Volatile cues from *Corcyra cephalonica* larva elicit behavioural responses in parasitoid, *Habrobracon hebetor*

G. Basana Gowda[1,*,†], Totan Adak[1,†], P. D. Kamala Jayanthi[2], P. Saravan Kumar[2], G. Guru-Pirasanna-Pandi[1], Naveenkumar B. Patil[1], M. Annamalai[1] and P. C. Rath[1]

[1]Division of Crop Protection, ICAR-National Rice Research Institute, Cuttack 753 006, India
[2]Division of Crop Protection, ICAR-Indian Institute of Horticultural Research, Bengaluru 560 089, India

The rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae), is a serious pest of grains in storage systems resulting in immense losses but is also widely used as a factitious host for mass rearing of many important natural enemies of crop pests. Given the role of kairomones, the aim of this study was to isolate and identify potential cues from the larval body wash of *C. cephalonica,* which could attract its gregarious ecto-parasitoid, *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae). Gas chromatography with electroantennography (GC-EAG) and olfactory assays were used to demonstrate the attraction of female *H. hebetor* to different larval body volatiles. A total of 15 EAG-active compounds were discovered in the body wash of *C. cephalonica* larvae that triggered a response in female *H. hebetor*. Among them, four compounds (*p*-xylene, naphthalene, *n*-eicosane and *n*-tricosane) were bioassayed for the behavioural response of parasitoids and found that *n*-eicosane significantly attracted a higher number of parasitoids than others. Our work establishes the attraction of *H. hebetor* to volatile kairomone cues emanating from the factitious host larval body, which offers an opportunity for its parasitoid, *H. hebetor* to improve the mass rearing efficiency.

Keywords: Behavioural assays, GC-EAG, GC-MS, larval volatiles, olfactometer.

CEREAL grains are very likely to be attacked by many storage pests resulting in quantitative as well as qualitative losses during storage[1]. Among the 600 insect species that infest stored products globally, nearly 100 species representing the families of Lepidoptera and Coleoptera are known to cause substantial economic losses[2]. Of these, the rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae), is an important storage grain pest throughout the world[3,4]. *C. cephalonica* larvae cause severe damage to stored food commodities while feeding, leaving silken threads that are later converted into a webbed mass[5,6]. Apart from rice, the larvae are also found to infest other products, including corn, sorghum, cocoa, almond, beans and dry fruits[7]. The infestation of rice moths in storage results in the loss of nearly milled rice by 3–7% (ref. 8). Being a serious storage pest having a wide host range, this insect has caught the attention of several scientific groups.

Synthetic chemical insecticides majorly dominate the control of storage insects to date. Despite insecticide's effectiveness, its widespread and uncontrolled usage frequently leads to pesticide resistance and detrimental non-target consequences[9]. Due to these shortcomings, non-chemical techniques of stored-product protection, such as biological control, have recently got a lot of attention[10–12].

Besides being a pest insect, *C. cephalonica* is also widely used as a factitious host for mass-rearing several biocontrol agents[13]. The gregarious ecto-larval parasitoid *Habrobracon hebetor* Say (Hymenoptera: Braconidae) is an effective natural enemy of several lepidopteran pests of stored grains and grain products[14,15]. It is found to be specific against multiple lepidopteran pests, including *C. cephalonica*, and provides exceptional biological control services in terrestrial agroecosystems[6,16]. *H. hebetor* females sting their host larvae to paralyse them before laying a varied number of eggs on or near the surface of the paralysed hosts[17]. Understanding the role of chemical cues that mediate the attraction of the female parasitoids, *H. hebetor*, to the *C. cephalonica* larvae could pave the way for enhancing the mass production efficacy of these parasitoids.

Semiochemicals are largely underappreciated in the field of insect pest management. Semiochemicals have a better chance of preventing insect pests from infesting stored products[18]. They play an important role in foraging female parasitoids' host habitat location, host acceptance and oviposition[19]. Intensified searching by the parasitoids is usually accompanied by the host body volatiles, mainly consisting of species-specific compounds such as saturated long-chain hydrocarbons, fatty acids and proteins[20,21]. The host searching followed by the parasitization capacity of *H. hebetor* is influenced by several factors in the storage

*For correspondence. (e-mail: basanagowda.g@icar.gov.in)
†Equally contributed.

environment. Further, the movement pattern of the parasitoid females is greatly influenced by the host stimuli. It has been reported that *H. hebetor* follows the chemical cues originating from the host larvae, frass and adults to locate its host[22]. The stimulatory effect of larval body volatiles on the oviposition activity of parasitoids has been proved by many scientists[23,24]. To date, there are no reports on the biological activity of *C. cephalonica* larval body volatiles against its parasitoid, *H. hebetor*. Hence, the present study was conducted to identify the potent larval body volatiles of *C. cephalonica* that have stimulatory effects on the attraction behaviour of its gregarious ecto-parasitoid, *H. hebetor*. Using gas chromatography-mass spectrometry (GC-MS), coupled gas chromatography-electroantennogram (GC-EAG) and olfactometer assays, we were able to identify and bioassay the constituents that attracted *H. hebetor*. The output of the present study would prove relevant for understanding the chemically mediated behavioural interactions between the host insect, *C. cephalonica* and its parasitoid, *H. hebetor*. Further, it helps to increase the efficiency of the conventional mass rearing of the parasitoid as these volatiles would stimulate the parasitoid to lay more eggs.

Material and methods

Rearing of rice moth, C. cephalonica

The rice moth, *C. cephalonica* colony, was received from the biocontrol laboratory, ICAR-National Rice Research Institute (NRRI), Cuttack, India. The insects were raised using Lalitha and Ballal's modified approach[25]. To mass rear *C. cephalonica*, 0.25 cc (5000 eggs) of eggs were inoculated on a medium containing insecticide-free and oven-sterilized broken maize kernels (2.5 kg), multivitamin powder (5 g), finely ground roasted peanut seeds (50 g), streptomycin sulphate (0.2 g), yeast (2 g) and formalin (0.1%; 10 ml)[26,27]. This setup was kept undisturbed until the larval and pupal development was complete for 30–35 days. Moths were collected after adult emergence using a vacuum suction apparatus (motor power: 120 W) that consisted of an outer plastic circular container (50 L) and an interior oviposition chamber (10 L) with wire mesh on the base to facilitate egg collection. To provide supplemental nourishment to the adult moths, a cotton swab dipped in 50% honey was put on the inside wall of the oviposition chamber. For raising, the eggs were gathered and kept on a fresh diet. Before larvae were utilized in the different experiments, they were reared for 10 generations, and 10th-generation larvae were used for experiments.

Source and rearing of parasitoids

An initial mother colony of parasitoids, *H. hebetor* was collected as pupae (~100) from the biocontrol laboratory, ICAR-NRRI (National Accession number: NBAIR-NRRI-HAB-01). A pair of parasitoids was placed in a plastic container (1000 ml) for mating shortly after adult emergence. Following a 24 h period of mating, five late instar larvae (4th and 5th instar)[28] of *C. cephalonica* were put between two layers of fine muslin cloth and placed over the mouth of the container (referred to as the sandwich method)[29]. Adult parasitoids were able to feed on honey delivered via sterilized cotton swabs. Female parasitoids were permitted to attack host larvae for 48 h before being incubated under regular laboratory conditions for further development (temperature 25° ± 1°C; relative humidity 70% ± 5%; and 14 h light: 10 h dark). Adult female parasitoids (0–12 h old) were reared for five continuous generations before they were used in experiments to avoid any carry-over effect.

Larval volatile extraction

To obtain *C. cephalonica* larval body wash, the solvent-assisted extraction (SAE) method[30] was followed. For this, analytical grade hexane (99.9%) was used. In a 25 ml test tube, ten numbers of *C. cephalonica* fifth instar larvae (weight = 0.53 g) were placed, then hexane (4 ml) was poured until the larvae were completely dipped in the solvent and manually agitated at room temperature of 26° ± 2°C for 2 min. Sodium sulphate (2 g) was added to the body wash to remove moisture. The content was filtered through a polytetrafluoroethylene (0.22 μ) filter paper. The filtrate was concentrated using a nitrogen evaporator (Nitrovap–1LV, Parker Hannifin, USA, and Nitrovap, Athena Technologies, India), then reconstituted in 2 ml hexane and stored at –20°C until further use.

Gas chromatography-electroantennogram studies

Using a gas chromatography-coupled electroantennogram detection method (GC-EAG), female *H. hebetor* reactions to *C. cephalonica* larval body volatiles were examined. A flame ionization detector (FID) was installed on the GC (Agilent 7890 B), which was connected to a Syntech EAG IDAC-4. To separate the body wash volatiles, a non-polar Hewlett-Packard-5 bonded phase fused silica capillary column with dimensions of 30 m length × 0.320 mm internal diameter × 0.25 μm film thickness was utilized. The thermal programme began with a 60°C oven temperature for 1 min, then climbed at 15°C/min to 240°C and held for 2 min in splitless mode at a constant pressure of 8.3 psi, for a total run time of 26 min with nitrogen as a carrier gas. The effluent from the GC column was split in half and directed to the antennal preparation and the GC detector simultaneously for the coupled GC-EAG analysis. If a peak eluting from the GC column evoked EAG activity in three or more runs, it was termed active. The data were examined with Chemstation software.

Electroantennogram (EAG) recordings were made using 1–2 days old mated *H. hebetor* females, as described by a

standard protocol[31]. Empty air and honey were employed as negative and positive controls respectively, in this bioassay. The olfactory stimuli were created by impregnating separate filter paper strips (Whatman No. 1, 6 cm length × 0.5 cm breadth) with 10 µl of distinct chemical compounds identified in *C. cephalonica* larval body wash. After allowing the solvent to evaporate for 1 min, the filter paper was placed inside the glass pasture pipettes (10 cm length and 6 mm outer diameter). Using a highly conductive electrolyte gel (Signa gel, Parker Laboratories, Inc.), the antenna of a sexually matured adult wasp was removed from the head and inserted across the positive and negative electrodes of an EAG combi-probe. A regulated airflow (300 ml/min) through the pipette with filter paper was used to stimulate antennal preparation. The odour stimulation was delivered, intensified, and recorded using Autospike software by delivering a puff of pure air (0.5 sec) (Syntech EAG Model IDAC-4, Intelligent Data Acquisition Controller). The test stimuli were administered in order, with interspersed control stimulation, to measure stimulus-response. Purified air was blown over the antennal preparation for at least 30 sec between stimulus presentations. The EAG Probe was set to a 100 Hz sampling rate with a 0–32 Hz filter rate. The normalized mean of all recorded antennal depolarizations was used to express the responses (amplitudes) to the host larval volatiles.

Gas chromatography coupled mass spectrometry analysis

Gas chromatography-mass spectrometry (GC-MS) was used to determine the chemical composition of the larval body extracts, with the same temperature programming as in the GC-EAG experiment (previous section). An Agilent 7890 B gas chromatography system was used to perform the mass spectral analysis, coupled with a mass spectrometry detector (Agilent 5977 B). The MS detector was tuned to a scan range of 40–450 mz^{-1} and held at 280°C in full scan mode (70 eV). One microlitre of each sample was injected through a 270°C injection port using a splitless mode (40 ml min^{-1}). Furthermore, long-chain alkanes have been suggested to play a function in plant–insect interactions[32,33]. The earlier GC method could detect compounds till *n*-C_{20}. To understand the presence of long-chain alkanes in larval body washes, there was a need to increase the GC oven temperature. Another temperature programming of the GC oven was done as follows: GC oven started at 50°C and was raised at 5°C min^{-1} to 200°C, then increased to 250°C at 10°C min^{-1} and held for 2 min, and later it increased to 280°C at 15°C min^{-1} and held for 5 min. A Shimadzu GC 2010 gas chromatogram (GC) linked to a TQ8040 mass spectrometer was used for the analysis (Shimadzu Corporation, Kyoto, Japan). A non-polar capillary column (SH-Rtx-5MS, 30 m length, 0.25 mm inner diameter, 0.25 µm film thickness; cross band 5% diphenyl/95% dimethyl polysiloxane) was used in GC. The carrier gas was helium, which had a constant linear velocity of 47.2 cm s^{-1}. A total of 1 µl sample was injected in splitless mode at 250°C in the injector. The MS was tuned to a scan range of 40–500 mz^{-1} and operated in electron impact (EI) mode at 70 eV. The volatile chemicals were identified by comparing their mass spectra to those kept in the National Institute of Standards and Technology 11 (NIST 11) mass spectral library, as well as their retention indices to those reported in the literature.

Chemicals

Authentic chemical standards (>96.99% pure) of compounds studied were procured from Sigma-Aldrich (Bangalore, India). Of the 15 EAG-active compounds detected, the olfactometer bioassay was conducted with available authentic standards (10 µl of 100 ng/µl) in our laboratory. The studied chemical compounds were dissolved in hexane for an olfactometer assay.

Olfactometer bioassay

The olfactometer bioassays were carried out according to an earlier procedure[34]. The behavioural effect of *C. cephalonica*, larval body wash volatiles on female adult *H. hebetor*, was investigated using a Y-tube olfactometer. The bioassays were conducted involving a single-choice option where the test sample (treated arm) was in one arm of the olfactory apparatus, whereas hexane was in the other (control arm). The experiment was carried out at room temperature (27° ± 1°C). Both the test sample as well as solvent control (hexane) were applied to filter paper strips (1 µl) and inserted in the corresponding arms. Each test insect (individual mated adult female) was given 10 min to decide. The number of *H. hebetor* that responded to the treated arm and control was recorded (N = 30).

Statistical analysis

The data from Y-tube olfactometer assays were subjected to a paired *t*-test. All analyses were carried out using SPSS 19.0 software, with Tukey's honestly significant difference (HSD) test used to compare treatment means. Further, the selection rate was calculated by the following formula[35].

$$\text{Selection rate} = \frac{\text{Insect number in treated arm}}{\text{Total number of insects}} \times 100.$$

Results and discussion

Gas chromatography-electroantennogram studies

The preliminary results indicated that crude extract of larval body wash of *C. cephalonica* attracted the parasitoids

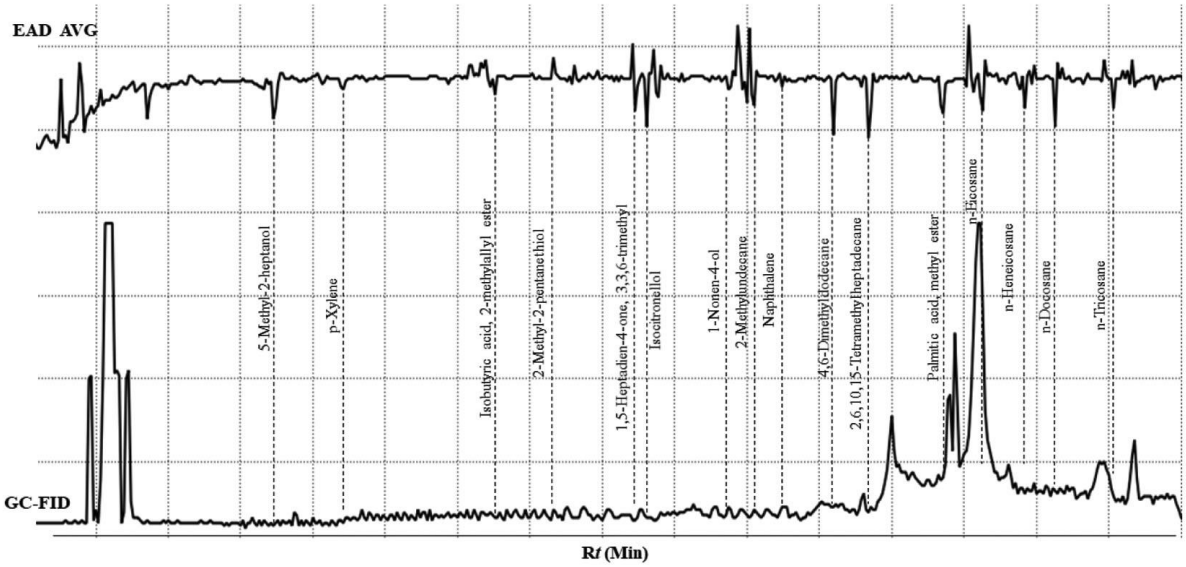

Figure 1. GC-EAG chromatogram showing the antennal responses of *Habrobracon hebetor* females to *Corcyra cephalonica* larval body volatiles. The serial numbers indicate compounds that elicited an antennal response in *H. hebetor* wasp and are listed in Table 1.

Table 1. Electrophysiologically active compounds of *Corcyra cephalonica* larval body volatile sample that elicited antennal responses in *Habrobracon hebetor*

Peak numbers	Compounds	Retention time (RT) (min)	Area (%)	EAG response (mV)
1	5-Methyl-2-heptanol	3.52	3.28	0.57
2	*p*-Xylene	4.24	0.41	0.16
3	Isobutyric acid, 2-methylallyl ester	5.05	4.72	0.24
4	2-Methyl-2-pentanethiol	5.18	0.63	0.14
5	1,5-Heptadien-4-one, 3,3,6-trimethyl	6.00	8.37	0.59
6	Isocitronellol	6.34	1.56	0.74
7	1-Nonen-4-ol	6.49	0.37	0.17
8	2-Methylundecane	7.3	0.21	0.52
9	Naphthalene	9.35	1.36	0.09
10	4,6-Dimethyldodecane	11.19	0.65	0.78
11	2,6,10,15-Tetramethylheptadecane	17.19	1.48	0.92
12	Palmitic acid, methyl ester	18.21	8.32	0.58
13	*n*-Eicosane	18.96	31.17	0.57
14	*n*-Heneicosane	19.86	1.15	0.55
15	*n*-Docosane	20.76	1.93	0.78
16	*n*-Tricosane	21.63	4.67	0.54

when tested with a Y-tube olfactometer. Hence the extract was subjected to GC-EAG to ascertain the response of *H. hebetor* to individual compounds of the extract. A total of 16 compounds from *C. cephalonica* larval body volatiles, viz. [5-methyl-2-heptanol; *p*-xylene; isobutyric acid, 2-methylallyl ester; 2-methyl-2-pentanethiol; 1,5-heptadien-4-one, 3,3,6-trimethyl; isocitronellol; 1-nonen-4-ol; 2-methylundecane; naphthalene; 4,6-dimethyldodecane; 2,6,10, 15-tetramethylheptadecane; palmitic acid, methyl ester; *n*-eicosane; *n*-heneicosane; *n*-docosane; *n*-tricosane] elicited an EAG response in *H. hebetor* (Figure 1 and Table 1). The GC-MS/total ion chromatogram of *C. cephalonica* larva body volatiles, along with the alkane hydrocarbon mix, is presented in Supplementary Figure 1.

Behavioural response of H. hebetor to different synthetic larval body volatiles

Female parasitoids wandered around in the olfactometer without displaying any distinct direction after being introduced. When the parasitoid detected a cue after the initial walking, it demonstrated arrestment. Antennal drumming and upwind movement towards the stimulus source were two other behavioural tendencies found. Among the four compounds tested, *n*-eicosane recorded a significantly higher parasitoid response towards the treated arm than the control (hexane) (0.733 ± 0.082; $t = 2.841$, d$f = 29$, $P = 0.008$). The remaining three compounds did not elicit any significant response (*p*-xylene: 0.466 ± 0.09; $t = -0.360$, d$f = 29$,

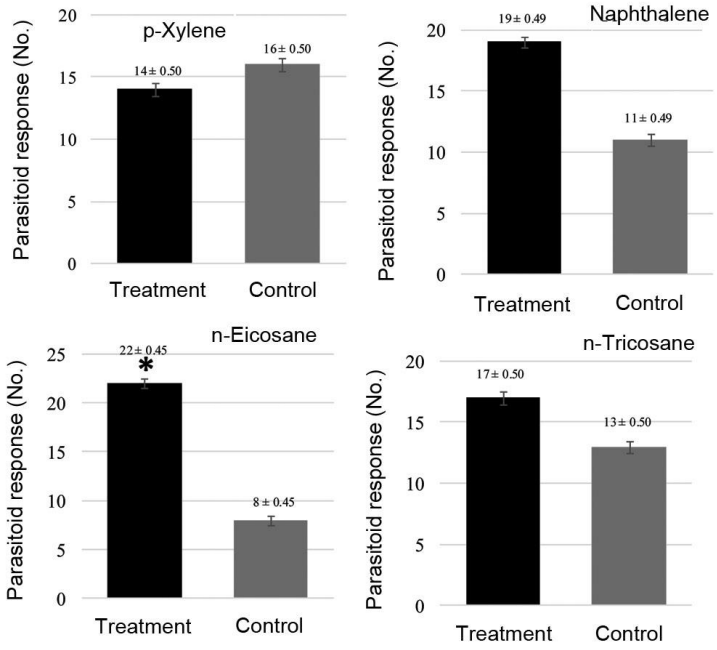

Figure 2. Response of *H. hebetor* females to different synthetic GC-EAG compounds of *C. cephalonica* larval body volatiles in olfactometer assays. *Denotes significant difference between treatment and control.

$P = 0.722$; naphthalene: 0.633 ± 0.08, $t = 1.490$, d$f = 29$, $P = 0.147$; *n*-tricosane: 0.566 ± 0.09, $t = 0.724$, d$f = 29$, $P = 0.475$) (Figure 2). Among the four compounds, the highest selection rate was recorded for *n*-eicosane (73.33%), followed by naphthalene (63.33%), *n*-tricosane (56.67%) and *p*-xylene (46.67%).

Semiochemicals are linked to a variety of insect behaviours, including feeding, mating and egg-laying[36]. Host insects have been observed to emit chemical cues containing unique hydrocarbons, fatty acids and proteins to encourage natural enemies to locate their host[37]. Only host-associated volatiles may be appealing or ecologically relevant to parasitoids for final host location and recognition[38]. Kairomones are chemical cues that aid interspecific interactions to benefit the species that detect them. In this study, we uncovered volatile cues that a natural enemy (*H. hebetor*) uses to exploit olfactory information acquired from its host (*C. cephalonica*). According to our findings, the volatiles generated by the host larval bodies (*C. cephalonica*) acted as kairomones to attract females of the larval parasitoid *H. hebetor*. A study by Ram *et al.*[39] reported that hexane extract of the *C. cephalonica* cuticle elicited a host-seeking response in female *Bracon brevicornis* Wesmael (Hymenoptera: Braconidae) adults. However, this is the first study to show that volatiles emitted by the factitious host's larval body, *C. cephalonica*, can attract females of a larval parasitoid, *H. hebetor*, and hence serve as a signal for host detection and location. Several previous studies have reported the role of volatiles/washings of host insects (other than the larval stage of *C. cephalonica*) in eliciting attraction in natural enemies. The parasitoid, *Bracon hebetor*, used the 2-acylcyclohexane-1,3-diones produced in the mandibular glands of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) fifth instars as arresting and host-trail tracking kairomones[40]. Application of host extract (kairomone) to the host larvae, *E. kuehniella* had reduced the host location time and enhanced the parastitism rate of its parasitoid, *B. hebetor*[41].

Of the four compounds (*p*-xylene, naphthalene, *n*-eicosane and *n*-tricosane) bioassayed for behavioural response, it was found that female *H. hebetor* was significantly attracted to *n*-eicosane. In the present study, we report *n*-eicosane as a kairomone from *C. cephalonica* larval body washes to female *H. hebetor*. Of the 16 compounds detected in GC-EAG, *n*-eicosane had the highest peak area of 31.17%, indicating its proportion in the *C. cephalonica* larval body wash. The compounds detected in the GC-EAG analysis of the present study have been reported earlier as potent insect attractants in various flora and fauna. The *n*-eicosane has been earlier reported to be a kairomone from various insect species such as *Hyphantria cunea* (Lepidoptera: Erebidae) pupae[42], lac insect (*Kerria lacca* Kerr.) (Hemiptera: Kerriidae) whole body extracts[43], and *C. cephalonica*[44]. Eicosane was found in the highest concentration in adult scale extract of *Earias vitella* ((Lepidoptera: Nolidae), which had kairomonal activity for *Chelonus blackburni* Cameron (Hymenoptera, Braconidae)[45]. *p*-Xylene is an aromatic hydrocarbon and has been reported from volatiles of various plant species to elicit responses in different insects like *Adelphocoris* spp. (Hemiptera: Miridae)[46],

braconid parasitoid, *Microplitis mediator* (Hym.: Braconidae)[47], *Saperda populnea* L. (Coleoptera Cerambycidae)[48] and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae)[49].

Habrobracon hebetor responded selectively to previously identified pheromone components of *Galleria mellonella*, nonanal and undecanal[50,51]. Naïve mated females *B. hebetor* were attracted significantly to two different blends of nonanal and undecanal in Y-tube tests[52]. In the present study, *n*-tricosane, a solid *n*-alkane reported earlier as an insect repellent, elicited a non-significant attraction response in the female *H. hebetor* during olfactory bioassay. The common predatory backswimmer, *Notonecta maculata* Fabricius (Hemiptera: Notonectidae), releases *n*-tricosane, which repels ovipositing females of *Culiseta longiareolata* (Macquart) (Diptera: Culicidae)[53]. Nakashima *et al.*[54] found that a terrestrial predatory beetle, *Coccinella septempunctata* (Coleoptera: coccinellidae), emitted various hydrocarbons like *n*-tricosane, which repelled an aphid parasitoid, *Aphidius ervi* (Hymenoptera: Braconidae). Extracts of female *C. cephalonica* bodies were found to be more attractive than males to *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae) and *Chrysoperla zastrowi sillemi* (Esben-Peterson) (Neuroptera: Chrysopidae), due to the presence of large amounts of attractive hydrocarbons, such as tricosane[55]. *A. ervi* and *Praon volucre* (Hymenoptera: Braconidae) avoided the predators *C. septempunctata* (L.) and *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) because the chemical trails of these predators had *n*-tricosane [$C_{23}H_{48}$] (ref. 56). Similarly, repellency of naphthalene to parasitoids were also reported in earlier studies[57]. In some cases of plants, UV absorption of accumulated naphthalene in the floral parts might attract insects to pollinate[58].

In the present study, it was demonstrated that out of the four compounds bioassayed for a behavioural response, two were alkanes (*n*-eicosane and *n*-tricosane). Generally, alkanes found in plant leaf surface waxes have been shown to play an important role in plant–insect interactions serving as oviposition stimulants[32,33]. We found that females of *H. hebetor* used alkanes such as *n*-eicosane as olfactory cues for oviposition on *C. cephalonica* larvae. Sarakar *et al.*[59] also reported long-chain alkanes from a host plant, *Momordica charantia* L. (Cucurbitaceae), as an attractant to pest insect, *Epilachna dodecastigma* (Coleoptera: Coccinellidae). Our bioassay results revealed that *H. hebetor* responded to alkanes like *n*-eicosane, which are low-volatile compounds that could serve as close-range cues. However, volatiles derived from the host, including low-molecular-weight aldehydes, alcohols, ketones and esters, may also act as long-distance indicators of the insect's host location[60].

Insect pests are increasingly being controlled biologically with synthetic kairomone-based lures. The use of host signals (kairomones) to improve entomophage effectiveness has long been proposed[23,61]. Natural enemies' olfactory associative learning for improved biological control has gotten a lot of attention recently, especially when training or conditioning them to kairomones during the mass-raising phase before field release[57,62]. Kairomone-based lure technologies can be used in field crops where background odours help natural enemies in locating herbivores[63,64]. Volatile compounds released by herbivores or hosts may often be distinguished from plant-field background odours, and they are the most reliable sources of information for natural enemies[65].

Conclusion

Overall, this study, for the first time, characterized the host (*C. cephalonica*) larval volatiles that elicited a behavioural response in its parasitoid (*H. hebetor*), emphasizing the importance of parasitoid host habitat locating signals from the host larva. The findings of this study may have potential implications. The possible kairomones revealed in the study may be employed to improve biological control tactics in the field by attracting and retaining natural enemies (attract and reward strategies), which helps reduce insect pest populations and is also ecologically beneficial. Secondly, since *H. hebetor* is an important parasitoid in managing a variety of lepidopteran pests, it can be commonly mass-produced in the laboratory on *C. cephalonica* as its factitious host. The kairomones identified in the present study could be employed to condition the parasitoids during their mass-rearing in the laboratory to improve biocontrol efficiency later in the field. Future studies should concentrate on preparing a stable volatile blend by combining diverse volatile compounds in appropriate ratios, as well as testing them in more natural situations and determining their effects on non-target organisms.

1. Hagstrum, D. and Subramanyam, B., *Fundamentals of Stored-Product Entomology*, St Paul. Woodhead Publishing and AACC International Press, 2006.
2. Anukiruthika, T., Jian, F. and Jayas, D. S., Movement and behavioral response of stored product insects under stored grain environments-A review. *J. Stored Prod. Res.*, 2021, **90**, 101752; https://doi.org/10.1016/j.jspr.2020.101752.
3. Coelho, M. B., Marangoni, S. and Macedo, M. L. R., Insecticidal action of *Annona coriacea* lectin against the flour moth *Anagasta kuehniella* and the rice moth *Corcyra cephalonica* (Lepidoptera: Pyralidae). *Comp. Biochem. Physiol. Part C*, 2007, **146**(3), 406–414; https://doi.org/10.1016/j.cbpc.2007.05.001.
4. Rani, A., Chand, S., Thakur, N. and Nath, A. K., Alpha-amylase inhibitor from local common bean selection: effect on growth and development of *Corcyra cephalonica*. *J. Stored Prod. Res.*, 2018, **75**, 35–37; https://doi.org/10.1016/j.jspr.2017.10.009.
5. Allotey, J. and Azalekor, W., Some aspects of the biology and control using botanicals of the rice moth, *Corcyra cephalonica* (Stainton), on some pulses. *J. Stored Prod. Res.*, 2000, **36**(3), 235–243; https://doi.org/10.1016/S0022-474X(99)00045-4.
6. Gowda, G. B. *et al.*, Insecticide-induced hormesis in a factitious host, *Corcyra cephalonica*, stimulates the development of its gregarious ecto-parasitoid, *Habrobracon hebetor*. *Biol. Control*, 2021, **160**, 104680; https://doi.org/10.1016/j.biocontrol.2021.104680.
7. Hashem, M. Y., Ahmed, A. A., Ahmed, S. S., Mahmoud, Y. A. and Khalil, S. S., Impact of modified atmospheres on respiration of last instar larvae of the rice moth, *Corcyra cephalonica* (Lepidoptera:

Pyralidae). *Arch. Phytopathol. Plant Prot.*, 2018, **51**(19–20), 1090–1105; https://doi.org/10.1080/03235408.2018.1560022.
8. Osman, N. (ed.), Assessment of damage by the rice moth *Corcyra cephalonica* (St.) on different grains at four levels of moisture content. In Proceedings of 7th ASEAN Tech Seminar on Grain Post Harvest Technology, 1984.
9. Champ, B. and Dyte, C., FAO global survey of pesticide susceptibility of stored grain pests. *FAO Plant Prot. Bull.*, 1977, **25**(2), 49–67.
10. Adarkwah, C. *et al.*, Effectiveness of the egg parasitoid *Trichogramma evanescens* preventing rice moth from infesting stored bagged commodities. *J. Stored Prod. Res.*, 2015, **61**, 102–107; https://doi.org/10.1016/j.jspr.2015.01.002.
11. Adarkwah, C., Obeng-Ofori, D., Opuni-Frimpong, E., Ulrichs, C. and Schöller, M., Predator–parasitoid–host interaction: biological control of *Rhyzopertha dominica* and *Sitophilus oryzae* by a combination of *Xylocoris flavipes* and *Theocolax elegans* in stored cereals. *Entomol. Exp. Appl.*, 2019, **167**(2), 118–128; https://doi.org/10.1111/eea.12760.
12. Stejskal, V., Kosina, P. and Kanyomeka, L., Arthropod pests and their natural enemies in stored crops in northern Namibia. *J. Pest Sci.*, 2006, **79**(1), 51–55; https://doi.org/10.1007/s10340-005-0109-2.
13. Vincent, A., Singh, D. and Mathew, I. L., *Corcyra cephalonica*: a serious pest of stored products or a factitious host of biocontrol agents? *J. Stored Prod. Res.*, 2021, **1(**94), 101876.
14. Akinkurolere, R., Boyer, S., Chen, H. and Zhang, H., Parasitism and host-location preference in *Habrobracon hebetor* (Hymenoptera: Braconidae): role of refuge, choice and host instar. *J. Econ. Entomol.*, 2009, **102**(2), 610–615; https://doi.org/10.1603/029.102.0219.
15. Mbata, G. N., Eason, J., Payton, M. and Davis, M., Putative host volatiles used by *Habrobracon hebetor* (Hymenoptera: Braconidae) to locate larvae of *Plodia interpunctella* (Lepidoptera: Pyralidae). *J. Insect Behav*., 2017, **30**(3), 287–299; https://doi.org/10.1007/s10905-017-9619-z.
16. Ghimire, M. N. and Phillips, T. W., Mass rearing of *Habrobracon hebetor* Say (Hymenoptera: Braconidae) on larvae of the Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae): effects of host density, parasitoid density, and rearing containers. *J. Stored Prod. Res.*, 2010, **46**(4), 214–220; https://doi.org/10.1016/j.jspr.2010.05.003.
17. Antolin, M. F., Ode, P. J. and Strand, M. R., Variable sex ratios and ovicide in an outbreeding parasitic wasp. *Anim. Behav.*, 1995, **49**(3), 589–600; https://doi.org/10.1016/0003-3472(95)80192-8.
18. Cox, P., Potential for using semiochemicals to protect stored products from insect infestation. *J. Stored Prod. Res.*, 2004, **40**(1), 1–25; https://doi.org/10.1016/S0022-474X(02)00078-4.
19. Sharma, A., Sandhi, R. K. and Reddy, G. V., A review of interactions between insect biological control agents and semiochemicals. *Insects*, 2019, **10**(12), 439; https://doi.org/10.3390/insects10120439.
20. Lockey, K. and Metcalfe, N., Cuticular hydrocarbons of adult *Himatismus* species and a comparison with 21 other species of adult Tenebrionid beetle using multivariate analysis. *Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol.*, 1988, **91**(2), 371–382; https://doi.org/10.1016/0305-0491(88)90156-3.
21. Tumlinson, J. H., Lewis, W. J. and Vet, L. E., How parasitic wasps find their hosts. *Sci. Am.*, 1993, **268**(3), 100–106; https://www.jstor.org/stable/24941408.
22. Darwish, E., El-Shazly, M. and El-Sherif, H., The choice of probing sites by *Bracon hebetor* Say (Hymenoptera: Braconidae) foraging for *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). *J. Stored Prod. Res.*, 2003, **39**(3), 265–276; https://doi.org/10.1016/S0022-474X(02)00078-4.
23. Lewis, W., Jones, R. L., Nordlund, D. A. and Gross, H., Kairomones and their use for management of entomophagous insects: II. mechanisms causing increase in rate of parasitization by *Trichogramma* spp. *J. Chem. Ecol.*, 1975, **1**(3), 349–360.
24. Tilden, R. and Ferkovich, S., Kairomonal stimulation of oviposition into an artificial substrate by the endoparasitoid *Microplitis croceipes* (Hymenoptera: Braconidae). *Ann. Entomol. Soc. Am.*, 1988, **81**(1), 152–156; https://doi.org/10.1093/aesa/81.1.152.
25. Lalitha, Y. and Ballal, C. R., Influence of seasons and inoculum dosages on the production efficiency of *Corcyra cephalonica* Stainton. *J. Biol. Control*, 2015, **29**(1), 25–30.
26. Chaudhuri, N. and Senapati, S., Development and reproductive performance of rice moth *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) in different rearing media. *J. Saudi. Soc. Agric. Sci*., 2017, **16**(4), 337–343; https://doi.org/10.1016/j.jssas.2015.11.004.
27. Gadratagi, B. G. *et al.*, Performance of *Trichogramma japonicum* under field conditions as a function of the factitious host species used for mass rearing. *PLoS ONE*, 2021, **16**(8), e0256246; https://doi.org/10.1371/journal.pone.0256246.
28. Hagstrum, D. W. and Smittle, B. J., Host-finding ability of *Bracon hebetor* and its influence upon adult parasite survival and fecundity. *Environ. Entomol.*, 1977, **6**(3), 437–439; https://doi.org/10.1093/ee/6.3.437.
29. Basana Gowda, G. *et al.*, Insecticide-induced hormesis in a factitious host, *Corcyra cephalonica*, stimulates the development of its gregarious ecto-parasitoid, *Habrobracon hebetor*. *Biol. Control*, 2021, **160**, 104680; https://doi.org/10.1016/j.biocontrol.2021.104680.
30. Venugopal, U., Jayanthi, P. D. K., Kumar, P., Jagadeesh, K. and Mohan, K. M., Host larval body odours AID host location of *Apanteles machaeralis*, a braconid larval endoparasitoid of cucumber moth *Diaphania Indica*. *Indian J. Entomol*., 2021, **83**(3), 315–320; http://dx.doi.org/10.5958/0974-8172.2021.00107.3.
31. Subramani, V. *et al.*, Volatile chemical signals underlying the host plant preferences of *Tuta absoluta*. *Entomol. Exp. Appl.*, 2021, **169**(11), 997–1007; https://doi.org/10.1111/eea.13099.
32. Das, S., Koner, A. and Barik, A., A beetle biocontrol agent of rice-field weeds recognizes its host plants by surface wax long-chain alkanes and free fatty acids. *Chemoecology*, 2019, **29**(4), 155–170; https://doi.org/10.1007/s00049-019-00285-1.
33. Mitra, S., Sarkar, N. and Barik, A., Long-chain alkanes and fatty acids from *Ludwigia octovalvis* weed leaf surface waxes as short-range attractant and ovipositional stimulant to *Altica cyanea* (Weber) (Coleoptera: Chrysomelidae). *Bull. Entomol. Res.*, 2017, **107**(3), 391–400; https://doi.org/10.1017/S0007485316001012.
34. Kamala Jayanthi, P. D. *et al.*, Specific volatile compounds from mango elicit oviposition in gravid *Bactrocera dorsalis* females. *J. Chem. Ecol.*, 2014, **40**(3), 259–266; https://doi.org/10.1007/s10886-014-0403-7.
35. Bertschy, C., Turlings, T. C. J., Bellotti, A. C. and Dorn, S., Chemically-mediated attraction of three parasitoid species to mealybug-infested cassava leaves. *Fla. Entomol.*, 1997, **80**(3), 1–14.
36. Murali-Baskaran, R. K. *et al.*, Role of kairomone in biological control of crop pests – a review. *Physiol. Mol. Plant Pathol.*, 2018, **101**, 3–15; https://doi.org/10.1016/j.pmpp.2017.07.004.
37. Rani, P. U., Sambangi, P. and Sandhyarani, K., Impact of plant phenolics as semiochemicals on the performance of *Trichogramma chilonis* Ishii. *J. Insect Behav*., 2017, **30**(1), 16–31; https://doi.org/10.1007/s10905-016-9595-8.
38. Dweck, H. K., Svensson, G. P., Gündüz, E. A. and Anderbrant, O., Kairomonal response of the parasitoid, *Bracon hebetor* Say, to the male-produced sex pheromone of its host, the greater waxmoth, *Galleria mellonella* (L.). *J. Chem. Ecol*., 2010, **36**(2), 171–178; https://doi.org/10.1007/s10886-010-9746-x.
39. Ram, A., Tiwari, L. D., Dass, R. and Mehrotra, K. N., Evidence for the presence of a kairomone in *Corcyra cephalonica* Staint. larvae for *Bracon brevicornis* Wesm. (Hymen., Braconidae). *J. Appl. Entomol*., 1982, **93**(1–5), 338–341.
40. Strand, M. R., Williams, H. J., Vinson, S. B. and Mudd, A., Kairomonal activities of 2-acylcyclohexane-1,3 diones produced by *Ephestia kuehniella* Zeller in eliciting searching behavior by the parasitoid *Bracon hebetor* (Say). *J. Chem. Ecol.*, 1989, **15**, 1491–1500.

41. Shonouda, M. L. and Nasr, F. N., Impact of larval-extract (kairomone) of *Ephestia kuehniella* Zell. (Lep., Pyralidae), on the behaviour of the parasitoid *Bracon hebetor* Say. (Hym., Braconidae). *J. Appl. Entomol*., 1998, **122**(1–5), 33–35.
42. Zhu, G. *et al.*, Chemical investigations of volatile kairomones produced by *Hyphantria cunea* (Drury), a host of the parasitoid *Chouioia cunea* Yang. *Bull. Entomol. Res.*, 2017, **107**(2), 234–240; https://doi.org/10.1017/S0007485316000833.
43. Mohanasundaram, A. *et al.*, Electroantennography and behavioral studies of *Eublemma amabilis* [Moore] and *Pseudohypatopa pulverea* [Meyr] in relation to volatiles of lac insect (*Kerria lacca* Kerr.) and its associated products. *Int. J. Trop. Insect Sci*., 2022, **42**(3), 2313–2324; https://doi.org/10.1007/s42690-022-00754-1.
44. Paul, A., Madhu, S. and Singh, D. B., Kairomonal effects of different host body washings on parasitism by *Trichogramma brasiliensis* and *T. japonicum*. *Int. J. Trop. Insect Sci.*, 1997, **17**(3–4), 373–377; https://doi.org/10.1017/S1742758400019214.
45. Trang, T. T. and Dey, D., Electroantennogram responses of *Chelonus blackburni* Cameron, egg-larval parasitoid of spotted boll worm *Earias vitella* to infochemicals on cotton ecosystem. *Omonrice*, 2013, **19**, 118–130.
46. Xiu, C.-L. *et al.*, Perception of and behavioral responses to host plant volatiles for three *Adelphocoris* species. *J. Chem. Ecol.*, 2019, **45**(9), 779–788; https://doi.org/10.1007/s10886-019-01102-3.
47. Li, M., Xia, S., Zhang, T., Williams III, L., Xiao, H. and Lu, Y., Volatiles from cotton plants infested by *Agrotis segetum* (Lep.: Noctuidae) attract the larval parasitoid *Microplitis mediator* (Hym.: Braconidae). *Plants*, 2022, **11**(7), 863; https://doi.org/10.3390/plants11070863.
48. Chi, D., Li, X., Yu, J., Xie, X. and Wang, G., The EAG response and behavior of the *Saperda populnea* L. to volatiles from poplar branches. *Acta Ecol. Sin.*, 2011, **31**(6), 334–340; https://doi.org/10.1016/j.chnaes.2011.09.003.
49. Guleria, N., Nebapure, S. M., Jayanthi, P. K., Suby, S. and Kumar, P. S., Identification of male-specific active host plant volatiles for maize stem borer, *Chilo partellus* Swinhoe. *Curr. Sci.*, 2021, **121**(4), 578.
50. Barth, R., Bau und Funktion der Flugel drusen einiger Mikrolepidopteren. Untersuhungen an den Pyraliden: *Aphomia gularis*, *Galleria mellonella*, *Plodia interpunctella*, *Ephestia elutella* und *E. kuehniella*. *Z. Wiss Zool.*, 1937, **150**, 1–37.
51. Roller, H., Bieman, K., Bjerke, J. S., Norgard, D. W. and Mcshan, W. H., Sex pheromone of pyralid moth. I. Isolation and identification of the sex-attractant of *Galleria mellonella* L. (Greater wax moth). *Acta Entomol. Bohemoslo.*, 1968, **65**, 208–211.
52. Dweck, H. K., Svensson, G. P., Gündüz, E. A. and Anderbrant, O., Kairomonal response of the parasitoid, *Bracon hebetor* Say, to the male-produced sex pheromone of its host, the greater waxmoth, *Galleria mellonella* (L.). *J. Chem. Ecol.*, 2010, **36**, 171–178.
53. Silberbush, A., Tsurim, I., Margalith, Y. and Blaustein, L., Interactive effects of salinity and a predator on mosquito oviposition and larval performance. *Oecologia*, 2014, **175**(2), 565–575; https://doi.org/10.1007/s00442-014-2930-x.
54. Nakashima, Y., Birkett, M. A., Pye, B. J., Pickett, J. A. and Powell, W., The role of semiochemicals in the avoidance of the seven-spot ladybird, *Coccinella septempunctata*, by the aphid parasitoid, *Aphidius ervi*. *J. Chem. Ecol.*, 2004, **30**(6), 1103–1116; https://doi.org/10.1023/B:JOEC.0000030266.81665.19.
55. Parthiban, P., Chinniah, C., Kalyanasundarm, M. and Baskaran, R., Kairomonal effect of acetone extracts of groundnut on foraging activities of *Trichogramma chilonis* (Ishii) and *Chrysoperla zastrowi* sillemi (Esben-Peterson). *Legume Res.*, 2017, **40**(2), 393–396; http://10.0.73.117/lr.v0iOF.11191.
56. Nakashima, Y., Birkett, M. A., Pye, B. J. and Powell, W., Chemically mediated intraguild predator avoidance by aphid parasitoids: interspecific variability in sensitivity to semiochemical trails of ladybird predators. *J. Chem. Ecol.*, 2006, **32**(9), 1989–1998; https://doi.org/10.1007/s10886-006-9123-y.
57. Hung, K. Y., McElfresh, J. S., Zou, Y., Wayadande, A. and Gerry, A. C., Identification of volatiles from plants infested with honeydew-producing insects, and attraction of house flies (Diptera: Muscidae) to these volatiles. *J. Med. Entomol.*, 2020, **57**(3), 667–676; https://doi.org/10.1093/jme/tjz232.
58. Yasukawa, S., Kato, H., Yamaoka, R., Tanaka, H., Arai, H. and Kawano, S., Reproductive and pollination biology of *Magnolia* and its allied genera (Magnoliaceae): I. Floral volatiles of several *Magnolia* and *Michelia* species and their roles in attracting insects. *Plant Spec. Biol.*, 1992, **7**, 121.
59. Sarkar, N., Mukherjee, A. and Barik, A., Long-chain alkanes: allelochemicals for host location by the insect pest, *Epilachna dodecastigma* (Coleoptera: Coccinellidae). *Appl. Entomol. Zool.*, 2013, **48**(2), 171–179; https://doi.org/10.1007/s13355-013-0168-4.
60. Schoonhoven, L. M., Van Loon, J. J. and Dicke, M., *Insect-Plant Biology*, Oxford University Press on Demand, 2005.
61. Giunti, G. *et al.*, Parasitoid learning: current knowledge and implications for biological control. *Biol. Control*, 2015, **90**, 208–219; https://doi.org/10.1016/j.biocontrol.2015.06.007.
62. Kruidhof, H. M., Kostenko, O., Smid, H. M. and Vet, L. E., Integrating parasitoid olfactory conditioning in augmentative biological control: potential impact, possibilities, and challenges. *Front Ecol. Evol.*, 2019, **7**, 84; https://doi.org/10.3389/fevo.2019.00084.
63. Ayelo, P. M., Pirk, C. W., Yusuf, A. A., Chailleux, A., Mohamed, S. A. and Deletre, E., Exploring the kairomone-based foraging behaviour of natural enemies to enhance biological control: a review. *Front. Ecol. Evol.*, 2021, **9**, 641974; https://doi.org/10.3389/fevo.2021.641974.
64. Randlkofer, B., Obermaier, E., Hilker, M. and Meiners, T., Vegetation complexity – the influence of plant species diversity and plant structures on plant chemical complexity and arthropods. *Basic Appl. Ecol.*, 2010, **11**(5), 383–395; https://doi.org/10.1016/j.baae.2010.03.003.
65. Rodriguez-Saona, C. R. and Stelinski, L. L., Behavior-modifying strategies in IPM: theory and practice. In *Integrated pest management: Innovation-Development Process*, Springer, Berlin, Germany, 2009, pp. 263–315.

ACKNOWLEDGEMENTS. We acknowledge Director, ICAR-National Rice Research Institute (NRRI), Cuttack, India for constant support and providing all the facilities. For this research work, funding was received from Rashtriya Krishi Vikas Yojana (RKVY), Government of Odisha, India. We thank Mr Harmohan Pradhan, Mr Narendra Biswal, Mr Anshuman Nath and Mr Bidyadhar Bhoi for helping in various phases of the study.

Received 15 November 2022; revised accepted 21 April 2023

doi: 10.18520/cs/v125/i2/183-190