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HOST FINDING BEHAVIOUR OF *LIPOLEXIS OREGMAE* (GAHAN) (HYMENOPTERA: BRACONIDAE)

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ABSTRACT: From a series of experiments, the host finding behaviour of by *Lipolexis oregmae* are studied through perception of various stimuli, viz., olfactory, contact of host substrate, visual and gustatory. Olfactory and contact receptors in parasitoid are located in her antennae. Only when 5 segments of both the antennae are amputed or waxed, the parasitoid took significantly more time to make first contact with the host. Similarly, time taken between first contact with the host and oviposition only vary significantly when 5 antennal segments were nullified their function. Oviposition by blind parasitoid suggests that sight does play a part in host finding but has a greater role in the proper orientation of the parasitoid to hosts during oviposition. Normal females were found to spend more time (452 ± 156 s) on the infested (with hosts) leaf of host plant followed by on leaves from which the aphids were shortly removed (235 \pm 125 s) and on uninfested leaves (fresh and healthy) ($107 \pm 31 \text{ s}$). The additional time was spent by the normal females in the licking of the honeydew. Licking of honeydew not only provides necessary nourishment to the parasitoids but also it assures them of the presence of their hosts. The presence of kairomones in the honeydew cannot be ruled out. Therefore, gustation (tasting honeydew) is partly helpful in host finding by reducing the host approaching period. The data presented herein demonstrated that the olfaction of chemical cues (kairomones) secreted by the aphid, vision and gustation of honeydew excreted by the aphid play major role in host finding by the parasitoid, *Lipolexis oregmae* and its antennae play major role in this process.

KEYWORDS: Aphid parasitoid, host finding, *Lipolexis oregmae*, *Aphis gossypii*, biological control.

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1. INTRODUCTION

Aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) are important components of the natural enemy guild which helps to control pest aphid populations in a variety of crops [1]. The impact of aphidiines on aphid populations in some major ecosystems, and in different geographical regions have been reviewed in past [2], [3]. Most of the aphidiine parasitoids used in aphid biocontrol belong to the genera: Aphidius, Binodoxys, Diaeretiella, Ephedrus, Lipolexis, Praon, and Trioxys [4], [5], [6]. Lipolexis oregmae (Gahan) is an aphid parasitoid on a number of host aphids in various countries, i.e., Malaysia [7], Taiwan [8], India and its adjacent countries [9-13], far East [14], Bangladesh [15], Vietnam [16], Thailand [17], Taiwan [18] and Guam [19]. The host selection is an essential and complex behavioural component of any host-parasitoid relationship, and it determines the capacity of the species to influence the population density of its host. It is now widely accepted that the parasitoid is first attracted to its host-habitat and then to host, which may be accepted or rejected [20-21]. The recognized phases of the process which results in successful parasitism, comprise the following: 1. host-habitat finding, 2. host-location, 3. host acceptance, 4. hostsuitability and 5. host-regulation [22-24]. After a female parasitoid has found a suitable host habitat and before it accepts or rejects a host, that host must be located. Female parasitoids rely on a hierarchy of physical or chemical stimuli to locate suitable hosts [22], [25-28]. Such host searching behaviour is strongly influenced by natural selection, and has been used in tests of optimal foraging theory [29]. The small size of many parasitoid species, however, makes field observations difficult, often restricting such work to the laboratory [20], [30-31]. Inside a host habitat, the parasitoids have evolved a variety of strategies to detect and orient to hosts from a considerable distance. This ability, called host location or finding, is defined as the perception and orientation by parasitoids to their host or its products. The important components of this definition are as follows: the stimuli must be associated with the presence of the host or with its secretions or excretions, and the parasitoid must perceive such stimuli at a distance from the host. Stimuli derived directly from the host are generally the most reliable source of information because they can inform the parasitoid of the presence, identity, availability and suitability of the host. Although this type of stimuli has a high reliability, it is often limited by low detectability. Low detectability of host derived information has two inherent constraints that limit its use as stimuli for host location. In terms of mass, hosts are small components of a complex environment and if they produce any information at all, it will be small in amount. Secondly, there should be constant selection on the hosts for inconspicuousness as a way avoid parasitization and predation (defense behaviour) [32]. It should also be kept in mind that host finding is a complex process, with its own hierarchy of behaviours. In a single species of parasitoid it can involve long-distance orientation via chemicals, sound or light; short-range intensive searching of hosts traces; and direct detection of hosts through integumentary chemicals or physical characterstics. In general, the factors associated with the host location comprise various stimuli, viz.,

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Prasad RJLBPCS 2018 www.rjlbpcs.com Life Science Informatics Publications vision, olfaction, gestation and acoustic [20]. Relatively few definitive studies involving physical stimuli have been done. Chemical sense are highly developed and extensively exploited by parasitoids for host location [32]. Numerous volatile chemicals are produced by the hosts themselves and also, in most cases, by their food plants. Therefore, olfactory perception has to be considered as the major sense involved in host location. Host finding behaviour of the aphid parasitoids had been studied in past for several aphid parasitoids, such as *Aphidius colemani* Viereck [33-34], *Aphidius ervi* Haliday [35], *Aphidius matricarae* Haliday [24], *Aphidius nigripes* Ashmead [36-37], *Aphidius rhopalosiphi* de Stefiani-Perez [38], *Binodoxys indicus* (Subba Rao and Sharma) [20], *Diaeretiella rapae* (M'Intosh) [39-40], *Lysiphlebus testaceipes* (Cresson) [33], [41] and *Lysiphlebia mirzai* Shujauddin [27]. The present paper deals with the host finding behaviour of less known aphid parasitoid, *Lipolexis oregmae*.

2. MATERIALS AND METHODS

2.1. The test insects (Lipolexis oregmae, Aphis gossypii)

The parasitoid *Lipolexis oregmae* were cultured in the laboratory on its host aphid, *Aphis gossypii* Glover on the brinjal plants grown in claypots.

2.2. The plants (Solanum melongena, the egg plant or brinjal)

The brinjal plants (var. Pusa Purple Long) used in experiments were cultivated in the field laboratory by adopting necessary agricultural practices except insecticide treatments. At 30 days of age, these plants were 15–20 cm high and had 4-6 leaves. At this stage they were infested with parthenogenetic females of *Aphis gossypii* and these females were allowed to reproduce for 10 days before the test. All plants tested were thus 40 days old.

2.3. Experimental sets up

2.3.1. Role of olfaction/taction

The female aphid parasitoids perceive olfactory stimuli as well as contact (touch) stimuli with their antennae like most of the insects. Therefore, to test the role of olfaction in host selection, 1, 5 and 10 segments of antennae (right and left separately and both) were either amputed or coated with wax. Treated parasitoids were introduced individually into Petri dishes having infested leaf of brinjal having about 50 aphids of mixed age put on moistened filter paper which was placed at the bottom of the dishes. Following behaviour of the female parasitoid, *Lipolexis oregmae* was recorded for 30 min.

- a. time taken between introduction and first antennal encounter with host aphid,
- b. time taken between introduction and oviposition, and
- c. number of antennal contact and pricking until oviposition.

In addition, the aqueous extract of *Aphis gossypii* was placed on Whatman filter paper No.1 and put on a Petri dish. A female *Lipolexis oregmae* was introduced inside the Petri dish and was covered with a glass plate and observed her host selection response.

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2.3.2. Role of vision

The female aphid parasitoids perceive visual stimuli with their compound eyes like other insects. Therefore, to test the role of vision in host selection, the eyes of the female parasitoids were blackened with Indian ink. Treated parasitoids were introduced individually into Petri dishes having infested leaf of brinjal having about 50 aphids of mixed age put on moistened filter paper which was placed at the bottom of the dishes and behaviour of the female parasitoid, *Lipolexis oregmae* was recorded for 30 min as mentioned above. In addition, in one experiment, the about 50 aphids of mixed age put on a host plant leaf and were separated from the parasitoids by a thin glass plate cutting off the chemical and tactile stimuli. The behaviour of the parasitoids was recorded.

2.3.3. Role of gustation

The female aphid parasitoids perceive gustatory stimuli with their mouthparts like most of the insects. Therefore, to test the role of gustation in host selection mouthparts are either amputed (maxillary and labial palps) or waxed. Treated parasitoids were introduced individually into Petri dishes having infested leaf of brinjal having about 50 aphids of mixed age put on moistened filter paper which was placed at the bottom of the dishes. Following behaviour of the female parasitoid, *Lipolexis oregmae* was recorded for 30 min as mentioned above.

3. RESULTS AND DISCUSSION

The host finding by aphid parasitoids are performed through perception of various stimuli, viz., olfactory, contact of host substrate, visual and gustatory.

3.1. Role of olfaction and contact

Olfactory and contact receptors are located in the antennae of the parasitoids like other insects. The response of the parasitoid for host location until its acceptance are displayed in Table 1. There was no significant effect of amputation or waxing of either of the 1, 5 or 10 antennal segment of one antenna of the parasitoid on the host location parameters. It implies that there was no difference between right and left antenna as for as perception of olfactory stimuli was concerned. However, when 5 segments of both the antennae are amputed (F=4.36; d.f. = 2, 12; P < 0.05) or waxed (F=3.90; d.f. = 2, 12; P < 0.05), the parasitoid took significantly more time to make contact with the host. When 10 segments of both antennae are amputed, no response of parasitoid was noticed for host aphids, but when waxed, then the parasitoid took much more time to make a contact with the host, but this contact was positively not by olfaction (directed) but seems to be random (Table 1). Similarly, time taken between first contact with the host and oviposition also did not vary significantly when only one segment of antennae are amputed or waxed. However, when 5 antennal segments were nullified their function, the female took much more time ($F_{amputed}$ =29.74; d.f. = 2,12; F_{waxed} =12.07; d.f. = 2,12; P < 0.01) (Table 1). Likewise, the third parameter of parasitoid's response, i.e., the number of antennal contacts till oviposition also varied significantly by differential nullification of the antennal segments (F=14.41; d.f. =

Prasad RJLBPCS 2018 www.rjlbpcs.com Life Science Informatics Publications 2,12; P < 0.01). The number of antennal encounters with and number of pricks to the hosts are significantly more when more than 5 segments of antennae are amputed or waxed (Table 1).

Table 1: Olfactory response of *Lipolexis oregmae* to *Aphis gossypii* after differential nullification of the function of its antennae in host location

Nullification	Number of	Time taken	Time taken	Number of	Number of
of function	amputed (a)	between	between first	antennal	pricks till
of antennae	or waxed (w)	introduction and	touch to	contacts till	oviposition
	segments	first touch to the	oviposition (s)	oviposition	
		hosts (s)			
Right	1a	216±63	55±16	4.2±1.9	3.6±2.3
	1w	208±28	49±21	4.4±1.9	3.6±2.2
	5a	268±54	69±20	3.6±2.1	2.8±1.3
	5w	242±64	70±26	3.4±1.7	4.0±1.6
	10a	306±44	102±20	6.2±2.2	3.8±2.2
	10w	336±54	114±21	6.4±1.7	3.4±1.7
Left	1a	203±62	73±25	3.6±1.8	4.0±2.1
	1w	175±62	69±15	3.6±1.5	3.6±2.3
	5a	297±27	76±15	3.0±1.7	3.4±1.7
	5w	247±73	80±14	4.2±1.9	3.6±2.3
	10a	326±95	109±36	6.2±1.5	3.2±1.8
	10w	324±39	109±29	6.6±1.1	3.6±2.3
Both	1a	254±54	64±22	4.4±2.1	8.8±2.6
	1w	243±75	71±25	3.2±1.3	10.0±3.9
	5a	353±53	269±77	4.6±1.8	12.4±3.8
	5w	347±64	234±98	4.0±1.6	11.0±2.9
	10a	-	-	-	-
	10w	552±81	-	-	-
Normal parasitoid	-	65±25	36±14	2.8±0.8	2.4±0.9

The above finding points out that the parasitoid is attracted towards host by olfaction. This is further confirmed by the attraction of the female parasitoid to the extract of *Aphis gossypii* placed on Whatman filter paper No.1. The attracted female touches the place of the extract with her antennae and bends her abdomen in the same fashion as she does during the pricking of host for oviposition, of course, no egg is laid. This behaviour reveals that it is the chemical cue (kairomone) which helps the parasitoid in finding and locating her host whereas the antennal

Prasad RJLBPCS 2018 www.rjlbpcs.com Life Science Informatics Publications contacts (tactile receptors) induces and activates her to lay eggs. This provides sufficient evidence about the presence of chemoreceptors both olfactory as well as tactile on the antennae.

3.2. Role of vision

How the vision is responsible in host-finding by *Lipolexis oregmae* is evident from Table 2, as blind parasitoids approach and make first contact ca. 60 s after their release later than the normal ones (when confined in area of ca. 20 cm²). Not only this, the number of antennal contacts and prickings prior to oviposition is significantly more in treated than in case of normal ones. Oviposition by blind *Lipolexis oregmae* suggests that sight does play a part in host finding but has a greater role in the proper orientation of the parasitoid to hosts during oviposition. When hosts were separated from the parasitoids by a thin glass plate (chemical and tactile stimuli thus cut off) the parasitoids neither stop nor examine the glass at the sites where the aphids lay directly underneath but are continuously on their legs.

Table 2. Visual and gustatory responses 1 of *Lipolexis oregmae* to *Aphis gossypii* in host-location after nullification of the function of respective sensory organs of the parasitoid

Function-nullified organs	Eyes	Mouthparts		Normal parasitoid
Amputed (a) or waxed (w) or coated with Indian ink (c)	С	a	w	-
Time taken between introduction and first touch to the hosts (s)	156±32b	137±49b	126±46b	65±25a
Time taken between first touch to oviposition (s)	78±26b	58±27a	56±21a	36±14a
Number of antennal contacts till oviposition	5.0±1.6b	3.0±1.0a	3.2±0.8a	2.8±0.8a
Number of pricks till oviposition	5.6±1.1b	3.0±1.0a	3.6±1.1a	2.4±0.9a

3.3. Role of gustation

Table 2 demonstrated that the parasitoids with amputed or waxed mouthparts needed about 100% more time in approaching the host than that taken by the normal ones. Further a normal/treated female (introduced in to a Petri dish containing hosts on the leaf) before contacting the leaf not only stayed for a while but also stopped her antennal movement. Thereafter she directed her antennae in the direction of the host, moved slowly and finally contacted the leaf with her antennae. On ascending the leaf, she began to tap the same with her antennae (bent in a C-posture) and the normal female licked the honeydew if encountered in the way. Normal females were found to spend more time on the infested leaf (with hosts) of host plant for 452 ± 156 s and on leaves from which the aphids were shortly removed for 235 \pm 125 s than on uninfested leaves (fresh and healthy) for 107 ± 31 s. The additional time was

Prasad RJLBPCS 2018 www.rjlbpcs.com Life Science Informatics Publications spent by the normal females in the licking of the honeydew. Licking not only provides necessary nourishment to the parasitoids but also it assures them of the presence of their hosts. The presence of kairomones in the honeydew cannot be ruled out. The inability of licking of honeydew by the treated specimens delays their approach to the host. As soon as the host is touched with the antennae of treated parasitoids, all the behavioural responses and events in such members are like the normal ones. Therefore, gustation (tasting honeydew) is partly helpful in host-location by reducing the host approaching period.

4. DISCUSSION

Olfactory and tactile senses are most commonly used by the aphid parasitoids in their hostfindings [20], [22], [27], [42-44]. It is now known that the initial searching and recognition behaviour is under chemical influence [25], [41], [45-48]. Although, the role of kairomones in different host-parasitoid systems has been worked out [49-60], its isolation, identification and synthesis is still unknown in aphids. The female *Binodoxys indicus* touches the place of the host extract with her antennae and bends her abdomen in the same fashion as she does during the pricking of host for oviposition, of course, no egg is laid [48]. This behaviour reveals that it is the chemical cue (kairomone) which helps the parasitoid in finding and locating her host whereas the antennal contact (tactile receptors) induces and activates her to lay eggs. This provides sufficient evidence about the presence of chemoreceptors both olfactory as well as tactile on the antennae. The source of kairomone may be haemolymph [59], [60] and cornicle [61]. Aphid sex pheromone may also serve as kairomones [21], [32], [62-63]. Kairomones have been found to be advantageous in the management of entomorphagous insects [52-53], [64]. Similar to the present findings, it has been demonstrated that the vision of parasitoids does play a part in host finding but has a greater role in their proper orientation to hosts during oviposition [20]. Similar findings have also been reported by other workers [65-68]. It has also been observed that the parasitoid (Binodoxys indicus) at several occasions during her search, passes past at a distance of ca. 5 mm from the host, but does not show any sign of recognitional aptitude [20]. Therefore, it may be concluded that the role of sight in host finding is of less importance, though is helpful in proper orientation with respect to hosts, of course, after she has recognised the hosts [24]. Honeydew often accumulates on the upper surfaces of leaves below them, as well as in the colonies. These accumulations of honeydew are attractive to parasitoids and can determine the pattern of within-plant foraging [69-70]. Licking of honeydew not only provides to the parasitoid but also it assures them of the presence of their hosts [20]. Also, the retention time on the host patch increased in presence of honeydew. Apparently the overall retention of parasitoids on honeydew is reflected through several different mechanisms shown by the parasitoids as arrest, decreased walking speed (orthokinesis) and increased turnings (klinokinesis). Honeydew, therefore, appears to be an arrestant, which keeps the parasitoid in the honeydew contaminated area, increasing the chance of contact of

Prasad RJLBPCS 2018 www.rjlbpcs.com Life Science Informatics Publications host aphids [71]. Honeydew acts as a kairomone for parasitoids [20], [36], [71]. Responses to honeydew can be very general; for example, Aphidius rhopalosiphi responded to the honeydew of a range of aphid species [71-72] and Aphidius nigripes responded to the honeydew from its host and two other species [36]. In the field, these responses may be significant in host habitat location. Aphidius rhopalosiphi searched longer on the leaves and ears of wheat plants contaminated with honeydew compared with non-contaminated plants [73]. While in the species Ephedrus cerasicola, individuals were found to accumulate on contaminated plants rather than fresh ones [74]. Most parasitoids respond to honeydew, even if it is from an aphid species outside the normal host range. However, the effects of honeydew on individuals are complex. Studies on Aphidius rhopalosiphi show that the searching time of females increases with the concentration of honeydew and that searching times are of a similar duration if the source of the honeydew is from aphids feeding on either plants or artificial diets. This suggests that honeydew acts as a within patch searching stimulant [71]. On the other hand, parasitoid searching time decreases after confinement in environments containing honeydew; indicating that individuals can habituate to these cues [71] The aphid parasitoid, Diaeretiella rapae uses honeydew emitted by its host, the cabbage aphid Brevicoryne brassicae, as a kairomone, the activity of which decreased gradually and lost completely within 3 days [75]. Also, the parasitoid is able to assess the number of aphids in the colony. The honeydew of host aphids attracts the parasitoids from a distance then arrests the parasitoid in the contaminated area. Slowing down the increased antennae examination would increase the possibility of the parasitoid encountering the host. The klinokinatic response would increase intense searching throughout the contaminated area, further increasing the chance of finding host [36].

CONCLUSION

The data presented herein furnish considerable insight into the host finding process of *Lipolexis oregmae*. It demonstrated the olfaction of chemical cues (kairomones) secreted by the aphid, vision and gustation of honeydew excreted by the aphid play major role in host location by the parasitoid. Parasitoids' antennae play major role in this process.

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CONFLICT OF INTEREST

I declare that no financial interest or any conflict of interest exists.

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