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Some Behavioural Characteristics of *Lysiphlebus fabarum* (Hym: Aphidiidae) Parasitizing *Aphis fabae* (Hom: Aphididae) under Laboratory Conditions

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Abstract: Some behavioural characteristics of *Lysiphlebus fabarum* (Marshall) as an important parasitoid of *Aphis fabae* was studied under laboratory conditions (21±1°C, a relative humidity of 70±5% and a photoperiod of 14:10 L:D h.) using different stages of *Aphis fabae* and its host plant. In this study, mating behaviour, sequences of oviposition, time spent for an adult parasitoid to emerge from its mummified aphid host and the effect of temperature and relative humidity on the percentage of adult emergence were determined. Average mating and oviposition handling times lasted 37.1±2.8 and 77.2±3.8 sec, respectively. It took 19.8±1.19 min for an adult wasp to emerge from its mummified aphid host. Females oviposited on or between sternal segments of aphid host thorax, usually on second or third segments. The time intervals between ovipostion to mummification and to the emergence of adult parasitoids were inversely dependent on host stages and decreased as the aphid nymphs grew to adult stage. The time interval between mummification and adult emergence decreased as the temperature increased. Results showed that temperature and relative humidity influenced percentage of adult emergence. Highest percentage was observed at 25°C and the lowest at 30°C with 50±5% of relative humidity, while the highest and lowest percentage of adult emergence was observed at 15°C and 30°C with RH of 70±5%, respectively.

Key words: *Lysiphlebus fabarum*, *Aphis fabae*, mummification, parasitoid emergence

INTRODUCTION

The black bean aphid, *Aphis fabae* (Scope) is a major pest on sugar beet (*Beta vulgaris*) and broad bean (*Vicia faba*). On sugar beet and also on fodder beet, it acts as an important vector of virus yellows. On broad bean, the main damage is caused directly by its sucking activity which may lead to a reduced crop yield and death of the whole plant[1,2].

The most abundant parasitoid of *Aphis fabae* in agroecosystems is *Lysiphlebus fabarum*[3]. This parasitoid has the potential to cause a drastic reduction in *A. fabae* population and hence could be useful in the biological control of this pest species[3,4].

Aphidiine wasps can be classified into six groups according to their oviposition behaviour; Type I are those with unspecialized oviposition behaviour, they lack special adaptations for host capture and oviposition. They have moderately long oviposition times, varying between 5 and 10 s (e.g., species in *Ephedrini*). Types II and III are those that display various morphological and behavioural adaptations. Females are able to grasp and immobilize a potential host for oviposition (e.g., species in Trioxini). Type IV are those with a relatively high flexibility of female abdomen and moderately to very long oviposition times (e.g., species in the genus *Pauesia*). Type V are characterized by a short oviposition time of less than 5 sec. termed a quick sting. Females typically avoid direct contact with a potential host and hence the risk of injury due to host defenses (e.g., species of *Aphidius* Nees). Type VI are those species that have evolved chemical counter strategies to the guarding behaviour of trophobiotic ants (e.g., species in *Adialytus* and *Lysiphlebus* genera[5].

Ovipostion times ranged between less than 0.5 (in *Aphidius* Nees) and 219.9±10.4 sec in *Pausia unilachni* (Gahan). It was 51.2±5.1 sec for *Lysiphlebus fabarum*[5]. The duration of an oviposition varies as aphidiids encounter different stages of their aphid hosts, for example in *Ephedrus cerasicola* Stary parasitizing *Myzus persicae* (Sulzer) oviposition time increased with the age of nymphs, being 13, 18, 21, 22 and 17 sec from 1st instars to adults, respectively[6].

Females in aphidiids mate once in their lifetime, while males are able to mate with several females. Mating time varies between 20- 40 sec and the number of mating is affected by temperature[7-9]. Average mating time for *L. fabarum* and *L. testaceipes* parasitizing *Toxoptera* sp. has been reported 18.8 and 60.8 sec, respectively[10].

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The objectives of the present study were to determine: a) the behavioural sequence exhibited by female *L. fabarum* at the time of mating and oviposition and b) the influence of host stage, temperature and humidity on the time interval between oviposition and emergence of adult parasitoid and on the percentage of adult emergence.

MATERIALS AND METHODS

Mating behaviour: In this experiment 4 unmated females were exposed to an adult male in a test tube (1x10 cm) replicated seven times. Average mating time was calculated using a stop watch. Prior to study mating behaviour, wasps were fed on a 30% of honey solution.

Oviposition behaviour: In this experiment, 12 different stages of *A. fabae* were presented to a mated female parasitoid on a cut of apical part of bean plant which was replicated 10 times. Ovipostion behaviour was observed under a stereomicroscope and using a magnifier. Oviposition handling time was measured using a stop watch. Females were fed as before and had no experience of ovipostion.

Parasitoid emergence from mummified aphids: In order to study the mechanism of parasitoid emergence from mummified body of its aphid host, the mummified aphids were carefully observed several times a day in a plastic petridish (8x1.5 cm). The time spent for an adult to emerge from its cocoon was measured using a stop watch.

In order to determine the time interval between oviposition to mummification and to the adult emergence, different stages (20 of each stage) of *A. fabae* were separately released on a potted bean plant. Each infested potted plant was then placed in a wooden framed cage (30x25x35 cm) with sides covered with muslin. The bottom of the cage consisted of a ply wood sheet. Five 1 day old females, along with 5 males were introduced into the cage for 24 h. Subsequently, the parasitoids were removed from the cage and parasitized aphids were kept until mummification. When the mummified aphids were appeared, they were separated from host plant and placed in a plastic petridish (8x1.5 cm) and transferred to a growth chamber (21±1°C, 70±5% RH and 14:10 L:D) until adult emergence. Then mummified aphids were observed daily in order to determine their activities and also the time interval to adult emergence.

Effect of temperature on the time interval between mummification and adult emergence: This experiment was conducted at 5, 10, 15, 20 and 25°C with a relative humidity of 50±5. Ten mummified aphids at the same age were placed in a petridish (1.5x8 cm) and replicated five times at each level of temperature. By daily observation, number of emerged adults were recorded and the experiment continued to the last adult parasitoid emergence.

Effects of temperature and humidity on percentage of adult emergence: Two levels of relative humidity (i.e., 70±5% and 50±5%) and four levels of temperatures (15, 20, 25 and 30°C) for each level of humidity were used to determine the effects of these two factors on the percentage of adult emergence from mummified aphids. Each experiment was replicated five times and at each replication, 20 mummified aphid at the same age were placed in a petridish (1.5x8 cm). Petridishes were observed daily and number of emerged parasitoids were recorded. The observation continued till the last parasitoids were emerged.

Data analysis was done using SAS[11] and when data were in percentage, arcsine transformation was performed for their normalization.

RESULTS

Mating behaviour: Male wasp when encounters his mate moves to the back of the female, while vibrating his wings drums the female antennae. Female does not frequently responds properly to the male. The male beats the female antennae by his forelegs trying to keep his position. If mating does not occur, male tries again. Female sometimes avoids mating by bending her abdomen downward and forward to pretend ovipositing. In a successful mating, male jumps to the back of the female and touches female antennae by his wings and legs and by a rapid movement approaches the tip of his abdomen to the female genitalia. The average mating time is shown in Table 1. Male while mating drums the female antennae that are held upward, by his antennae.

Female cleans end of her abdomen by her antennae after mating. Females of *L. fabarum* mate once in their lifetime, but males are polygamous and are capable of mating with several females. Therefore, mated females by bending their abdomen prevent males to mate again.

Table 1: Average and range of mating time in *L. fabarum* (Sec)

Replicates	No. of females	Average of mating time (±SE)	mating time (ranges)
1	4	38.0±1.3	35-42
2	4	39.5±1.67	35-43
3	4	46.0±6	40-52
4	4	25.0±2.89	20-30
5	4	32.0±1.33	29-33
6	4	30.0±5	25-35
7	4	49.0±0.67	48-50
Total	28	37.1±2.8	20-52

Oviposition behaviour: Female parasitoid while keeping her antennae upward walks around, drumming with the antennae on the surface of the plant in front of her. She continues searching for the host by bending and returning her antennae. On encountering the host, stops walking. Antennal contact is necessary in host recognition, after this, an encounter with an aphid host is initiated. Prior to oviposition, female parasitoid stabs the aphid host with the tip of her trebrum for a few seconds and stands on her legs, while bending her abdomen between legs and under sternite. She uses tip of her trebrum and antennae to find out the right site for oviposition on the host thoracic segments. Oviposition usually takes place on or between thoracic segments, generally on second or third segments of aphid host sternum. After oviposition, female cleans herself, preparing her for another oviposition. Average ovipostion handling time and other relevant data are given in Table 2.

Parasitoid emergence from mummified aphids: Mature adult parasitoid in order to emerge from its mummified aphid host, starts cutting a circular hole in the host tegument using its mandibles. Cuticular disc is then pushed out by head pressure of the wasp. Emergence hole in *L. fabarum* is made on the tergite of the host usually before cornicles. When cuticle is cut down in half, adult wasp emerges from mummy in a few seconds and in most of the times the cuticular disc is completely separated from mummy, however, sometimes remains attached to it. Newly emerged wasps clean themselves rapidly and start fly around in short ranges with regular stops. Emergence time of an adult parasitoid from mummy is shown in Table 3.

The time intervals between ovipostion to mummification and to emergence of adult parasitoids are inversely dependent on host stages, they are decreased as the aphid nymphs grow to adult stage. Results are shown in Table 4.

Effect of temperature on the time interval between mummification and adult emergence: Analysis of variance showed that temperature influences significantly the time interval between mummification and adult emergence ($p<0.05$, $F = 126.62$, $df = 4, 241$). Comparison of means using Duncan's Multiple Range Test showed that between 5 and 10°C which shows the longest time interval there is no significant differences. The time interval decreases as the temperature increases to 25° (Table 5).

Effects of temperature and relative humidity on percentage of adult emergence: Nested analysis of variance showed that the temperature influences

Table 2: Average oviposition handling time (Sec±SE) of *L. fabarum* parasitizing *A. fabae*

Replicates	No. of aphids exposed to	No. of ovipostion times	Average of ovipostion handling time	Ranges
1	12	8	70.0±1.2	60-73
2	12	7	50.0±1	49-53
3	12	5	62.0±0.97	59-63
4	12	10	105.0±2.6	103-107
5	12	6	82.0±3.1	79-84
6	12	6	100.0±0.5	99-102
7	12	8	65.0±2.13	60-66
8	12	9	90.0±0.43	89-92
9	12	10	55.0±1.3	54-66
10	12	11	93.0±2	80-94
Total	120	80	77.2±3.8	49-107

Table 3: Mean emergence time of *L. fabarum* from a mummified aphid host (in minutes)

Replication	Time to emerge
1	25
2	23
3	20
4	18
5	26
6	28
7	17
8	18
9	15
10	15
11	16
12	25
13	15
14	15
15	20
Mean±SE	19.8±1.19

percentage of adult emergence ($p<0.05$, $F = 42.07$, $Df = 3,16$). Highest percentage was observed at 25°C and the lowest at 30°C with 50±5% of RH. Comparison of means using Duncan Multiple Range Test showed that there was no significant difference between 15 and 30°C (Table 6).

Highest and lowest percentage of adult emergence was observed at 15 and 30°C, respectively, when RH was 70±5%. Percentage of adult emergence at 15 and 20°C was higher than that of 50±5% of RH. There was no significant difference between 25 and 30°C (Table 6). This experiment showed that relative humidity also affects the percentage of adult emergence ($p<0.05$, $F = 30.74$, $df = 1, 31$).

DISCUSSION

Study of behavioural characteristics of aphidiine parasitoids will help us to distinguish different species in this family. Mating time and mating behaviour of *L. fabarum* were the same as those obtained and observed by Tremblay[12] and Stary[13], but mating time obtained here was longer than that measured by Marullo[10]. According to Stary[13] mating times in Aphidiidae varies between 20-40 sec and temperature

Table 4: Mean time required (±SE) for mummification of a parasitized aphid and wasp emergence (in days) at different stages of *A. fabae*

Host stages	Oviposition to mummification	Mummification to Adult emergence	Mummification to Adult emergence
1st instar nymph	9.4±0.78	8.36±0.58	17.5±0.82
2nd instar nymph	8.73±0.27	6.52±0.94	14.21±0.47
3rd instar nymph	7.21±0.62	6.33±0.46	13.75±0.68
4th instar nymph	6.5±0.32	6.22±0.13	11.18±0.42
Adults	6.0±0.19	4.13±0.26	10.0±0.26

Table 5: Mean time interval (days) between mummification and adult emergence of *L. fabarum* at different temperatures

Temperature (°C)	5	10	15	20	25
Time interval±SE	8.6±0.25a	8.3±0.2a	6.2±0.21b	6.12±0.14b	3.36±0.16c

Data with the same letter are not significantly different at 0.05

Table 6: Effect of temperatures on mean percentage of adult parasitoid emergence at two levels of RH (50±5% and 70±5%)

Temperature (°C)	15	20	25	30
Percentage of emergence±SE 50±5%(RH)	41.47±2.83c	54.412±1.87c	66.570±1.23c	35.638±1.12c
Percentage of emergence±SE 70±5% (RH)	63.80±2.6b	79.17±4.7a	59.02±3.03ab	34.38±1.57d

Data with the same letter are not significantly different at 0.05

also influences the number of mating[9]. Bellows *et al.*[14] by studying intraspecific and interspecific mating behaviour and by genetical studies have determined new strains in this family.

Aphidiine wasps have been classified into six groups according to their oviposition behaviour[5]. Present studies showed that *L. fabarum* had a type VI oviposition behaviour. Oviposition behaviour and the mechanism by which the parasitoid subdues its host are important in the identification of Aphidiidae at genus and even species level. Females of *L. fabarum* attack their hosts and insert their ovipositor in the venteral side of the host body and oviposit on or between sternal segments of aphid host thorax, usually on second or third segments. This is quite different in *Ephedrus* spp. that females insert their ovipositor in the dorsal surface of the host body close to their heads. This is also different in *L. testaceipes* females which insert their ovipositor in the segment just under cornicles[5,15]

According to Stary[13], Volkl and Mackauer[5] oviposition handling time varies between species in Aphidiidae and it ranges between 0.5-220 sec. Oviposition handling time of *L. fabarum* is 77.2±3.8 sec that is in the ranges mentioned above.

The emergence behaviour of adult parasitoids from mummified aphid hosts is another characteristic that is important in species recognition in the family. Stary[16] divided Aphidiidae into four groups according to the location of their emergence hole. *L. fabarum* is placed in the fourth group which the emergence hole is opened in the dorsal side of the aphid host usually prior to the cornicles. Emergence time was at the ranges that has been reported for the family[17].

The time interval between oviposition and adult emergence was found to be inversely dependent on the host stages, it decreased as the aphid nymphs grew up to adult stage. Similar results have been found on *Ephedrus cerasicola* developing in different stages of *Myzus persicae*[18].

Temperature had a significant effect on the development of *L. fabarum* after aphid mummification. This period was longest at 5 and 10°C and shorter as the temperature increased to 25°C. A similar result was found on this parasitoid in the field conditions[17]. Lower temperatures can be used for the storage of parasitoid at mummified stage when it is supposed to be applied against a pest as an biological control agent. The best temperature for the storage of mummified parasitoids is 7 to 10°C (Stary, Personnel Communication).

Both temperature and relative humidity affected variably percentage of adult emergence from mummified aphid hosts. When relative humidity was 70±5%, highest and lowest percentage of adult parasitoid emergence were observed at 15 and 30°C, respectively. This was relatively different at 50±5% of RH. Tremblay[17] in an experiment using two ranges of relative humidity (40-65 and 60-80%) with different levels of temperatures found variable results of adult emergence for *L. fabarum* as observed in this study. He observed lowest percentage of emergence at 32°C and 40-65% of relative humidity and the highest at 18-20°C and a relative humidity of 60-80%.

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