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Biology of *Hemiptarsenus varicornis* (Hymenoptera: Eulophidae), A Parasitoid Wasp of the Leafminer *Liriomyza trifolii* (Diptera: Agromyzidae)

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Dipteran leafminers belonging to the family Agromyzidae are serious insect pests for many vegetable and ornamental crops in Asia including Vietnam and Japan. The parasitoid wasp *Hemiptarsenus varicornis* (Girault) has recently been recognized as an effective biological control agent of such leafminers including *Liriomyza trifolii* (Burgess) and *L. bryoniae* (Kaktenbach). However, the biology of *H. varicornis* is not fully understood. In this paper, we investigated development time, adult longevity, egg production and parasitism of *H. varicornis* in the laboratory, examining basic life history characteristics of this parasitoid. At a constant 25 °C condition, females survived significantly longer than males when fed on honey solution in the absence of hosts. The mean longevity were 7 and 12 days for males and females, respectively. Development time from egg to adult emergence of males was significantly shorter than that of females (adv. 10.4 vs. 10.9 days at 25 °C). Dissection experiments showed that females emerged with no or only a few mature and immature eggs. Fecundity of females remained steady when no hosts were provided (2–4 mature eggs), suggesting that *H. varicornis* have to feed on hosts to enhance egg production. Cage experiments were conducted to evaluate reproductive capacity of females. The results demonstrated that female *H. varicornis* killed on average 31 leafminer larvae by parasitism during 5 consecutive days since emergence. The female showed destructive host-feeding, and killed on average 11 leafminer larvae by host-feeding in addition to by parasitism. There was a positive relationship between numbers of hosts fed upon and those parasitized, suggesting again that host-feeding related to enhancement of egg production.

INTRODUCTION

In recent years, dipteran leafminers belonging to the genus of *Liriomyza* of the family Agromyzidae are recognized as serious and widespread pests in Europe, America, and many Asian countries including Vietnam and Japan (e.g., Wardlow, 1985; Minkenberg and van Lenteren, 1986; Sheng *et al.*, 1989; Spencer, 1989; Ohno *et al.*, 1999a, b; Thang, 1999; Rauf *et al.*, 2000). They attack numerous vegetable crops like beans, cucumber, potato and crucifers, and also a number of ornamental crops. *L. sativae* Blanchard may cause yield losses of up to 70% in tomato crops (Waterhouse and Norris, 1987; Spencer, 1989). In Japan, *L. trifolii* (Burgess) has been one of the most serious pest leafminers since its accidental introduction. *L. trifolii* has historically been a major pest of a wide variety of greenhouse crops in the world (Parrella *et al.*, 1984; Wardlow, 1985; Minken-

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berg and van Lenteren, 1986).

In agricultural systems using frequent pesticide application, leafminers have developed resistance to pesticides quickly, and therefore, control of leafminers exclusively with pesticides is difficult (Parrella *et al.*, 1984; Wardlow, 1985). Alternative methods such as biological control or IPM program are thus required to effectively suppress leafminer populations. Accordingly, research has been conducted to test various IPM strategies and combinations of different control methods against pest dipteran leafminers (e.g., Minkenberg and van Lenteren, 1987; Heinz and Parella, 1990; Weintraub and Horowitz, 1998; Saito *et al.*, 1995; Ohno *et al.*, 1999a, b; Ozawa *et al.*, 1998, 1999, 2001; Weintraub, 2001).

So far, parasitoid Hymenoptera have proven success as agents of biological control of a variety of insect pests developing insecticide resistance (Greathead, 1986; Waterhouse and Norris, 1987). More recently, several studies have shown that biological control can be a promising method to control leafminer pests (e.g., Johnson and Hara, 1987; Parrella *et al.*, 1987, 1989; Sheng *et al.*, 1989; Saito *et al.*, 1995; Ozawa *et al.*, 2001) .

Although there are many hymenopteran parasitoids attacking *L. trifolii* (Konishi, 1998; Arakaki and Kinjo, 1998; Ohno *et al.*, 1999a; Ozawa *et al.*, 2001), *Hemiptarsenus varicornis* (Girault) (Hymenoptera: Eulophidae), a solitary larval ectoparasitoid, is suggested to be a promising agent (Saito *et al.*, 1995; Ozawa *et al.*, 2001). However, relatively few studies have examined the basic biology of this parasitoid. A brief description of *H. varicornis* development time when reared on *L. trifolii* was given (Bordat *et al.*, 1995; Saito *et al.*, 1997). Bordat *et al.* (1995) provided information on parasitism of *L. trifolli* larvae of different stages (i.e., larval instars) by *H. varicornis*. However, other information such as adult longevity, fecundity etc. are lacking. Basic information about the biology of this parasitoid is still limited.

Thus, the biological characteristics of *H. varicornis* are not fully understood. Consequently in this paper, we investigated biological parameters including development time, adult longevity, egg production and reproductive capacity of *H. varicornis* in the laboratory, examining basic life history characteristics of the parasitoid. Our study provides additional information on the biology of *H. varicornis*, which may allow better use of this species as a control agent against agromizid leafminers.

MATERIALS AND METHODS

Cultures

The leafminer *Liriomyza trifolii* used in our study was originated from Fukuoka, Kyushu, Japan. The populations have been maintained for generations in the laboratory, by using Kidney bean plants as a host plant (Ohno *et al.*, 1999a). The parasitoid wasp *Hemiptarsenus varicornis* was originated from Shizuoka, Honshu, Japan (Saito *et al.*, 1997). Leafminers and parasitoids were mass–reared and maintained at 25±1 °C, 60–70% humidity under a constant light in a rearing room.

General rearing procedure

Seeds of kidney bean were planted in plastic pots (7.5 cm in diameter). After one week of germination, a pan (32 cm*44 cm*6 cm) containing 24 potted plants was then

located on a large shelf covered with a fine meshed nylon. Leafminer adults were released into the shelf for oviposition. After a 24 h exposure for oviposition, the potted plants were removed from the shelf and maintained at 25±1 °C until all leafminer larvae were matured. Leaves containing matured larvae were cut off from the plants, and were collected to gain adult leafminers.

Hemiptarsenus varicornis was reared on the *L. trifolii*. Kidney bean plants infested with large numbers of second and third instars of *L. trifolii* and female parasitoids were introduced into a transparent plastic box (20 cm*20 cm*35 cm), one side of which was covered with a fine meshed nylon. Tissue paper saturated with a 30% honey solution was also placed in the box as a food source for female parasitoids. Female parasitoids were allowed to attack and parasitized leafminer larvae for 24 hours. After 24 hours for parasitism, plants with parasitized hosts were taken away, and were kept in the rearing room for 5–7 days. Leaves containing parasitized larvae were then cut off from the plants, and were placed in a rearing plastic box (19 cm*25 cm*9 cm) for parasitoid emergence.

Adult longevity

Longevity of adult parasitoids was investigated under a laboratory condition at 25± 1 °C. Newly emerged adults were individually placed in plastic cups (4.5 cm in diameter, 3.0 cm in height) together tissue paper saturated with a honey solution (30%). Tissue paper was replaced daily to provide parasitoids with fresh food. Parasitoid mortality was recorded every day until all parasitoids had died. In total, 44 females and 81 males were used in this experiment.

Development time

Development time from eggs to adult emergence of *H. varicornis* was examined at a constant 25 °C condition. Parasitized hosts were obtained as with the methods mentioned in the general rearing procedure. Potted plants with parasitized leafminers were removed from the cage after a 24–hours–exposure. The plants were maintained at 25±1 °C for 7 days in a rearing room. Then, leaves of the plants were cut off, and were placed singly in plastic cups until parasitoid emergence. The day of parasitoid emergence was recorded every day, and developmental time required from egg (oviposition) to adult parasitoid emergence in day was calculated.

Egg production

Egg production rate of *H. varicornis* was assessed in this experiment. For this purpose, dissection experiments were performed. Newly emerged females were collected and were allowed to mate with males. Mated females were placed in plastic cups (4.5 cm in diameter, 3.0 cm in height) together with tissue paper saturated with a 30% honey solution. The cups were then kept at 20±1 °C. In this experiment, females were not allowed access to hosts.

Females of 1, 2, 3, 4, 5 and 6 days old were dissected under a binocular microscope, and the number of mature and immature eggs a female carried were counted. A total of 36 females were used in this experiment. Mature eggs are elongate oval and opaque with the smooth surface, and can hence be distinguished from immature eggs with the rough surface.

Oviposition and host-feeding

The aim of this experiment was to examine the potential of *H. varicornis* as a natural enemy of *L. trifolii*. For this purpose, we investigated how many leafminer larvae a female of *H. varicornis* could kill. Preliminary observations showed that *H. varicornis* killed *L. trifolii* larvae by parasitism and host-feeding, the numbers of hosts fed upon as well as those parasitized were counted.

Kidney bean plants infested with large number of second and third instars of *L. trifolli* were used in this experiment. Plants with leafminers were individually placed in a transparent plastic box (see the General rearing procedure) and then one female *H. varicornis* was carefully introduced into the box. Tissue paper saturated with a 30% honey solution was also placed in the box to provide female wasps with food.

Female *H. varicornis* was allowed to attack leafminers for 24 hours. After a 24 h exposure, plants were removed from the cage, and leafminers within leaf tissues were checked under a binocular microscope. Number of parasitoid eggs and leafminer age (second and third instars) were recorded. Care was taken to examine whether body fluids of host leafminers was exuded on the body surface or not. When host body fluids was found to be exuded on a host, we regarded that the host was fed upon by a female wasp.

This treatment was repeated five consecutive days; thus a total of five plants with leafminers were given to each female wasp (female age: 1-5 days). The experiment was conducted at 25±1 °C under constant light. A total of seven females and 1383 host leafminers were used in the experiment.

Data analyses

Statistical treatments were made with the aid of StatView (SAS Institute, 1998). Normality and equal variance of data were checked, and the subsequent statistical analyses were then performed. Longevity and development time were analyzed with ANOVA or Welch's ANOVA. Regression analyses were used to analyze egg production data.

RESULTS AND DISCUSSION

Longevity

Males and females lived on average for 7.05 and 12.41 days, respectively when they were allowed to feed on a honey solution (Table 1). Parasitoid longevity differed significantly between the sexes (ANOVA, F=5.13, $P<0.05$); therefore females survived longer than males. In many parasitoids, life expectancy in females is nearly always greater than

Table 1. Longevity and development time of *Hemiptarsenus varicornis* at 25 °C

Parasitoid sex	Longevity*	Development time*
Male	7.05±5.69 (23)	10.4±0.9 (258)
Female	12.41±7.96 (25)	10.9±1.0 (134)

* Data were shown as mean±SD. Longevity and development time in females were both longer than those in males (ANOVA, P<0.05). Numbers in parentheses indicate sample size.

males (e.g., Ooi, 1980; Olmi, 1994). Our study has shown that this holds true for *H. varicornis*.

Nutritional factors have been shown to be important in the modification of longevity and lifetime reproductive success in parasitoids (Leius, 1960, 1961; Syme, 1977; van Lenteren *et al.*, 1987; Jervis *et al.*, 1993; Jervis and Kidd, 1996; Olson *et al.*, 2000; Schmale *et al.*, 2001). Carbohydrate intakes cause an increase in life span of many insects (e.g. Slansky and Rodriguez, 1987). In our study, male and female *H. varicornis* were allowed access to a 30% honey solution. This is because when only water is provided, they die within a few days (personal observations). Thus, as with the case for many parasitoids, carbohydrate sources are important for *H. varicornis* to increase reproductive lifetime.

Development time

Mean development time of *H. varicornis* from egg to adult emergence when reared on *L. trifolii* under a constant 25 °C was 10.4 and 10.9 days for males and females, respectively (Table 1). Based on the result of Bartlett test (F=3.70, P=0.054), Welch's ANOVA was applied to examine a mean difference between the sexes. Mean development time significantly differed between the sexes (Welch's ANOVA, F=29.19, $P<0.01$), and thus males developed faster than females did (Table 1).

Comparable values are reported by Saito *et al.* (1997) for *H. varicornis* developing on *L. trifolii*; in their study, mean development time for females and males are 8.8 day and 8.6 day, respectively. The mean values appear to be smaller than those in our study. Part of the reasons may be that, in our rearing systems, humidity is low (around 60%),

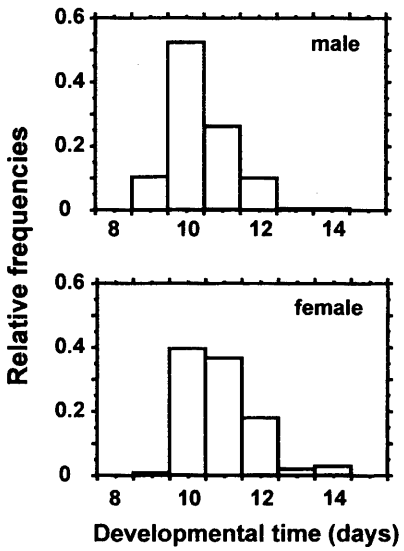

Fig. 1. Variation in development time in male and female *Hemiptarsenus varicornis*.

leading to longer development time.

Male parasitoids often develop faster than females, and, hence, emerge from the host earlier. This phenomenon is called protandry, and protandry is known to exist in many insect species including most parasitic Hymenoptera (Doutt, 1964; Fagerstrom and Wiklund, 1982; Hirose *et al.*, 1988; Quicke, 1997) as does in *H. varicornis* (Fig. 1).

In addition, an examination of frequency distributions of male and female development time showed that male development time varied significantly less than female development time (Kolmogorov–Smirnov test; df=2, P=0.0003) (Fig. 1). Because male parasitoid wasps can usually mate many times while females of many parasitoids mate only once, shorter development time may enable a male to increase its chances of contacting virgin females.

Egg production

In the present study, we investigated fecundity of female *H. varicornis* when hosts were not given. In the condition, females had only 2–4 mature eggs with a few immature eggs in the ovary. For both mature and immature eggs, there were no significant relationships with female age (Fig. 2; Regression analysis; N=35, df=1, r^2=0.02, t=0.89, P=0.38 for mature eggs; r^2=0.08, t=1.74, P=0.09 for immature eggs). The number of mature and immature eggs remained steady between the first and sixth days after wasp emergence.

Given that female *H. varicornis* had a potential to lay more eggs in cage experiments (also see Bordat *et al.*, 1995), the results of the dissection experiment are not consistent

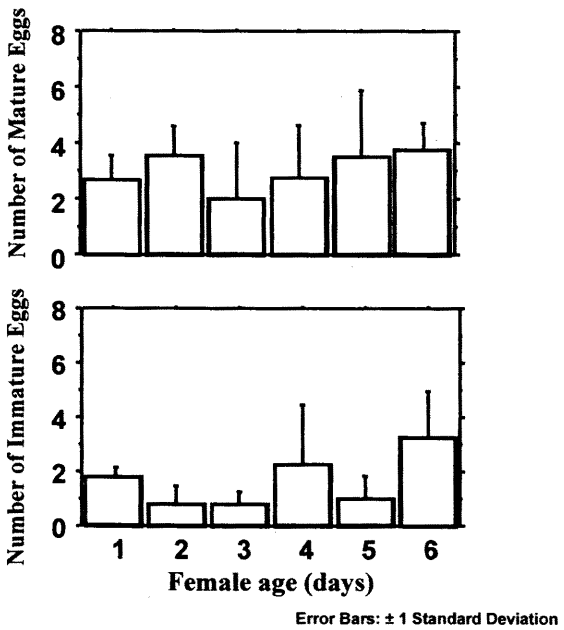

Fig. 2. Egg production by *Hemiptarsenus varicornis* in relation to female age.

with the results of the oviposition and host-feeding experiment (see below). A most likely explanation is that *H. varicornis* enhances egg production in the presence of hosts. Eulophid parasitoids attacking dipteran leafminers are known to feed on hosts to produce eggs (Minkenberg and van Lenteren, 1986). Likewise, *H. varicornis* is said to feed on hosts. If this holds true, the maximum egg production of *H. varicornis* would be attained only when sufficient numbers of hosts are given. To test this, relationships between host-feeding behavior and egg production should be examined (see below).

Oviposition and host-feeding

Female *H. varicornis* parasitized 14–43 host leafminers during the experimental period (Table 2). The female killed 6–18 host leafminers by host-feeding (Table 2). Mean numbers of hosts parasitized and fed upon were 31.4±0.6 and 11.4±4.9, respectively. Mean numbers of hosts killed (i.e., # parasitized plus # host-fed) during the 5-day-experimental period was 42.9±14.8.

Table 2. Numbers of hosts used for oviposition and host-feeding among seven female *Hemiptarsenus varicornis* in the cage experiment

Wasp no	No. of hosts parasitized	No. of hosts fed upon	Total no. of hosts killed
1	42	15	57
2	26	10	36
3	34	6	40
4	14	6	20
5	24	9	33
6	43	18	61
7	37	16	53
Average *	31.4±10.6	11.4±4.9	42.9±14.8

* Mean values were indicated with standard deviations.

Numbers of hosts parasitized considerably varied among parasitoid individuals. The reasons were unclear. One possible factor would be variation in the size of female parasitoids. For many parasitoids, it is often found that female size closely relates to fecundity of each female (e.g. Godfray, 1994; Quicke, 1997). In our study, unfortunately, the size of test females was not measured.

Because larger individuals are generally more fecund and have longer life span, larger individuals are more likely to work better as a biocontrol agent. If this is the case for *H. varicornis*, production of large females will be required during mass-rearing process. Factors affecting the size of *H. varicornis* and the relationship between female size and fecundity should be examined in the future study.

H. varicornis killed considerable numbers of hosts by host-feeding (Table 1). There was a significant relationship between total numbers of hosts fed upon and those parasitized (Fig. 3) (Regression analysis: N=7, r^2=0.63, t=2.89, P=0.034). This result suggests that females that feed more can parasitize more. Host-feeding is an essential means of obtaining protein sources for a number of parasitoid wasps, and feeding activities

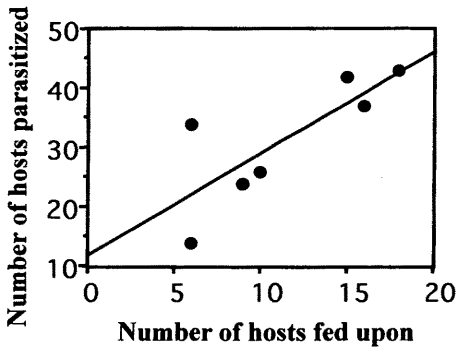

Fig. 3. Relationship between the numbers of host-feedings and ovipositions in *Hemiptarsenus varicornis*.

correlate to female fecundity (e.g. Leius, 1961; van Lenteren et al., 1987; Jervis and Kidd, 1996; Morales-Ramos et al., 1996; Ueno, 1999). This is the case for *H. varicornis*.

The result also suggests that about 60% variations in numbers of hosts parasitized by each test female can be explained by differences in numbers of hosts each test female feed on. However, why there is a variation in numbers of hosts fed upon remains unsolved. Again, relationships between female size and host-feeding should be investigated.

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