

**SOME BIOLOGICAL PARAMETERS OF *LYSIPHLEBUS FABARUM* (HYMENOPTERA: APHIDIIDAE)
A PARASITOID OF *APHIS FABAE* (HOMOPTERA: APHIDIIDAE) UNDER LABORATORY CONDITIONS**

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ABSTRACT: Some biological traits of *Lysiphlebus fabarum* (Marshal) an important parasitoid of *Aphis fabae* were studied under laboratory conditions (21 ± 1° C, a relative humidity of 70 ± 5 % and 14:10 L:D h. of photoperiod). Different stages of *Aphis fabae* and its host plant were used. Average preadult period of male and female adult parasitoids were 13.18 ± 0.28 and 13.68 ± 0.27 days, respectively. There were no significant differences between male and female wasps longevity (P > 0.05). There was a significant difference between preadult period of female and male parasitoids on different life stages of *Aphis fabae* (P < 0.05). Diet affected significantly the female parasitoid longevity (P < 0.05). Longevity was longer when the females were fed on 30% honey solution and the shortest when they had no access to host plant, host, water and honey solution. Sex ratio changed towards males as temperature increased. Data analysis revealed that female parasitoids prefer *Aphis fabae* over *Aphis craccivora* Koch and *Aphis nerri* for oviposition (P < 0.05). The lowest oviposition preference was shown for *Aphis nerri*. Mean lifetime fertility of *Lysiphlebus fabarum* was 122 ± 27.28 offsprings/ female on *Aphis fabae*. The intrinsic rate of increase (r_m), mean generation time, doubling time and rate of increase per week (R_w) were 0.28, 16.31 days, 2.47 days, and 7.11, respectively.

KEY WORDS: Diet, Host preference, Population growth, Preadult period, Sex ratio

Aphidiids as endoparasitoids of aphids oviposit in the host body in a way that is specific in these wasps. The larvae hatch into their hosts bodies after incubation. They develop as solitary endoparasitoids. After aphid mummification, they spin cocoons beneath the emptied bodies of their hosts. Prepupal, pupal and adult stages are completed inside the cocoon and the mummified body of the aphid. Mature adults emerge from their mummified aphid host by cutting a circular hole in the host tegument (Stary, 1970).

The lifespan of adult aphidiids is influenced by many factors, such as temperature, humidity, food, presence or absence of hosts, etc. (Stary, 1988). This period generally takes 13-16 days from oviposition to adult emergence in *Lysiphlebus testaceipes* (Weeden & Haffman, 1995) and 10 ± 0.26 days in *L. fabarum* (Baghery-Matin et al., 2005). Development time in *L. testaceipes* ranges between 53.53 ± 0.48 at 10° C to 8.86 ± 0.06 days at 26° C (Welling et al., 1986). This period has been reported as 33.7 days at 15 °C, 21.1 days at 21.6 °C and 19.9 days at 24 °C for *Ephedrus cerasicola* (Hofsvang & Hagvar, 1977). Adult females of *Aphidius sonchi* Marshal, a parasitoid of the sowthistle aphid, *Hyperomyzus lactucae* (L.) lived longer in the absence of hosts than in their

presence and also longer than males. Those supplied with water and honey lived longer than those without honey (Liu & Carver, 1985).

The aphidiid wasps vary in their preference for different stages of their aphid hosts. Although the female wasps prefer the second and the third instar nymphs for oviposition, they all attack four instar nymphs (Stary, 1988). The females of *Aphidius sonchi* Marshal, oviposited in all nymphal instars and both apterous and alate adults of the host, *Hyperomyzus lactucae* (L.) (Liu & Carver, 1985). The females of *Ephedrus cerasicola* oviposited in four nymphal stages and newly emerged adults of *Myzus persicae*, but they preferred the third instar nymphs to others (Tremblay, 1964). It has been found that female *Lysiphlebus fabarum* prefers the second and the third instar nymphs of *Aphis fabae* to other ones and the rate of parasitism is related to the movement of the aphid host and the parasitoid itself (Tremblay, 1964). According to Rakhshani et al. (2004), *Trioxys pallidus* also showed a greater preference for the third and fourth instar nymphs of the walnut aphid, *Chromaphis juglandicola* (Kalt.) than the others. Studies on the biology of *Aphidius sonchi* revealed that it was a specific parasitoid of species of *Hyperomyzus* (Mackauer & Stary, 1967). However, observations showed that the parasitoid also laid eggs in *Macrosiphum euphorbiae* (Thomas), an aphid occurring commonly on *Sonchus* with *H. lactucae* (L.) but the development of parasitoid larvae were never found in *M. euphorbia*, indicating that development of the parasitoid in *Macrosiphum euphorbiae* ceased during the egg stage (Liu & Carver, 1985).

The sex ratio in aphidiids is in favor of females in the field conditions, but it is variably influenced by environmental and genetical factors (Stary, 1988). Host size as an environmental factor affects parasitoid sex ratio, as smaller aphid hosts result in male parasitoids and the larger ones in females. Wellings et al. (1986) also found that smaller hosts produced male parasitoids in *Aphidius ervi*. Age in adult females is another important factor that influences sex allocation and progeny production in *Lysiphlebus delhiensis* (Kouame & Mackauer, 1991; Serivastava & Singh, 1995). The sex ratio (male/female) in *Ephedrus cerasicola* and *L. fabarum* has been reported 1: 1 and 1:1.8, respectively (Stary, 1988).

The intrinsic rate of increase (r_m) for *Trioxys pallidus* reared on *Chromaphis juglandicola* has been studied by Rakhshani (2001) and for *Ephedrus cerasicola* by Hagvar and Hofsvang (1990).

In this research some biological parameters of *L. fabarum* reared on *A. fabae* were studied.

MATERIALS AND METHODS

Preadult period of the parasitoid

In order to determine preadult period of the wasp, colonies of different stages of *A. fabae* were separately established in clip cages (60 x 10 mm). Each colony was then transferred to broad bean, *Vicia fabae*, which were inserted into a glass vial (90 x 50 mm) filled with water. The plant was fixed with nonabsorbent cotton into test tube. This tube then was placed in a transparent plastic dish (300 x 180 mm) on sides of which four circle holes (each 2 cm in diameter) were made for aeration and adult parasitoid releases. Three of them were covered with muslin and the fourth one was plugged with a cork. On the top of the dish cover, a hole (80 mm in diameter) was also made to feed wasps. Dishes were kept in growth chambers (21±1°C, a relative humidity of 70 ± 5 % and 14: 10 L: D). Five pairs of female and male parasitoids were introduced to each dish for 24 hrs. The different stages of aphid in dishes were observed daily. After mummification, mummified

aphids from each dish with a plant part were placed in a Petri-dish (80 x 15 mm) and they were kept until adult parasitoid emergence. The number of adult parasitoid emerged each day was recorded and the observation was continued until all adults that emerged.

Effect of different diets on parasitoid longevity

Mated females were fed on (i) 30% of honey solution (sprayed as tiny droplets on the cover of the plastic dish), (ii) 30 % of honey solution with aphids and host plants, (iii) aphids and host plants without honey solution, and (iv) without food in the absence of aphids and host plants. Thirty five mated females (as replications) were released into a plastic dish (80x120 mm) for each treatment and by daily observation, number of dead female was recorded till the last wasp died.

Effect of temperature on sex ratio

Twenty mummified aphids at the same age (third instar nymphs of *A.fabae*) were placed in a Petri-dish (15 x 80 mm) and were reared to adult stage at four levels of temperatures (15, 20, 25, and 35° C and a relative humidity of 70 ± 5%). Each experiment was replicated five times at each temperature level. Petri-dishes were observed daily and the number of adult parasitoids emerged was recorded and they were sexed under a stereomicroscope (the abdomen tip in female is sharper than male).

Host species preference

In order to determine host species preference of the parasitoid, 20 third instar nymphs of *Aphis fabae*, *A. craccivora* and *A. nerri* were established on a part of *Vicia fabae*, *Robinia pseudoacacia*, and *Nerium oleander* plants. These were then transferred to a transparent plastic box as above. A pair of 1 day-old male and female parasitoid already fed on 30 % of honey solution, was introduced into each box. This experiment was replicated 6 times. After 24 hrs. wasps were removed from the cages using a pooter, and the infested host plant parts were transferred to transparent plastic dishes (120 x 80 mm). They were then kept in a growth chamber (25 ± 1° C, 65 ± 5% RH.) for 72 hrs. Aphids were then transferred in a deep freezer and were then dissected to determine the number of parasitoid eggs laid.

Analysis of variance was used for data analysis and means were compared with Duncan's multiple range test using SAS (1995) software. All experiment were performed in a completely randomized design.

Population growth parameters

In order to determine fertility lifetable of the parasitoid, adults emerging from mummified aphids were used. For this, 15 pairs of 1 day-old female and male parasitoids were introduced into transparent plastic boxes (70 x 110 x 200 mm) containing 50 third instar nymphs of *A. fabae* established already on apical parts of host plant (*Vicia fabae*). After 24 hrs, plant parts bearing parasitized hosts inserted in a glass vial (as above) filled with water were removed and transferred to transparent plastic dishes (80 x 120 mm) and were kept in a growth chamber (21±1°C and 70 ± 5 of RH, 10D: 14L) until adult parasitoids emerged. Fifty third instar nymphs were presented separately to each female parasitoid until they died. At the end of each experiment, the sex ratio of offsprings was determined and used to obtain female percentage at each age class. Population growth rates were calculated according to Andrewartha and Birch (1954) and Carey (1993):

Intrinsic rate of increase ($1 = \sum e^{-rx} l_x m_x$),
 where, x = age in days, r = intrinsic rate of increase, l_x = age-specific survival, m_x = age-specific number of female offspring.

RESULTS

Preadult period

Average preadult period of male and female adult parasitoids reared on third instar nymphs of the aphid hosts were 13.18 ± 0.28 and 13.68 ± 0.27 days, respectively. There was no significant differences between this period for male and female wasps ($P > 0.05$). Preadult periods of adult parasitoids on different life stages of *A. fabae* are presented in Table 1. There was a significant difference between preadult period of female parasitoids on different life stages of the host ($P < 0.05$). The same results were found for male parasitoids ($P < 0.05$). This period was shorter than female parasitoids.

Effect of different diets on parasitoid longevity

Data analysis showed that diet had a significant effect on female adult parasitoid longevity ($P < 0.05$). The longevity was longer when the females were fed on 30% honey solution (12.83 ± 0.77 days) and the shortest (1.57 ± 0.15 days) when they had no access to host plant, host, water and honey solution. The female longevity on host plant, aphid, honey solution and on host plant & aphid was 8.86 ± 0.38 and 4.28 ± 0.256 day, respectively.

Effect of temperature on sex ratio

The sex ratio of *L. fabarum* at different levels of temperatures are shown in Table 2. The percentage of females decreased as the temperature increased.

Host species preference

Data analysis on the mean percentages of parasitism revealed that female parasitoids prefer *A. fabae* ($52.33 \pm 3.53\%$), over *A. craccivora* ($34.63 \pm 2.61\%$) and *A. nerri* ($10.56 \pm 3.33\%$) for oviposition ($P < 0.05$). As it is clear the lowest oviposition preference was shown for *A. nerri*.

Population growth parameters

Fertility life table parameters are shown in Table 3. Mean lifetime fertility of *L. fabarum* was 122 ± 27.28 offsprings/female (with a range of 62-141) on *A. fabae*. Age-specific survival (l_x) and age-specific fecundity of the parasitoid population (m_x) are illustrated in Fig.1.

DISCUSSIONS

Average preadult period of *L. fabarum* decreased as the host stages grew older. Similar results were found by Stary (1986). According to Hofsvang and Hagvar (1986) preadult period of *Ephedrus cerasicola* was influenced by species and age of the host and the temperature. Similar results were also found on *Aphidius matricariae* Hal. parasitizing *Myzus persicae* Sulz. (Rabasse & Shalaby, 1980).

Successful biological control is partly dependent on the longevity and reproductive success of beneficial insects. Availability of carbohydrates can improve the nutrition of parasitic insects, and thereby increase their longevity and realized fecundity. Evidence suggests that individual fitness benefits afforded by

food sources are important for a time-limited parasitoid (Williams & Roane, 2007). In this study, food provision of female parasitoids affected their longevity significantly. Providing parasitoids with food will result in increased longevity and subsequent parasitism rates (Wäckers, 2001; Azzouz et al., 2004; Irvin et al., 2007). Similar results were found by Hofsvang and Hagvar (1986) on *Ephedrus cerasicola*. Longevity is generally influenced by searching activity, body size, mating, oviposition, temperature, humidity, photoperiod and diet (Jervis & Copland, 1996). The adult parasitoid of *Trioxys pallidus* lived shorter when fed only on water and honey solution compared to those kept with hosts and fed upon honeydew and first instar nymphs of *Chromaphis juglandicola*. It was even shorter when they were kept without hosts and food (Rakhshani, 2001). In this research, the longevity of adult females of *Lysiphlebus fabarum* with hosts was shorter than *E. cerasicola* with *M. persicae* and *E. californicus* with *Acyrtosiphon pisum* (Hofsvang & Hagvar, 1975a).

The sex ratio changed towards males as temperature increased. According to Tremblay (1964) at higher temperatures, most activities of the parasitoid including mating, oviposition, flight and searching declined and that in turn resulted in the reduction of offspring number and an increase in the number of males in the population. This result is similar to those found by Tremblay (1964). As females grow older, daily oviposition rate decreases leading to an increase in male offsprings (Hofsvang & Hagvar, 1975b, Hagvar & Hofsvang, 1990). Parasitized nymphs of *Aphis fabae* start to mummify prior to maturity and reproduction. Therefore, by eliminating reproduction in younger instars and parasitization of fourth instar nymphs and adult stages by the parasitoid will eventually decrease aphid reproduction and its population considerably (Tremblay, 1964; Hofsvang & Hagvar, 1986; Hagvar & Hofsvang, 1991).

Data analysis on host species selection showed that *A. fabae* was the most preferred host for the parasitoid. This has been shown to be related to the colour of the aphid host, as this parasitoid prefers aphids with darker colours to others (Tregubenko, 1980). Carver (1984) in a study on the host ranges of *L. fabarum* and *L. testacipes* found that the percentage of adult parasitoids emergence on *A. nerii* was very low as compared with those on *A. craccivora* and *A. citricidus*. The toxic substances in *Nerium oleander* leaf tissues affect the growth of parasitoid inside the body of the aphid host (P. Stary, unpubl. data, 2002).

The intrinsic rate of increase (r_m) obtained for *L. fabarum* was similar to those obtained for *Ephedrus cerasicola* (Hagvar & Hofsvang, 1990) and *Trioxys pallidus* (Rakhshani, 2001). The r_m values in these insects were 0.38 and 0.28, respectively. Hagvar and Hofsvang (1990) state the intrinsic rate of increase in Aphidiid wasps generally ranges between 0.29 and 0.38. Net reproductive rate obtained here was less than the reproductive rate for *Ephedrus cerasicola* on *M. persicae* and more than that calculated for *T. pallidus* on *Chromaphis juglandicola*. The generation time of *L. fabarum* was greater than those obtained for two above mentioned was species, but the doubling time was lower than *T. pallidus* and higher than *Ephedrus cerasicola*.

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Table 1. Average (\pm Se) preadult periods (in days) of *L.fabarum* reared on different life stages of *A. fabae* at $21\pm 1^{\circ}\text{C}$.

Host stages:	1 st instar	2 nd instar	3 rd instar	4 th instar nymph	adults
Females:	17.38 \pm 0.33 a (n 17)	15.76 \pm 0.19b (n 20)	14.21 \pm 0.27bc (n 32)	11.3 \pm 0.4 cd (n 25)	9.73 \pm 0.14d (n 9)
Males :	17.06 \pm 0.17 a (n 35)	14.37 \pm 0.04b (n 35)	13.92 \pm 0.17bc (n 48)	11.25 \pm 0.82cd (n 34)	9.3 \pm 0.19d (n 12)

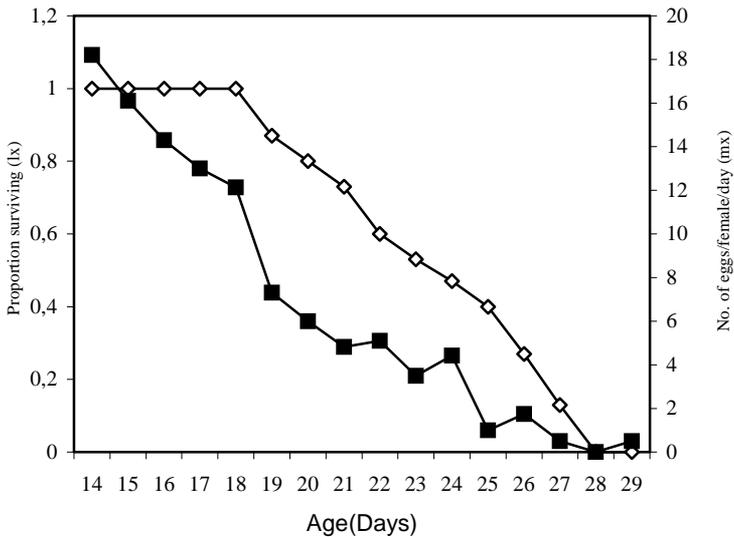
Data with the same letter are not significantly different at 0.05

Table 2. Sex ratio of *L. fabarum* reared on *A. fabae* at different temperatures.

Temperature (oC)	No. of males	No. of females	Female%
15	53	147	73.5
20	57	160	73.7
25	56	123	68.7
30	38	58	60.4

Table 3. Population growth parameters of *L. fabarum* reared on *A. fabae* under laboratory condition .

Parameters	Values
Net reproductive rate ($R_0 = \sum l_x m_x$)	94.34
Intrinsic rate of increase (r_m)	0.28
Mean generation time ($T = \ln R_0 / r_m$)	16.31
Doubling time ($Dt = \ln 2 / r_m$)	2.47
Rate of increase per week ($R_W = e^{r_m}$)	7.11

Fig. 1. Age-specific survival rate (l_x) and age-specific fecundity (m_x) of *L. fabarum* reared on *A. fabae*.