Biology of the Bethylid Parasitoid Goniozus legneri Gordh (Hymenoptera: Bethylidae)

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ABSTRACT

The bethylid parasitoid Goniozus legneri Gordh was introduced from U.S.A in 1997 as a bio-control agent against the pyralid pest Palpita unionalis (Hb.) attacking olive trees in Egypt. The present paper deals with its biology. The four constant temperatures, 15, 20, 25 and 30 °C; each combined with 60 ±5 % R.H. were used to estimate the durations of immature stages and activity of adult reproduction. The temperature showed a significant effect on each parameter. The most favourable hygrothermic condition are 30 °C and 60 %R.H.

The longevity of mated and unmated parasitic individuals were also estimated under different temperatures and feeding regimes. It was significantly affected by such factors.

1. INTRODUCTION

The bethylid *Gonoizus legneri* Gordh is one of the bio-agents of olive leaf moth, *Palpita unionalis* (Hb.) attacking olive trees in Egypt. It is known as a primary ectoparasitoid of lepidopterous larvae; *e.g.*, the carob moth *Spectrobates ceratoniae* (Zeller), the naval- orangeworm *Amyelois transitella* (Walker) in Arizona and California (Butler and Schmidt, 1985) and *A. transitella* in California (Legner and Workentin, 1988). Other lepidopterous larvae are used as laboratory hosts for this bethylid; *e.g., Pectinophora gossypiella* (Saunders) (Bulter and Schmidt, 1985); *Ephestia kuehniella* (Zeller) (Sarhan, 1989); *Manduca sexta* (L.) and *Galleria mellonella* (L.) (Skinner *et al.*, 1990).

The present paper deals with the biology of this bethylid as one of the bio- factors affecting the population densities of the olive leaf moth *P. unionalis* in Egypt.

2. MATERIALS AND METHODS

2.1. Host rearing:

Samples of olive branches infested with *P. unionalis* were collected from orchards and kept in glass jars of 20 x10 cm. under room conditions of 25 ± 2 °C and 60 ± 5% R.H. until pupation of larvae. Pupae were collected, sexed and isolated in tubes of 2 x10 cm till emergence. Couples of the newly emerged moths were kept in glass jars of 20x10 cm., provided with branches bearing fresh olive leaves for deposition. Branches were exchanged daily and droplets of honey solution were used for moths nutrition. Deposited eggs were reared under controlled conditions of 27 °C and 60 ± 5% R.H. Newly hatched larvae were transferred to another jars and provided daily with fresh young olive branches until pupation.

2.2. Parasitoid rearing:

Fertilized females of the parasitoid *G. legneri* were maintained at 25 ± 2 °C and 60 ± 5% R.H. in glass tubes (7x2 cm), stoppered with a piece of cotton with few droplets of honey as nutrition, together with 4th instar larvae of *P. unionalis* as a host. Parasitized hosts were removed daily and placed in Petri dishes until pupation and emergence of adult parasitoids. Healthy larvae were introduced daily to the parasitic adults until its death. The host larvae in the 4th instar are the most suitable for parasitoid rearing followed by the 5th one. However the earlier instars died before parasitoid emergence while, the oldest one (6th instar) is unsuitable because of the toughness of its cuticle.

2.3. Statistical analysis:

(F-test) was used for the data concerning with the effect of temperature on the duration of immature stages and adults longevity, while Chi ^{2} test was used for sex ratio.

3. RESULTS AND DISCUSSION

3.1. Duration of various immature stages Incubation period and hatchability rate:

The incubation period of *G. legneri* eggs was estimated under the four controlled temperatures 15, 20, 25 and 30 °C (each combined with 60 % ± 5 R.H.).The shortest mean incubation period (24.0 ± 0.2 hours) was reported for the highest temperature (30.0 ± 1 °C), while the longest one (66.0 ± 1.4 hours) was for the lowest degree (15.0 ± 1 °C) (Table 1). Intermediate records were reported for the in-between

degrees. On the other hand, hatchability rates ranged from 85.0 to 95.0 %; highest rates were reported for 25 and 30 °C.

Statistical analysis (F-test) of the data shows that temperature has a negative and highly significant effect on the incubation period.

Larval duration

The larval stage of *G. legneri* was passed through three instars. Associating with the afore-mentioned four temperatures, the duration of any of the larval instars increased with the decrease of temperature. Under the four temperatures, the first larval instar elapsed the shortest periods, while the third one occupied the longest ones (Table 1). The shortest period was 7.0 ± 0.2 hours reported for the first instar at 30 °C, while the longest one was 195.0 ± 0.7 hours for the third larval instar at 15 °C. Accordingly, the total larval durations were in respective 1.9 ± 0.6, 3.4 ± 0.7, 8.1 ± 1.6 and 14.8 ± 0.9 days at 30, 25, 20, and 15 °C; showing the longest period at 15 °C and the shortest period at 30 °C.

Statistical analysis of the data (F-test) shows that temperature has a negative and highly significant effect on the duration of the three larval instars and on the total larval duration.

Pre-pupal duration:

The shortest mean duration of the pre pupal stage was 1.5 ± 0.1 days, at 30 ± 1 °C; increasing to 2.5 ± 0.1 and 6.5 ± 0.1 days at 25 and 20 °C, respectively. However, at 15 °C the 3rd instar larvae failed to spin cocoons and to continue its development to pre-pupae. (Table 1).

Statistical analysis of the data (F-test) shows that temperature has a negative and highly significant effect on the duration of the pre-pupal stage.

Pupal duration:

The shortest mean duration was 4.0 ± 0.2 days, at 30 °C; increasing to 6.0 ± 0.2 days at 25 °C and 13.0 ± 0.2 days, at 20 °C; showing progressive increase with the decrease of temperature (Table 1).

Statistical analysis of the data (F-test) shows that temperature has a negative and highly significant effect on the duration of the pupal stage.

Total developmental period:

The shortest total developmental period (8.4 ± 0.2) days was obtained at 30 °C; increasing to 13.4 ± 0.3 and 29.5 ± 0.6 days at 25 and 20 °C., respectively.

In this concern, Butler and Schmidt (1985) recorded a total developmental period of 15.6 days at 25 °C and 9.2 days at 30 °C when the parasitoid was reared on the pink boll worm *Pectinophora gossypiella*. Sarhan (1989) reported that this period was 20 and 11.1 days at 25 and 30 °C, respectively when the parasitoid *G. legneri* was reared on *Ephestia kuehniella*. Abbas (1999) mentioned that the developmental period of *G. legneri* was 11.3 days at 27 ± 1 °C and 50-55 % R.H. when the parasitoid was reared on larvae of the navel orangeworm *A. transitella*.

Statistical analysis of the data (F-test) has shown that temperature has a negative and highly significant effect on the total developmental period of *G. legneri*.

3.2. Adult Stage

3.2.1. Oviposition

Process of oviposition:

Using its ovipositor, the parasitic female paralyses its host before deposition. During stinging, the host larva shows some struggle for a short time, after which it becomes completely quiet. It requires 10.0 (5-15) minute to paralyze its host larva. Under laboratory conditions, female parasitoid may sting several hosts without deposition. After complete paralysis, eggs are deposited on any part of the host body except the head capsule. On deposition, the females usually prefer the larval tergites than its pleurites or sternites. According to Lee (1992) it seems that, the parasitoid female regulates the number of eggs laid on the host as there is a linear relationship between the host body mass and this number.

In case of successful parasitism, the parasitized larva remains soft; otherwise it becomes dry and wrinkled. As in many other Hymenoptera, females must feed on the body fluids of the host to obtain the protein required for egg production. In case of *G. legneri* the female uses its mandibles to scratch the host's body integument to suck the oozing fluid.

Ovipositional period and fecundity:

The ovipositional periods and the number of eggs laid per female had been studied in both virgin and mated females reared and kept at 30 ± 1 °C, 25 ± 1 °C, 20 ± °C and 15 ± 1 °C and 60 ± 5% R.H., to assess the effect of mating and temperature; ten females were used in each case. Females were confined singly in (10 x 3 cm) glass tubes covered with a piece of cotton and fixed with a rubber band, and provided with a small droplet of honey to serve as adult's food. Each female was given 5 host larvae for 24 hour, and the parasitized host larvae were removed to be replaced by healthy ones until death of the female parasitoid. The removed parasitized host larvae were examined under a stereoscope binocular and the deposited eggs were counted every 24 hour. Obtained data are presented in Table (2).

Table (1): Duration of the immature stages of *G. legneri* at different constant temperatures and 60 ± 5 % R.H.

Biological parameters \ Temp. °C		30 °C	25 °C	20 °C	15 °C
Egg	Incubation period / hr	24 ± 2.0 (23-25)	36 ± 0.4 (34-38)	48 ± 0.4 (46-50)	66 ±1.4 (60-72)
	% hatchability	95.0	95.0	90.0	85.0
Larva	1st / hr	7±0.2 (6 – 8)	13±0.2 (12-14)	42±1.1 (36-48)	72±0.5 (70-74)
	2nd / hr	7.5 ±0.2 (6 -9)	13.5±0.3 (12-16)	44±0.7 (40-48)	89±1.5 (82-96)
	3rd / hr	30±1.5 (24 -36)	54±1.5 (48 - 60)	108±3.0 (96-120)	195±0.7 (192-198)
	Total larval period / day	1.9 ±0.6 (1.5-2.2)	3.4 ±0.7 (3.0 - 3.8)	8.1 ±1.6 (7.1– 9.0)	14.8±0.9 (14.3-15.3)
Pre-pupal / day		1.5±0.1 (1 - 2)	2.5±0.1 (2 - 3)	6.5±0.1 (6 – 7)	-
Pupa / day		4.0±0.2 (3-5)	6.0±0.2 (5-7)	13.0±0.2 (12-14)	-
Total developmental period / day		8.4±0.2 (6.4-10.2)	13.4±0.3 (11.4-15.4)	29.6±0.5 (27.0-32.1)	-

Pre-oviposition period:

In mated or virgin females, the pre-oviposition period prolonged with the decrease of temperature from 30 °C to 20 °C; reached to the longest period (8 ± 0.2 days) under the two cases at 20 °C. However, at 15 °C, this period extended to 1-3 months and therefore this degree of temperature was not suitable at all. On the other hand, this period was generally longer in virgin females than in mated ones, especially at 30 °C or 25 °C. The shortest period obtained was 1.5 ± 0.1 days reported for mated females at highest degree of temperature (30 °C) (Table 2).

Oviposition period

As in case of the pre-oviposition period, the oviposition period prolonged with the decrease of temperature, whether associated with mated or virgin females. On the other hand, this period was comparatively longer in mated than in virgin females. The longest period was 79.3 ± 3.9 days reported for mated females at 20 °C, while the shortest one was 30.6 ± 4.2 days for virgin females at 30 °C (Table 2)

Post-oviposition period:

This period showed a similar trend as that in the pre-oviposition period; being longer with the decrease of temperature and in virgin than in mated females. The longest period (9.1 ± 0.8 days) was reported for virgin females at 20 °C, while the shortest (2.2 ± 0.2 days) for mated females at 30 °C. (Table 2).

Statistical analyses (F-test) showed insignificant effect of temperature and fertilization on the ovipositional periods

Number of eggs per female:

Associating with any degree of temperature, the total number of eggs laid per female was comparatively greater in mated females than in virgin ones. On the other hand, the number deceased with the decrease of temperature (Table 2). Associating with the three degrees of temperature 30, 25 and 20 °C, the average numbers of eggs/female were 196.7, 193.6 and 126.9 eggs in mated females, opposed to 163.2, 158.1 and 99.4 eggs in virgin ones. The maximum number (196.7±20.4 eggs) was reported for the mated female at 30 °C, while the lowest (99.4 ±7.1 eggs) was for the virgin female at 20 °C. The daily rate of deposition showed in general, a similar trend as that of the total number laid; being greater in mated female than in virgin one and at higher temperature than at the lower one.

Statistically, (F-test) showed significant effect of temperature and fertilization on the daily and total number of eggs laid per female.

Number of parasitoids reared on a *P. unionalis* larva:

In a laboratory experiment conducted at 30 ± 1 °C and 60 ± 5% R.H., 20 larvae of *P. unionalis* were subjected individually for 24 hrs to a mated parasitic female and kept until adults' emergence. It was found that the number of adult' parasitoids could be reared on a larva ranged between 2 & 18 individuals, with an average of 9.9 individuals. The mean sex ratio (♂ : ♀) was 1: 3.8.

3-Number of *P. unionalis* larvae attacked by a single mated or virgin parasitoid female during its life span.

Each of 10 mated and 10 virgin females was provided daily with five *P. unionalis* larvae in a glass tube (7x2 cm). during its life span at 30 ± 1 °C, and 60 % R.H. For each female, the number of paralyzed larvae was calculated; (paralyzed larvae include parasitized and unparasitized larvae).The number of eggs laid by each female was also calculated. Obtained data revealed that the mean number of eggs laid per female during its life span was 197.4 ± 22.4 (121-328) in mated females, opposed to 160.2 ± 25.3 (90-320) in virgin ones. The mean number of paralyzed larvae was 40.3 ± 1.2 (37-49) larvae in mated female and 30.3± 1.4 (25-40) larvae in virgin females. The mean number of parasitized larvae was 30.7±1.4 (25-39) and 20.4±1.4 (15-31) larvae in mated and virgin females, respectively.

4-Adult's longevity:

Longevity of *G. legneri* adults was determined in mated males and females at three constant temperatures, 20, 25 and 30°C. Another experiment was conducted on unmated adults at 30±1 °C with four different diet treatments, *i.e.*, starvation, water, honey, *P. unionalis* larvae plus honey.

Effect of mating on longevity was also studied at 30±1 °C when adults were provided with honey and *P. unionalis* larvae.

A- Effect of temperature on longevity:

Data (Table 3) showed that whether in mated males or females, longevity are longer with the decrease of temperature; reaching its maximum to 46.9±1.9 and 91.7±3.8 in mated males and females, respectively. On the other hand, mated females lived longer than associated males under any of the tested temperatures.

Statistical analysis (F. test) shows that temperature has a negative and highly significant effect on the longevity of both mated males and females.

B- Effect of adult's diet on longevity:

The mean longevities of unmated males at 30±1 °C and feeding conditions of starvation, water, honey and larvae of *P. unionalis* plus honey were 5.2 ± 0.7 (3-7); 6.1± 0.6 (4-8); 11.5 ± 2.2 (2-22) and 12.0 ± 2.2 (7-22) days, respectively. The respective longevities for unmated females were 7.5 ± 0.7 (6-10); 7.6± 0.5 (6-11); 20.9 ± 1.0 (15-25) and 34.8±4.4 (14-65) days.

Data (Table 4) showed that under the four temperatures of feeding, females lived longer than males. On the other hand, providing the parasitoid by its host in addition to honey showed the longest longevity, whether in males or females; being in respective 12.0 ± 2.2 and 34.8 ± 4.4 days. Around the same item, feeding on honey only followed the first regimes and later feeding on water; the respective figures were 11.5±2.2 , 20.9±1.0 and 6.1±0.6, 7.6±0.5 in males and females, respectively. However, starved, males and females lived for 5.2±0.7 and 7.5±0.7 days only.

Statistical analysis (F-test) of the data shows that adult's diet has a positive and highly significant effect on the longevity of unmated males and females. Considering LSD values, in case of males and females, the differences between feeding on honey and larvae plus honey lack significant. However, in case of feeding on larvae plus honey and starvation or water the differences were significant.

C- Effect of mating on adults longevity:

In mated females and males, longevity was 43.7±4.6 and 13.7±1.9 days, opposed to 34.8±4.4 and 17.6±2.2 days for unmated individuals (Table 5).

Statistical analysis of data (T test) show that mating has a positive significant effect on the longevity of the female, but has a highly significant negative effect on males.

Effect of host species on sex ratio:

Larvae of nine lepidopterous species were subjected to mated females of *G. legneri* at 30 ± 1 °C and 60 ± 5 % R.H. until emergence of adult parasitoids that were sexed to estimate the sex ratio for each case.

Data (Table 6) showed that the sex ratio in *G. legneri* is affected by the used host species. When, *Corcyra cephalonica*, *Heliothis armigera*,and *Pectinophora gossypiella* were used , sex ratio was 1 male : 4 females; being deviated significantly (Chi Square test) than ratios reported for other hosts that showed in significant variations in between ; ranging from

1: 3 to 1:3.8.

Host range in laboratory:

Nine lepidopterous species were used to rear *G. legneri* in the laboratory. For each host species, 20 larvae were confined individually and exposed to parasitoid mated females for a period of 24 hours. Results (Table 7) showed that all the 9 tested species were susceptible for parasitism , but with varied rates. Rates of paralyzed larvae ranged from 40 to 75 %, while those of successful parasitism were from 25 to 60 %. Highest rates of either paralysis or parasitism were reported for the two hosts *P. gossypiella* and *E. kuehniella*, while low rates were reported for *G. mellonella*, *C. gnidiella* and *P. operculella*.

Table (3): Effect of temperature on longevity of mated adults of *G. legneri* at 20, 25 and 30 °C and (60 ± 5 % R.H).

Treatments	Longevity (days) x ± SE	
	Males	Females
20 °C	46.9 ±1.9 (37-56)	91.7 ± 3.8 (78-120)
25 °C	17.3 ±2.4 (9-32)	59.0 ± 6.2 (36-90)
30 °C	13.7 ± 2.3 (7-24)	46.6 ±4.4 (23 -74)

Table (4): Effect of diet on longevity of unmated males and females of *G. legneri* at 30 ± 1 °C and (60 ± 5 % R.H.).

Treatments		Longevity (days) x ± SE	
		Males	Females
Starvation		5.2 ±0.7 (3-7)	7.5 ±0.7 (6 –10)
Water		6.1 ±0.6 (4-8)	7.6 ± 0.5 (6 -11)
Honey		11.5 ± 2.2 (2-22)	20.9 ±1.0 (15-25)
P. unionalis larvae + Honey		12.0 ±2.2 (7-22)	34.8 ±4.4 (14-65)
LSD	0.05	4.6	6.4
	0.01	6.1	8.6

Table (5): Longevity of mated and unmated (males and females) of *G. legneri* at 30± 1 °C and (60 ± 5 % R.H.).

Treatments	Longevity (days) X ± SE	
	Males	Females
Mated	13.7 ±1.9 (7-24)	43.7 ±4.6 (23-74)
Unmated	17.6 ±2.2 (7-28)	34.8 ±4.4 (14-65)

Table (6): Sex ratio of *G. legneri* reared in the laboratory on different host species at (30± 1 °C & 60 ± 5 % R.H.).

No.	Host species	Total no. of parasitoids	Males	Females	Sex ratio ♂:♀
1	*Phthorimaea operculella*	20	5	15	1:3
2	*Galleria mellonella*	20	5	15	1:3
3	*Cryptoplabes gnidiella*	18	4	14	1: 3.5
4	*Plodia interpunctella*	33	7	26	1: 3.7
5	*Palpita unionalis*	34	7	27	1 : 3.8
6	*Ephestia kuehniella*	34	7	27	1 : 3.8
7	*Corcyra cephalonica*	20	4	16	1 : 4
8	*Heliothis armigera*	25	5	20	1 : 4
9	*Pectinophora gossypiella*	25	5	20	1 : 4

Table (7): Host range of *G. legneri* at 30 ± 1 °C and (60 ± 5 % R.H.).

No.	Species of larvae	Total No. of exposed larvae	Total No. of paralyzed larvae	Paralyzed larvae %	Total no of larvae with successful parasitism	% Para-sitism
1	***Pectinophora gossypiella***	20	15	75	12	60
2	***Ephestia kuehniella***	20	15	75	11	55
3	***Corcyra cephalonica***	20	13	65	9	45
4	***Palpita unionalis***	20	13	65	9	45
5	***Plodia interpunctella***	20	12	60	8	40
6	***Heliothis armigers***	20	12	60	8	40
7	***Galleria mellonella***	20	8	40	5	25
8	***Cryptoplabes gnidiella***	20	8	40	5	25
9	***Phthorimaea operculella***	20	10	50	5	25

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