Biological Aspects of the Ecto-larval Parasitoid Species, *Goniozus legneri* Gordh (Hymenoptera: Bethylidae) on Different Insect Hosts under Laboratory Conditions

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ABSTRACT

Major biological aspects of the ecto-larval parasitoid, *Goniozus legneri* Gordh (Hymenoptera, Bethylidae) were studied under laboratory conditions using three different insect hosts; *Ephestia kuehniella* (Zeller), *Pectinophora gossypiella* (Saund.) and *Phthorimaea operculella* Zeller. These pest species were chosen as they can serve as laboratory rearing hosts for mass rearing of *G. legneri*. Three distinct larval instars for the parasitoid were determined. Total developmental period ranged between 13.61±0.61 and 14.83±0.48 days on different hosts. Longevity of parasitoid males was much shorter than the parasitoid females; it ranged between 1.67±0.70 and 2.67±0.70 days in males and 6.00±1.46 -20.60±3.93 days in females. Insignificant differences in fecundity of the parasitoid females were found among the tested hosts. It ranged between 42.07±8.91 and 44.67±10.59 eggs/female. Number of paralyzed and parasitized larvae by *G. legneri* female of different hosts was counted. *E. kuehniella* was the most suitable laboratory host for mass- rearing of *G. legneri*.

Key Words: *Goniozus legneri*, biology, duration, longevity, fecundity, different hosts.

INTRODUCTION

Goniozus legneri Gordh (Hymenoptera, Bethylidae) is a primary gregarious ecto-larval parasitoid for a wide range of full grown larvae of lepidopterous hosts. It was introduced to California, USA from South America, Uruguay and Argentina, for biological control of the navel orange worm, *Amyelois transitella* (Walker) in 1977. Both sexes were described by Gordh (1982) for the first time. The navel orange worm is the original host of *G. legneri* (Grodh, 1982; Legner, 1983 a,b; Butler & Schmidt,1985; Hendricks,1995; Flint & Dreistadt,1998). Legner and Warkentin (1988) released *G. legneri* on almond to reduce *A. transitella*. Abbas (1999) reared *G. legneri* on its original host *A. transitella* in two rearing trails under laboratory conditions. *G. legneri* has been recorded also attacking the carob moth, *Ectomyelois ceratoniae* Zell. (Butler & Schmidt, 1985; Sarhan, 1989; Kaschef *et. al.,* 2002) and the pink bollworm, *Pectinophora gossypiella* (Saund.) (Butler & Schmidt, 1985).

Through the Egyptian-American collaborative National Agricultural Research Project (NARP) entitled "Cotton Integrated Pest Management with Emphasis on Biological Control of Pink Bollworm" which was carried out during 1991-1994, certain exotic parasitoid species were imported from Australia to provide additional mortality factors beside the indigenous parasitoid complex of pink bollworm. In Egypt as well in the USA. *G. legneri* was one of those exotic parasitoid species. The species was mass- reared successfully under the laboratory conditions at the Department of Biological Control (DBC), Agricultural Research Center (ARC), Giza, Egypt (Ellington and El- Heneidy, 1994).

In Egypt, *Perisierola* (= *Goniozus*) sp. was recorded as an external parasitoid of *P. gossypiella* (Hekal 1974 and Farrag 1976). In 1988, *G. legneri* was imported into Egypt from the USA and reared on the larvae of *Ephestia kuehniella* (Zeller) under laboratory conditions (Sarhan (1989). Abul Fadl (2001) and Kaschef *et. al.*, (2002) recorded that *G. legneri* parasitized *Ephestia cautella* (Walker) and *E. calidella* (Guenne) on date palm trees.

In general, *G. legneri* plays an important role for controlling navel orange worm, pink bollworm, codling moth, carob moth, oasis date moth and fig (almond) moth on their host plants as almonds, cotton, walnuts carob trees, dates, loquat, pecans, pistachio, prunes and most stored products (Abul Fadl, 2001).

The aim of this study is to highlight some of the major biological aspects of the larval parasitoid *G. legneri*, using three different insect hosts under laboratory conditions.

MATERIALS AND METHODS

Three insect hosts; *E. kuehniella*, *P. gossypiella* and *Ph. operculella* Zeller were used in the study. These pest species were chosen as they can serve as laboratory rearing hosts for mass rearing of *G. legneri*. All the experimental studies were carried out under the constant laboratory conditions of 27±1°C and 60±5 %R. H.

1-Rearing of insect hosts

a) *Ephestia kuehniella* (Zell.)

The rearing material (wheat) was sterilized at 200°C for 1–2 hours. After being in equilibrium with the atmospheric air, 1 gm of eggs of the host (obtained from DBC, ARC, Giza) was mixed into a quantity of 500 gm of the wheat flour placed into 2 kg plastic jars. The jars were examined daily till eggs hatching. After 3 weeks, larvae developed to pupae. Pupae were collected and placed into another jar till emergence of adults. The emerged adults were collected by means of an aspirator, connected to a vacuum pressure pump. Collected moths were placed in Zinc cylinder (21 X 11 cm) with top Zinc cover which has a hole to put moths inside the cylinder. The cylinder bottom was covered with mesh cloth to allow the drop of eggs, which were received in a half Petri-dish placed under the Zinc cylinder. Similar technique was applied by Daumal *et. al.* 1973. Rearing took place in a conditioned room at 22±1°C and 60±5% R.H.

b) *Pectinophora gossypiella* (Saund.)

To secure large numbers of the pink boll worm, *P. gossypiella*, moths and larvae in the laboratory, fresh larvae were collected from the cotton bolls during the cotton season and kept in plastic trays, 13x18 cm, with green cotton bolls in each tray till pupation. Pupae were

placed in glass vials, 4x11cm till emergence of adults. The emerged adults were transferred into 2 kg. plastic jars by means of an aspirator, the moths were provided with pieces of cotton moistened with honey solution. Eggs were deposited on tissue papers hanged inside the jars which were collected daily. Larvae were reared on the green cotton bolls until reaching maturity. Rearing took place during the cotton season in a conditioned room at 25±1°C and 60 ±5% R.H.

c) *Phthorimaea operculella* Zeller

To secure the large number of the potato tuber-moths and larvae in laboratory, the rearing method described by Abbas (1981) was followed. Establishment of a laboratory stock culture of *Ph. operculella* began with larvae collected from infested potato field. The larvae were kept in glass jars, 13 x 7 cm, with a small potato tuber, as food, in each jar, until pupation. Pupae were placed in glass vials,
7x2 cm., 20 pupae each, till the emergence of adults. The emerged adults were put in an oviposition cage. The moths were provided with a piece of cotton wool moistened with 10% honey solution. Eggs were deposited on the black sheets of cotton cloth which were removed daily. Larvae were reared on pieces of potato tubers until reaching maturity. Rearing took place in a conditioned rearing room at 25±1°C and 60 ±5 % R. H.

II- Rearing of the parasitoid, *Goniozus legneri* Gordh

Rearing method of G. *legneri* was described by Abul Fadl (2001). Parasitoid adults were obtained from the laboratory rearing stock culture of the parasitoid at DBC, ARC, Giza by means of an aspirator. Every three females and one male were confined together for mating in a plastic container (7x6 cm) covered with muslin and kept in position by means of rubber band. Droplets of honey on a paper stripes were added as food. After four days, five full grown larvae of *Ephestia* sp. were introduced to each confined mated parasitoid female in a Petri-dish (10 cm in diameter) provided with droplets of honey scattered on the inner surface of the lid. The female was transferred every 48 hours to a similar dish containing another five fresh full-grown larvae for egg deposition.

Biological studies

a- Determination of the duration for parasitoid immature stages

Parasitoid's immature stages were observed under dissecting stereo-microscope using a very fine needle and brush in drops of Ringer's solution to determine the duration of each stage (25 replicates for each stage were used). The study was carried out on the three host larvae of *E. kuehniella, P. gossypiella* and *P. operculella* at constant temperature of 27±1°C and 60 ±5% R. H.

b- Adult longevity

Longevity of *G. legneri* adults was determined for mated and unmated males and females reared on different diets at 27±1°C and 60±5% R.H. Twenty-five males and females were used in each case.

c- Fecundity and ovipositional periods of *G. legneri*

Ovipositional periods were estimated for 25 mated females at 27±1°Ç and 60±5% R. H. on two insect hosts only; *E. kuehniella* and *P. gossypiella* larvae. Each female was confined with 5 full grown larvae of both species in a Petri-dish (9 cm in diameter) with droplets of honey scattered on the inner surface of lid for 24 hrs till oviposition. The parasitized larvae of both species were replaced with similar number of larvae and examined daily for counting deposited eggs; number of the trials for oviposition/female was counted until death of the female. Number of paralyzed host larvae of each species was also counted.

Statistical analysis

T-test was used for analyzing the data of fecundity and ovipositional period experiments while F-test was used for analyzing the data for durations of immature stages and adult longevity experiments.

RESULTS AND DISCUSSION

Durations of immature stages:

Three distinct larval instars for the parasitoid, *G. legneri* were determined. The early first larval instar is of apodous hymenopterous form. It was difficult to distinguish between the egg and a newly hatched instar larva, it remained static by feeding. The parasitoid larvae usually make feeding punctures in the host integument with their mandibles, before feeding. Body of the second larval instar, is creamy in color, movements of internal organs can be seen. The parasitoid larva steadily increased in size and the host larva was relatively alert, based on its response to a sharp probe and microscopic observation of haemolymph flow and contraction of the circulatory system. Body of the third larval instar, was yellowish, the host was more sluggish and flow of the haemolymph was spasmodic. Successful development appeared contingent upon the host remaining alive. The parasitoid's larvae detached their mandibles from host's integument and began to spin their cocoons. Afterwards, the host became moribund and mummified when parasitoid larval feeding completed and the host died.

a- Incubation period (Egg stage)

The shortest incubation period averaged, 1.640±0.040 days, was obtained in case of *P. gossypiella* and the longest (2.190±0.045) days was in case of *E. kuehniella.* While in case of *Ph. operculella*, it was 1.800±0.040 days. Statistical analysis (F-test) showed a significant effect on the eggs incubation period of *G. legneri* among the three different host larvae (Table 1).

b- Larval stage

Average durations of the three larval instars and the total duration of the larval stage are presented in Table (1). The shortest duration of the total larval period (2.200±0.11 days) was obtained in case of *E. kuehniella* and the longest (2.990±0.120 days) was in case of *Ph. operculella.* While in case of *P. gossypiella*, it was 2.270±0.097 days. Statistical analysis (F-Test) showed a significant effect on the total duration of larval stage among the three host larvae of different species.

c- Pre–pupal stage

Average durations of the pre-pupal stages are presented in Table (1). The shortest period (0.730±0.045 day) was recorded in case of *E. kuehniella* and the longest (0.840±0.059 days) was in case of *Ph. operculella.* While in case of *P. gossypiella*, it was 0.810±0.050 day. Statistical analysis (F-Test) showed

a significant effect on the duration of the pre-pupal stage in relation to the three tested host species.

d- Pupal period

Average durations of the pupal period are presented in Table (1). The shortest value (8.500±0.490 days) was recorded in case of *E. kuehniella* and the longest duration (9.330±0.470 days) was recorded in case of *P. gossypiella*. While in case of *Ph. operculella*, it was 9.220±0.400 days. Statistical analysis (F-Test) showed a significant effect on the duration of the pupal stage among the host larvae of the tested three species.

e- Total developmental period

Total developmental periods of the parasitoid averages are presented in Table (1). The shortest duration (13.610±0.610 days) was obtained in case of *E. kuehniella* and the longest duration (14.830±0.480 days) was in case of *Ph. operculella*; meanwhile, it was 14.400±0.500 days in case of *P. gossypiella*. Statistical analysis (F-Test) showed a significant effect on total developmental period among the three different host larvae.

Obtained results are in agreement with the findings of Butler & Schmidt (1985) who determined the developmental periods of *G. legneri* on *P. gossypiella* at nine constant and three fluctuating temperatures. The duration from egg to pre-pupal stage varied from 12.8 days to 3.1 days at 17° C and 35° C, respectively. The pupal period required an additional 35 days and 5 days at 17° C and 35° C, respectively. Sarhan (1989) studied the development of different stages of *G. legneri* on the larvae of *E. kuehniella* at four constant temperatures. The duration from egg to pre-pupal stage varied from 14.2 days to 3.3 days at 17° C and 35° C, respectively. Pupal period varied from 37.1 days at 17°C to 6.0 days at 35°C.

Abbas (1999) studied the duration of different immature stages of *G. legneri* on larvae of the navel orange worm, *A. transitella* and found that the average egg incubation period was 24 hrs, the larval stage lasted 3.1 days. The pre-pupal stage lasted 26 hrs, and the pupal stage lasted 6.7 days. The total developmental period of the parasitoid averaged 11.3 days; the study was carried out under 27±2°C and 50-55% R.H. Abul Fadl (2001) studied the developmental periods of different immature stages of G. *legneri* on *Ephestia cautella* larvae, at three constant temperatures and 60±5% R.H. and found that the egg incubation period varied from 3.3 days at 20±1°C to 0.92 days at 30±1°C. The duration of the larval stage (3 larval instars) ranged from 3.9 days at 20±1°C to 1.9 days at 30±1°C. The duration of the pre-pupa varied from 1.7 days at 20±1°C to 0.56 days at 30±1°C. The pupal stage duration ranged from 23.9 days at 20±1°C to 6.7 days at 30±1°C. Meanwhile, the total developmental period ranged between 32.8 days at 20±1°C and 10.1 days at 30±1°C.

Adult longevity:

Data obtained are presented in Table (2). Longevity of adults varied significantly according to different diet regimes. Longevity of parasitoid males was much shorter than the parasitoid females. Although the sex ratio of the parasitoid adults was not estimated but it was obviously in favor of the females. The mean mated male longevity values were 1.67±0.70 and 2.67±0.70 days at starved and honey solution feeding diets, respectively. Meanwhile, the longevity of unmated male on the same diets lasted 1.53±0.72 and 2.33±0.70 days, respectively. Correspondent values of the mated females were 6.73±1.53 and 15.20±2.04 days at starved and honey solution feeding cases, respectively; while they were 6.00±1.46 days and 15.20±2.01 days for the unmated female, respectively.

Statistical analysis (F-Test) of data showed that significant differences appeared among the longevities of both males and females on different diet regimes. Insignificant differences were found between mated and unmated individuals of each sex (Table 2).

Obtained results agreed with those reported by Sarhan (1989) and Abul Fadl (2001) but disagreed with the records of Abbas (1999). Sarhan (1989) reported that the average longevities of *G. legneri* females were 18.2 days at 30°C and 13.3 days at 35°C. Abbas (1999) recoded 69.4 days at 27°C. Abul Fadl (2001) mentioned that the longevity of males ranged between 2.8 days at 20°C and 1.6 days at 35°C (male longevity was less than 4 days). Longevity of females varied from 46.27 days at 20°C to 11.47 days at 35°C. In case of the feeding adults at 25°C, the longevity of starved male was 1.33 days while that of male fed on honey solution was 2.67 days. The longevity of starved female was 8.53 days, extended to 21.73 and 29.33 days when fed on honey solution and honey solution + haemolymph, respectively.

Fecundity and ovipositional periods of *G. legneri*

The parasitoid usually paralyzes its host larvae before oviposition. The female parasitoid encounters the posterior end of the host, tries to sting its end, but it always attacks the first thoracic segment (Fig.1). After that, the female leaves the host and begins grooming. Female parasitoid has to sting more than one host larva per day and has to feed on the oozing haemolymph of these host larvae before oviposition. The female roams about its paralyzed host larvae; when it comes so near it stops and oscillation of antennae becomes evident. Then, the female darts towards the host larva and probates the most suitable ovipositing region. The female prefers to lay its eggs on a dorsal orientation (Fig. 2).

Fig. (1): The parasitoid female attacks *P. gossypiella*

Fig. (2): Development of immature stages of *G. legneri* on *P. gossypiella*

Data presented in Table (3) indicate that the mean duration of pre-ovipositional period of the parasitoid female on *E. kuehniella* larvae (2.60±1.02 days) was shorter than that on *P. gossypiella* (3.13±0.88 days). The mean duration of ovipositional period of the female parasitized *E. kuehniella* larvae (16.13±3.42 days) was longer than that on *P. gossypiella* (14.93±2.69 days). The mean duration of post-ovipositional period of the female

Table (1): Durations of the developmental stages of *G. legneri* on different host larvae under the laboratory conditions 27±1°C and 60±5% R. H.

Stage	Duration (days)			
	E. kuehniella	*P. gossypiella*	*Ph. operculella*	L .S. D. 0.05
Incubation period	2.190±0.045 A (2.13-2.25)	1.640±0.040 B (1.58-1.71)	1.800±0.040C (1.75-1.88)	0.110
1st larval instar	0.870±0.058 (0.79-0.96)	1.083±0.043 (1.00-1.17)	1.200±0.094 (1.083-1.380)	-
2nd larval instar	0.730±0.046 (0.67-0.79)	0.920±0.041 (0.83-1.00)	1.040±0.034 (1.00-1.08)	-
3rd larval instar	0.560±0.050 (0.46-0.63)	0.710±0.048 (0.63-0.79)	0.730±0.032 (0.71-0.79)	-
Total larval period	2.200±0.11 A (1.96- 2.29)	2.270±0.097 B (2.58-2.95)	2.990±0.120C (2.79-3.35)	0.051
Pre-pupa	0.730±0.045 A (0.67-0.79)	0.810±0.050 B (0.71-0.88)	0.840±0.059C (0.75-0.95)	0.027
Pupa	8.500±0.490 A (8.00-9.00)	9.330±0.470 B (9.00—10.00)	9.220±0.400C (9.00-10.00)	0.098
Total developmental period	13.610±0.610 A (12.88-14.24)	14.400±0.500 B (13.92-15.21)	14.830±0.480C (14.29-15.88)	0.402

Table (2): Longevity of *G. legneri* adults under different diet regimes at 27 ± 1°C and 60 ± 5% R. H.

Diet	Male		Female	
	Mated	Unmated	Mated	Unmated
Starved	1.67±0.70 A (1.0-3.0)	1.53±0.72 A (1.0-3.0)	6.73±1.53 A (5.0-9.0)	6.00±1.46 A (4.0-8.0)
Honey	2.67±0.70 B (2.0-4.0)	2.33±0.70 B (1.0-3.0)	15.20±2.04 B (13.0-20.0)	15.20±2.01 B (13.0-19.0)
Honey + haemolymph of larvae	2.67±0.70 B (2.0-4.0)		20.60±3.93 C (15.0-27.0)	
L.S.D. 0.05	0.360		4.930	

Table (3): Ovipositional periods, numbers of paralyzed and parasitized host larvae and fecundity on larvae of different hosts at 27 ±1°C and 60 ± 5% R. H.

Host larvae	Ovipositional period in days			Numbers of paralyzed host larvae	Numbers of parasitized host larvae	Numbers of eggs / female
	Pre-	Ovi.	Post-			
E. kuehniella	2.60±1.02 (2.0-5.0)	16.13±3.42 (10.0-21.0)	1.93±1.23 (0.0-4.0)	22.67±4.01 (17.0-31.0)	8.67±2.10 (6.0-13.0)	44.67±10.59 (29.0-62.0)
P. gossypiella	3.13±0.88 (2.0-5.0)	14.93±2.69 (10.0-19.0)	2.20±1.38 (1.0-5.0)	22.20±4.23 (14.0-29.0)	7.80±2.10 (5.0-12.0)	42.07±8.91 (27.0-59.0)

Table (4): Rate of oviposition in female parasitized host larvae per day and per time

Host	Ovipositional period	No. of parasitized eggs/ female/ day	No. of times for deposition eggs/ life	No. of deposited eggs/ time
E. kuehniella	16.13±3.42 (10.0-21.0)	2.80±0.17 (2.0-3.0)	6.90±1.60 (4.0-9.0)	6.40±0.72 (4.0-7.0)
P. gossypiella	14.93±2.69 (10.0-19.0)	2.80±0.44 (1.0-4.0)	6.50±1.40 (4.0-9.0)	6.50±0.58 (5.0-8.0)

parasitized *E. kuehniella* larvae (1.93±1.23 days) was shorter than that on *P. gossypiella* (2.20±1.38 days). The total number of eggs deposited per female averaged 44.67±10.59 eggs on *E. kuehniella* larvae which was more than that on *P. gossypiella* (42.07±8.91 eggs). Females did not lay eggs daily. The total number of paralyzed host larvae by the female of *G. legneri* was 22.67±4.01 for *E. kuehniella* and 22.20±4.23 for *P. gossypiella* larvae. The total number of parasitized host larvae by the female *G. legneri* was 8.67±2.10 *on E. kuehniella* and 7.80±2.10 on *P. gossypiella* larvae.

Statistical analysis (T-Test) showed that different host larvae had an insignificant effect on the ovipositional period, rate of oviposition or the number of paralyzed or parasitized host larvae.

Data in Table (4) indicate that the average number of eggs laid by parasitoid female was 2.80±0.17 eggs/day among 16.13±3.42 days in case of *E. kuehniella* and 2.80±0.44 eggs/day among 14.93±2.69 days in case of *P. gossypiella* throughout its oviposition period. The mated female laid eggs 6.90±1.60 times, with an average of 6.40±0.72 eggs each time on *E. kuehniella* larvae, while it laid eggs 6.50±1.40 times, with an average of 6.50±0.58 eggs each time on *P. gossypiella* larvae, through its life span. Insignificant effect on the number of egg-laying times or daily number of eggs laid on different host larvae was found.

Obtained results agreed with that reported by Butler and Schmidt (1985), Sarhan (1989) and Abul Fadl (2001) but disagreed with the records of Abbas (1999). Butler and Schmidt (1985) found that the female of *G. legneri* parasitized about 10.9 larvae producing 67.6 cocoons. Sarhan (1998) determined the number of larvae parasitized per female *G. legneri* as 2.2 larvae, 5.1 larvae, 7.6 larvae and 8.1 larvae of *E. kuehniella* at 17°C, 25°C, 30°C and 35°C, respectively. Abbas (1999) found that *G. legneri* female laid eggs 21.5 times during its life span, with an average of 13.4/ female and total number of eggs laid per female was 260.8 eggs. He also reported that the averages of the pre-oviposition, oviposition and post-oviposition periods were 4.4, 63.6 and 6.8 days at 27°C. Abul Fadl (2001) reported that the means of pre-ovipositional, ovipositional and post ovipositional periods of the *G. legneri* were 3.6, 26.27 and 2.2 days at 25°C. The daily mean number of eggs/female was 4.81 eggs while the mean total number of eggs laid/ female was 126.4 eggs at 25°C.

It could be concluded that *E. kuehniella* is the most suitable laboratory host for mass-rearing of *G. legneri. Ephestia* is used commonly as laboratory host for mass-rearing of several parasitoid species, mainly *Trichogramma* spp. On the other hand, *G. legneri* is recommended as a primary parasitoid species for serious insect pest species such as the pink bollworm and potato tuber moth larvae under field and store conditions.

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