2306.14	1664.91	4014.04
C17	Heptadecane	4231.66	2577.30	851.17	ND	695.89
C18	Octadecane	3554.20	ND	ND	ND	719.13
C19	Nonadecane	ND	ND	ND	ND	ND
C20	Eicosane	12775.57	3708.07	1487.13	ND	7446.32
C21	Heniecosane	24676.27	9228.40	593.84	ND	5313.80
C22	Docosane	9150.79	4189.34	446.71	418.93	2117.91
C23	Tricosane	ND	2299.40	ND	ND	ND
C24	Tetracosane	ND	ND	ND	ND	ND
C25	Pentacosane	ND	3398.87	ND	418.84	ND
C26	Hexacosane	10892.69	10886.33	59794.12	1463.43	11754.37
C27	Heptacosane	164295.91	8575.55	66058.99	2492.86	111841.01
C28	Octacosane	5287.37	12709.79	406.10	1271.52	4676.92
C29	Nonacosane	20461.25	7141.58	143233.55	12444.11	47532.04
C30	Triacotane	37322.83	10277.50	358.21	562.74	5339.41

ND- Not detected

Table 2. Hydrocarbon profile of kairomonal extracts of *E.vitella*,*P.gossypiella* and *C. cephalonica*

Carbon No.	Hydrocarbon	*C. cephalonica* Egg wash	*E.vitella* Egg wash	*E. vitella* Adult scale wash	*E. vitella* Frass	*C. cephalonica* Larval body wash
C10	Decane	ND	ND	ND	ND	ND
C11	Undecane	1240.42	ND	56888.18	ND	ND
C12	Dodecane	ND	ND	ND	ND	ND
C13	Tridecane	180.62	1719.08	16064.60	ND	ND
C14	Tetradecane	351.37	12196.50	76066.61	219.82	99.92
C15	Pentadecane	60.01	2036.21	ND	187.79	50.18
C16	Hexadecane	4589.47	131622.81	194007.87	1791.23	69.30
C17	Heptadecane	40.61	28581.45	5870.33	91.83	53.53
C18	Octadecane	297.20	102998.64	53111.56	60.07	70.46
C19	Nonadecane	ND	170.75	ND	70.93	ND
C20	Eicosane	3764.86	30034.61	123527.95	743.57	3699.20
C21	Heniecosane	11146.97	10328.70	306042.70	1614.70	6585.90
C22	Docosane	12677.44	3464.29	41421.32	781.18	2157.03
C23	Tricosane	116.04	ND	12620.14	150.52	505.20
C24	Tetracosane	3311.91	1931.19	107446.23	46.56	223.73
C25	Pentacosane	ND	662.06	17672.81	781.36	15790.58
C26	Hexacosane	26277.42	8068.36	130066.77	3119.24	21162.16
C27	Heptacosane	ND	54952.43	46503.33	7067.52	12315.03
C28	Octacosane	19841.77	5512.77	53076.40	2243.85	12840.35
C29	Nonacosane	42701.94	42418.03	65407.60	7693.74	46513.41
C30	Triacotane	24086.79	13353.79	56483.14	5146.49	2516.80

ND- Not detected

Table 3. Electroantennogram response of *C. blackburni* to synomonal extract from different parts of cotton variety

Mean relative EAG responses (%) ± SD							
Variety	**LEAF**	**Undamaged bud**	**Damaged bud**	**Undamaged boll**	**Damaged boll**	**AIR**	**HEXANE**
PS 8-6	151.4±12.5	130.3±16.2	144.3±12.7	121.1±11.5	137.7±18.4	84.4±13.5	107.1±10.1

(Mean of 15 observations)

	SE	CD (P<1%)
PS 8-6	2.409	4.025**

* * Significant at 1%

Table 4. Electroantenogram response of *C.blackburni* to different concentrations of various kairomonal extracts

STATS. INDEX	Mean realative EAG responses (%) ± SD											
	CcE 1	**EvA1**	**EvE 1**	**EvF1**	**CcE 3**	**EvA 3**	**EvE 3**	**EvF3**	**CcE 5**	**EvA5**	**EvE 5**	**EvF5**
MEAN	116.5	100.9	122.3	122.6	145.2	123.6	153.5	149.8	112.3	142.8	125.4	135.2
SD (±)	11.6	9.2	12.2	13.7	15.6	11.4	14.8	17.0	13.3	13.6	10.8	13.9
CD (P<0.001)	6.89**											
SE	2.676											

** Significant at 1%

CcE 1: Egg extract of C. cephalonica (conc. 1 ppm)

CcE 3: Egg extract of c. cephalonica (conc. 100 ppm)

CcE 5: Egg extract of C. cephalonica (conc. 104 ppm

EvE 1: Egg extract of E. vitella (conc. 1ppm)

EvE 3: Egg extract of E. vitella (conc. 100 ppm)

EvE 5: Egg extract of E. vitella (conc. 104ppm)

EvA 1: Scale extract of E. vitella (conc. 1 ppm)

EvA3: Scale extract of E. vitella (conc. 100 ppm)

EvA 5: Scale extract of E. vitella (conc. 104 ppm)

E vF 1: Feces extract of E. vitella (conc. 1ppm)

EvVF 3: Feces extract of E. vitella (conc. 100 ppm)

EvF 5: Feces extract of E. vitella (conc. 104 ppm)

Plate 1. EAG responses of *C. blackburni* to synomonal extracts of cotton variety Pusa 8-6 (Repeated with 5 individual wasps)

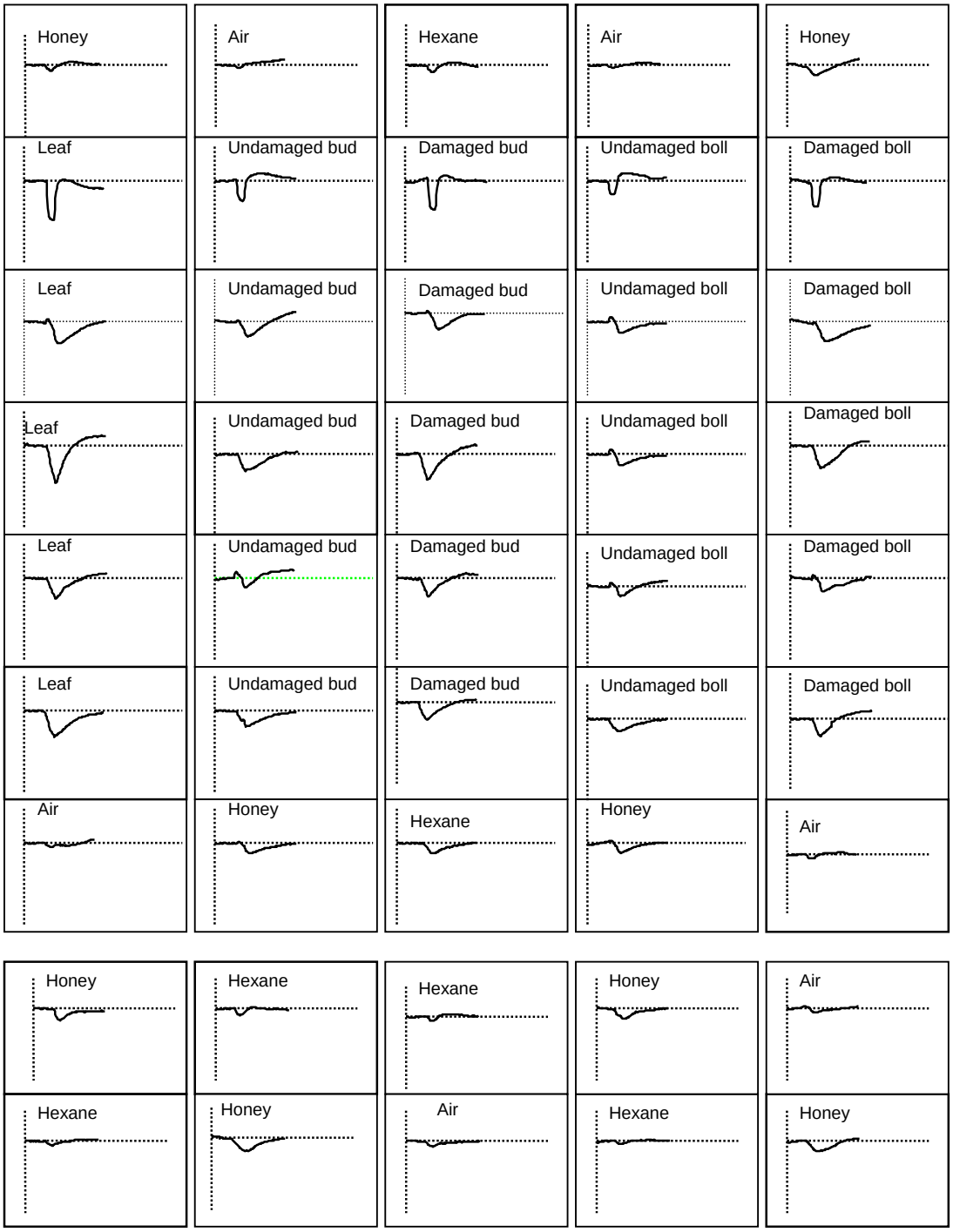
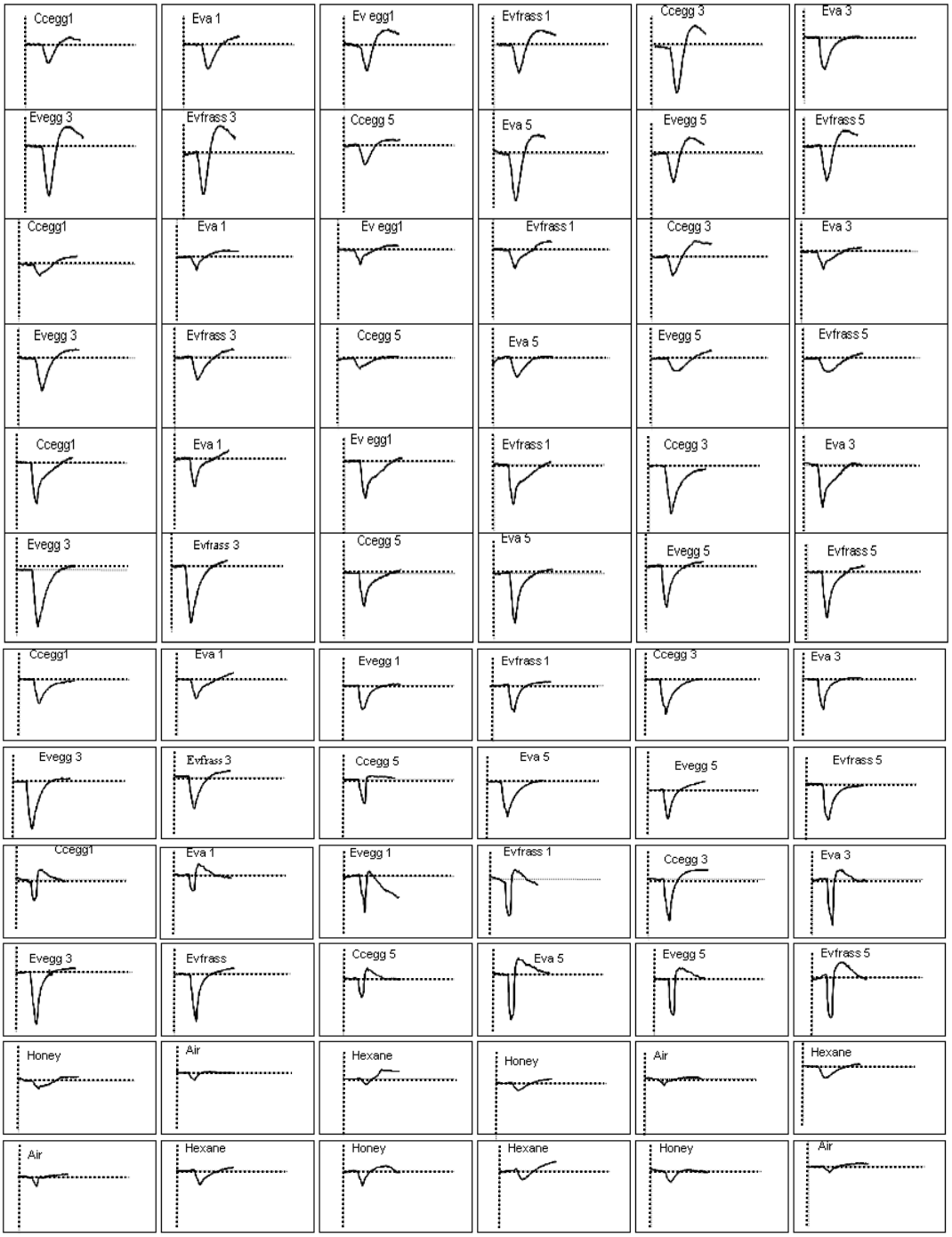


Plate 2. EAG response of C. blackburni to different concentraions of kairomonal extracts (Repeated with 5 individual wasps)



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PHẢN ỨNG EAG CỦA *Chelonus blackburni*, ONG KÝ SINH SÂU ĐỤC TRÁI BÔNG *Earias vitella* ĐỐI VỚI HỢP CHẤT HÓA TRUYỀN TIN (infochemical)

Kết quả phản ứng EAG của *C. blackburni* Cameron đối với cả hai hợp chất hóa truyền tin infochemical (hợp chất dễ bay hơi) bao gồm synomone từ lớp cutin của các bộ phận bị gây hại bởi sâu đục trái bông *E. vitella* và không bị gây hại trên giống bông vải Pusa 8-6 và kairomon (hợp chất hydrocarbon tiết ra từ lớp cutin của ấu trùng, trứng, chất thải và vẩy của *E. earias* và *C. cephalonica* cho thấy loài ong ký sinh nầy có khả năng phản ứng mạnh đối với lá và những bộ phận bị gây hại so với những bộ phận không bị gây hại, phản ứng của *C. blackburni* đối với synomone yếu hơn so với kairomone. Kairomone được biết là chất tiết ra từ ký chủ giúp ký sinh định vi nơi có ký chủ xuất hiện, EAG có biên độ cực đại (153,5%) đối với hợp chất từ vỏ trứng của *E. earias*, ở nồng độ 100ppm, so với hợp chất từ chất thải (149,8%) của cùng nồng độ và 142,8 % trên hợp chất từ lớp vẩy của thành trùng nầy ở nồng độ cao hơn (10^4ppm). Hầu hết hydrocarbon hiện diện trong những hợp chất này có nồng độ cao như Heptacosan, Hexacosane, Nonnacosane and Triacotane. Những synomone này được cho là hợp chất ưa thích đối của khả năng định vị ký chủ ong ký sinh. Ngoài ra, những hợp chất này được biết đóng vai trò quan trọng trong sự tương tác giữa cây trồng- sâu hại như hấp dẫn sự đẻ trứng, hấp dẫn hoặc xua đuổi côn trùng đến gây hại. Vì vậy, phản ứng EAG được sử dụng cho mục đích nghiên cứu phản ứng của các loài ong ký sinh đối với hợp chất infochemical trong thiên nhiên từ đó có thể tạo ra một số infochemical nhân tạo giúp gia tăng khả năng định vị ký chủ của chúng trong môi trường thiên nhiên nhằm đạt được hiệu quả cao trong phòng trừ sinh học.