ARRESTMENT OLFACTORY RESPONSES OF THE PARASITOID, *MICROPLITIS RUFIVENTURIS* TOWARDS CONTACT CHEMICALS PRODUCED BY THE HOST, *SPODOPTERA LITTORALIS* AND SOME OF ITS HOST PLANTS.

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ABSTRACT

The arrestment olfactory responses of the parasitoid, *M. rufiventuris* towards a contact chemical from its host larvae and also host diet are investigated. Female *M. rufiventuris* without previous exposure to host diet, host feeding damage, host faeces or host larvae and with no oviposition experience showed various arrestment responses. Inexperienced females exhibited searching responses towards host diet and non-diet in the following order of preference: (cotton = castor) > (berseem = root beet) > (rice, maize or wheat). No difference in responses however was obtained towards collected *S. littoralis* faeces after feeding on different diets. In response to different searching sources; the highest responses were obtained towards the host-damaged leaves and host larvae (4th instar) while the weakest response was towards normal and non-host damaged leaves. All the obtained results depended on the chemical stimuli of the searching source where there is any role for the visual stimuli of the parasitoid.

INTRODUCTION

The parasitoids comprise a large and diverse group of insects, primarily Hymenoptera and Diptera, which develop as larvae on or in the tissues of their insect hosts. *Microplitis rufiventuris* Kok. (Hymenoptera – Braconidae) is known to attack many lepidopterous species, one of them, *Spodoptera littoralis* Boisd. (Lepidoptera – Noctuidae) which attacks a wide variety of cultivated and non-cultivated plants. Many parasitic

Hymenoptera use olfactory cues and responses to orient first towards a potential host habitat and second towards their host (Vinson, 1981; Weseloh, 1981; and Vet *et al*., 1983). Host finding in parasitoids often takes the form of a sequence of responses involving several levels of stimuli. Such a sequence process has been observed by many authors; e.g. Doutt (1959 & 1964) and Noldus (1989) as one of "host-habitat finding" followed by "host finding". At each level in the sequence, two sorts of

stimuli operate: "attractant" stimuli which elicit orientation to areas containing olfactory sources, and "arrestant" stimuli which elicit orientation, a reduction in the distance or area covered per unit time by parasitoids moving within these source areas. The latter response has often been given such purposive labels as "intense search". Once arrested in a host area at one level of foraging, parasitoids may respond to further attractant and arrestant stimuli which tend to localize their movements in even smaller units of host distribution (Dethier *et al.*, 1960 and Waage, 1978). Thus for instance, the braconid, *Opius fletcheri* Silv., the ichneumonid *Diadromus pulchellus* Wesm. and the eucoilid *Leptopilina clavipes* (Harig) are attracted at one level to olfactory stimuli from the plants, they exhibit arrestment in areas of the plant containing a chemical produced by the host itself (Nishida, 1956; Noyes, 1974, and Vet, 1983).

Several lines of indirect evidence suggest the importance of the host plant for parasitoid searching. The amount of parasitism suffered by a polyphagous host species often depends on the food plant it attacks (Vinson, 1981 & 1985 and Nordlund *et al.* 1987).

The attractant stimuli involved in parasitoid foraging are of two sorts; those chemical stimuli produced by the host as host kairomone, host faeces, host feeding, sex pheromones and aggregation pheromones. The second sort include instances where the second action of the host on its environment generates attractants (Thorpe and Jones, 1937; Rice 1968;

Mitchell and Mau, 1971; Hendry *et al.*, 1973; Sternlicht, 1973 and Bragg, 1974).

So the present study examines the arrestment of the braconid *M. rufiventuris* in response to attractant cues produced by its host, the larvae of *S. littoralis* and those produced by its host feeding diets when the parasitoid was found. Also during investigations with the parasitoid, we became interested in the factors involved in the recognition of its host as those cues of host plant, faeces, damaged plant and also host larval instars.

MATERIALS AND METHODS

Susceptible strains of *S. littoralis* and *M. rufiventuris* were obtained as eggs and pupae respectively from the biological control laboratory, Entomology Department, Faculty of Science, Cairo University. After hatching, *S. littoralis* larvae were fed on fresh leaves of castor oil plant. From the beginning of the second instar, each 10 larvae were placed in a rearing plastic vial. Larvae were fed and observed daily for removing of uneaten food and faeces. After pupation, 10 males and 10 females were transferred to the ovipositional cage. Ovipositing egg batches were collected daily, placed in rearing cups till hatching where they were provided with fresh castor oil leaves.

After *M. rufiventuris* emergence, each five females with five males were placed in a plastic cup (12 x 6 cm), provided with few drops of honey on the inner side of its upper portion. For maintaining a culture of

M. rufiventuris, each adult parasitoid female was left with an adequate number of host larvae for about 2-hours and then transferred to a new cup containing another unparasitized host individuals. This method was repeated daily till at least 50 host larvae were parasitized. All cups were covered with plastic covers, and provided with freash leaves of castor oil plant daily and kept till emergence of the parasitoid adults.

The experimental host food plants used as searching sources were cotton (*Gossypium barbdense*; family Malvaceae), berseem (*Trifolium alexandrinum*; family Leguminasae), root beet (*Beta vulgaris* var. *rsbira*; family Chenopodiaceae) and castor oil plant (*Ricinus communis*; family Euphorbiaceae). maize (*Zea maize*), rice (*Oryza sativa*) and wheat (*Triticum vulgare*) family Graminae as non host plant foods were also used.

For bioassays, only virgin parasitoid females of 24 – 48h old were used, since previous ovipositional experience of the parasitoid may affect the recognition behaviour (Van Lanteren, 1976 and Strand & Vinsion 1982). All bioassays were conducted in a rectangular cage of 80 x 60 x 50 cm. Four sides of the cage were made of clear glass while both the bottom and the roof were made of wood. A small door to facil·tate handling of the experimental cups provided the roof. Glass cups of 5 x5 cm were used, each contains a searching source and covered with muslin cloth, fixed in position by a rubber band. The cups used only once then replaced by new cups ones, arranged in a circle in the experimental cage and separated by at least 5cm from each other. Ten parasitoids were placed in the center of the cage bottom surrounded by the searching sources. The searching behaviour of the parasitoid including the number of contacts (number of parasitoids come into contact with the searching source) and the number of examinations (an examination was scored if, after source contact, the female parasitoid began the characteristic examination behaviour by bending their antennae from side to side). The searching responses were recorded for 1 hour.

The different larval instars, host plant diet, damaged leaves and faeces used as searching sources had the same weight before exposure to the parasitoid. Empty clean cups covered with the same muslin cloth scared in position by rubber bands were used as a control.

All the experiments were carried out in the laboratory at room temperature of $24 \pm 2°C$ and relative humidity of 70 ± 5.

Data were analyzed by analysis of variance (one-way ANOVA) at significant level of $P = 0.05$.

RESULTS

The searching responses of the parasitoid, *M. rufiventuris* towards the different *S. littoralis*- diet and non-diet plants are shown in table (1). Female parasitoid exhibited an overall preference for its host diet. It was observed that the numbers of contact and examination responses to cups containing host diet were more significantly higher than those containing non-host diet ($P<0.05$). For host diet plants, higher searching

containing non-host diet (P<0.05). For host diet plants, higher searching responses (P < 0.05) were obtained towards cotton, and castor and than towards root beet and berseem. On the same way the higher percentages of exams were obtained towards the *S. littoralis* diets.

Table (2) shows the searching responses of the parasitoid towards *S. littoralis* faeces obtained from various host plants. Faeces from larvae fed cotton, castor, berseem or root beet elicited similar number of contacts and exams (P > 0.05). The highest percentage of exams 87.6% was obtained with the faeces collected from larvae fed on cotton.

Data obtained in table (3) show the effect of the host instars on the searching responses of the parasitoid, *M. rufiventuris*. The highest mean number of contacts and exams was obtained with the fourth instar (25.2 ± 0.86 and 24 ± 1.20 respectively. On the other hand the lowest searching responses were obtained with the 1st and 6th instars (6.8±0.66, 4±1.10 for contacts and 3±0.71, 1.4±0.51 for exams respectively.

The searching responses of the female towards the principal searching sources as normal and damaged plant leaves, host faeces and host larvae were compared (Table 4). Higher searching responses of contacts and exams (P<0.05) were obtained towards host larvae or its damaged cotton leaves than towards other sources. The highest percentage of exams 99.2% was obtained for searching responses of the parasitoid towards the host larvae.

DISCUSSION

Host selection and successful parasitism is a multistep process involving host habitat location, host acceptance, host suitability and host regulation (Doutt, 1959; Vinson, 1984; Tumlinson *et al.*, 1993 and Vinson *et al.*, 1998). Recent studies are beginning to reveal the complexity of the tritrophic interaction between host plant, insect host and the parasitoid. Though based on laboratory experiments, the present study indicates that, female *M. rufiventuris* have the ability of searching responses to locate the habitat of its potential host by an olfactory response emanating from *S. littoralis* host plants. Several lines of indirect evidences suggest the importance of the host plant odors for parasitoid searching based on behavioral studies in the laboratory by Vinson (1981 and 1985), Vet *et al.* (1983) and Drost and Dicke (1992).

Having arrived in a potential host habitat, a parasitoid begins the next phase in the search for hosts. Often insects show arrestment in response to contact with kairomones deposited by their hosts on the substratum. Materials containing such kairomones have been shown to include host salivary gland or mandibular gland secretion, host faeces and cuticular secretions (Jervis and Kidd 1996).

Other important sources of arrestant or of short-range attractant chemicals is the faeces. This study showed that the parasitoid, *M. rufiventuris* has the same responses towards *S. littoralis* faecs although host-diets were different. Same

responds of *M. croceipes*, a parasitoid of the corn earworm (*Helicoverpa zea*) to a chemical in the host faeces were observed by Lewis and Jones (1971) and Lewis and Martin (1990).

The most important result was the searching responses of the parasitoid to the different host larval instars. The highest responses were to the 4th instar and 3rd instar while the lowest responses were obtained with the 6th and 1st instars. This may indicate that the kairomone of each *S. littoralis* larval instar is different from that of other instars and may explain why the parasitoid, *M. rufiventuris* prefers 4th, 3rd, and 2nd instars for oviposition in the normal choice as observed by Younes (1999).

Comparing the searching responses of the parasitoid to different arrestant sources, it was found that, the weakest response was to the normal and non-host damaged cotton leaves. The higher response was to host faeces, but by far the most important response was to host-damaged leaves or to host larvae. The parasitoid responded much more strongly to leaves that have been damaged by the host than to artificially damaged leaves. This may indicate that, mandibular and labial gland secretion of *S. littoralis* are an important source of short-range attractants and arrestants. This agrees with the result of Turlings *et al.* (1991) concerning the arrestant responses of parasitoid *Cotesia* (*Apanteles*) *marginiventris* and host *S. exigua*.

This study indicated that, compounds such as host-chemical attractant (kairomones) are an important link in the search to develop acceptable artificial hosts for mass production of parasitoids. Further, recognition kairomones may prove effective in stimulating parasitoids to attack previously unacceptable hosts which can be more easily reared for mass production purposes in the laboratory.

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Table (1): Intense searching responses of the parasitoid, *M. rufiventuris* towards *S. littoralis*-host and non-host plants.

Plant	Searching responses (mean ± SE)		
	Contacts	Exams	% Exams
Cotton	20±1.58a	14.4±0.92a	72
Castor	18±1.14a	10.6±0.91a	59
Root beet	11±0.71b	7.4±0.51b	67
Berseem	12.2±1.20b	7±0.71b	57
Maize	3.8±0.86c	1.2±0.58c	31.5
Wheat	5±0.71c	0.6±0.04c	12
Rice	3±0.66c	0.4±0.04c	13.3
Control	3±0.70c	0.4±0.24c	13.3

- Means followed by the same letter in the same column are not significantly differs (ANOVA, P>0.05).

Table (2): Intense searching responses of the parasitoid, *M. rufiventuris* towards *S. littoralis* faeces, after feeding on different host plants.

Diet source	Searching responses (mean ± SE)		
	Contacts	Exams	% Exams
Cotton	25.8±0.86a	22.6±1.10a	87.6
Castor	27.2±0.85a	18.4±0.74a	67.6
Berseem	24.8±1.11a	19.6±0.97a	79
Root beet	26.4±1.71a	21.8±1.20a	82.6
Control	4.4±0.902b	1.2±0.58b	27.3

- Means followed by the same letter in the same column are not significantly differs (ANOVA, P>0.05).

Table (3): Intense searching responses of the parasitoid, *M. rufiventuris* towards different host instars.

Searching source	Searching response (mean ± SE)		
	Contacts	Exams	% Exams
1st larval instar	6.8±0.66a	3±0.71a	44.1
2nd larval instar	12±0.71b	9±0.72b	75
3rd larval instar	19.4±1.12c	16.4±0.92c	84.5
4th larval instar	25.2±0.86d	24±1.21d	95.2
5th larval instar	11.2±1.21b	8±0.71b	72.7
6th larval instar	4±1.12a	1.4±0.51a	35
control	3.8±0.35a	0.6±0.32a	15.8

- Means followed by the same letter in the same column are not significantly differs (ANOVA, P>0.05).

Table (4): Intense searching responses of the parasitoid, *M. rufiventuris* towards different searching sources.

Searching source	Searching responses (mean ± SE)		
	Contacts	Exams	% Exams
- Normal cotton leaves	19.8±0.96a	14±0.70a	70.7
- Damaged cotton leaves (not by *S. littoralis* larvae)	16.4±0.90a	12±0.61a	73.2
- Damaged cotton leaves by *S. littoralis* larvae	26.2±1.10b	24.4±1.11b	93.1
- Host faces	22.2±1.20c	19.4±1.21c	87.4
- Host larvae (4th instar)	26.6±0.93b	26.4±0.91b	99.2
- Control	2.4±0.74d	0.8±0.37d	33.3

- Means followed by the same letter in the same column are not significantly differs (ANOVA, P>0.05).