LIFE AND FERTILITY TABLES FOR THE CITRUS LEAFMINER PARASITOIDS, *CIRROSPILUS INGENUUS* GAHAN AND *PNIGALIO* SP.

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Abstract

Life and fertility tables for the parasitoids, *Cirrospilus ingenuus* Gahan and *Pnigalio* sp. on the citrus leafminer (CLM), *Phyllocnistis citrella* Stainton were conducted to study some of their biological aspects under laboratory conditions. The average egg incubation, larval and pupal periods for the parasitoid, *C. ingenuus* were 1.59±0.42, 3.85±0.57 and 4.04±0.57days, respectively, while these aspects were 1.00±0.25, 2.87±0.38 and 4.48±0.29 days for *Pnigalio* sp.. Pre-oviposition, oviposition and post-oviposition periods of these two parasitoids lasted 1.80±0.92 & 1.20±0.79, 8.90±0.88 & 5.60±0.84 and 1.90±0.74 & 1.50±0.53 days, respectively. *C. ingenuus* adult female longevity was twice (12.60±0.95 days) that of males (6.90±0.99 days). While, it was once and half (8.30±1.06 days) that of *Pnigalio* sp. males (5.40±1.07). Egg-laying behavior of the parasitoid females approved that both parasitoid species are synovigenic females. The average of fecundity were 30.90±3.81 and 52.30±13.13 eggs / female for *C. ingenuus* and *Pnigalio* sp.. The net reproductive rate (Ro), intrinsic rate of natural increase (r_m) and generation time (T) of the parasitoids, *C. ingenuus* and *Pnigalio* sp. and their host (CLM) were (14.64, 22.45 and 15.67), (0.18, 0.26 and 0.15) and (15.19, 11.80 and 18.84), respectively.

INTRODUCTION

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera; Gracillariidae) is an important pest of citrus orchards in several countries of the world (Heppner, 1995 and Smith & Hoy, 1995). In Egypt, CLM was found in July 1994 in many citrus orchards and nurseries in eastern side and middle of Nile Delta (Hashem, 1996 and Abo-Sheaesha, 1997) and eleven annual generations were recorded (Abdel-Rahman, 1998).

Citrus nursery production systems are especially susceptible to leafminer damage (Villanueva-Jimenez and Hoy, 1998). The damage is directly related to the ratio of the young leaves and the total canopy of the young trees (Argov and Rossler, 1998).

Parasitoids provide an opportunity for significant reduction in CLM populations (Batra and Sandhu, 1981; Browning and Pena, 1995 La Salle, 1996 and Schauff *et al.*, 1998). Many of these are indigenous parasitoids which have moved over onto *P. citrella* as it has spread (La Salle and Pena, 1997). Several of these species appeared to be capable of exerting substantial level of control (Ateyyat, 2002).

The parasitoids, *C. ingenuus* and *Pnigalio* sp. were the most numerous parasitoids attacking CLM in several governorates of Egypt (Hashem, 1996; Eid, 1998; Moustafa, 1999 and Hammad & Jasmin, 2000).

For the success of integrated pest management control program it is essential to know several information concerning the biology of the pest and their dominant natural enemies. The intrinsic rate of natural increase (r_m) is necessary to describe the growth potential of a population under given climatic and food conditions. It is an important parameter in inductive strategic and management models for insect pest population (Southwood, 1978).

So, the present work aims to know some biological aspects for the parasitoids, *C. ingenuus* and *Pnigalio* sp. on their host (CLM) by constructing survival and fertility life tables.

MATERIALS AND METHODS

To study the biology of the parasitoids, *C. ingenuus* and *Pnigalio* sp., a laboratory culture of each parasitoid as well as their insect host (*P. citrella*) was maintained under laboratory conditions.

1- Rearing techniques :

i- Host rearing technique :

The CLM was obtained in the pupal stage from the citrus orchard of the Experimental Farm of the Faculty of Agriculture, Mansoura University.

These pupae were collected in late June. Pairs of male and female pupae were deposited in 5 cm diameter Petri-dishes, with a piece of moistened cotton wool on a filter paper in the dish bottom, and kept until emergence. As the moths oviposited only on very young and tender leaves (Zhang *et al.* 1994), young navel orange seedlings planted in plastic pots were prepared for oviposition. Each seedling had a stem of about 1.0 cm in diameter and 50.0 cm in height. To have a source of *P. citrella* larvae homogenous in age, CLM females were released into a cylinder screen-cages (0.4 m in diameter and one meter in higher). Each cage contained three navel orange seedlings.

ii- Parasitoids rearing technique :

The two tested parasitoids, *C. ingenuus* and *Pnigalio* sp. were collected from their host, *P. citrella* on navel orange leaves in pupal stage. The pupae of each parasitoid species were sexed and kept into Petri-dishes (5 cm diameter) having suitable moisture until adult emergence.

To start a culture of each parasitoid species, pairs of parasitoid adults were introduced into caged navel orange seedlings infested with CLM for oviposition.

2- Parasitoids life and fertility table studies :

To have initial populations of *C. ingenuus* or *Pnigalio* sp. homogenous in age, pairs of parasitoid adults were introduced for a period of 12 hours into the cylinder screen cages. Each cage contained a three navel orange seedling having tender leaves infested with CLM 3rd instar larvae.

An initial population of each parasitoid was started as 200 newly deposited eggs (homogenous in age) on navel orange seedling under laboratory conditions. For each parasitoid species the eggs were tested individually by using lens (10x magnification) until hatching. The resulted larvae were inspected daily until pupation. The numbers of dead individuals of each stage were recorded daily and the duration of each stage was calculated. The pupae were sexed and kept in Petri-dishes (5 cm diameter) until emergence of parasitoid adults. The number of emerged adults were recorded.

To study the fertility table, thirty pairs of the newly emerged parasitoid adults were used. Each pair was confined with 15 larvae (third instar larvae) of the host in a glass test-tube (5 cm in diameter X 20 cm length) for oviposition, which provided with a piece of cotton wool moistened with 10 % honey solution to serve as parasitic food. Every 24 hours, the navel orange leaves carrying CLM parasitized larvae were removed and renewed by fresh larvae of the host and soon until the death of the parasitoid adults. The number of eggs laid per female was counted and recorded daily. In addition to the pre-oviposition, oviposition and post-oviposition periods were recorded.

3- CLM life table :

An initial population was started as 500 eggs deposited on ten seedlings under laboratory conditions by using a lens with 10X magnification. The daily numbers of dead individuals of each stage were recorded and the duration of each stage was calculated. When the eggs developed to the pupal stage, the resulting pupae were sexed and kept in the previously mentioned Petri-dishes until adult emergence. The number of emerged adults and the adult longevity were recorded.

To study the fertility table, newly emerged thirty pairs of moths were caged, each pair in a cage containing navel orange flushes for oviposition. The daily number of eggs laid per female was recorded.

To estimate the stage-specific survival (I_X) and the intrinsic rate of natural increase (r_m), life and age-specific fecundity tables were constructed as follow:

Life table was constructed with the following columns:

x : The pivotal age for the age class in days.

Ix : The number of survivals at the beginning of age class x.

dx : The number of deaths during the age interval x.

Qx : The mortality percentage per age interval as the rate per thousand.

Age specific fecundity table was constructed with the following columns:

x : Actual female age (time from egg stage).

m_X : The number of living females born per female in each age.

L_X : Represented the fraction surviving of females of an initial population of one.

The parameters, net reproductive rate (Ro), mean generation time (T) and the intrinsic rate of increase (r_m) were calculated according to Southwood (1978) as follow :

$$Ro = \Sigma L_X m_X$$

$$T = \Sigma x (L_X m_X) / Ro$$

$$r_m = In (Ro) / T$$

RESULTS

1- Life table of the parasitoids, *C. ingenuus* and *Pnigalio* sp. :

The age specific survival (Tables, 1 and 2) were studied for the parasitoids, *C. ingenuus* and *Pnigalio* sp. reared on navel orange seedlings infested with CLM larvae under laboratory conditions based on an initial cohort of 200 eggs.

1-1- *C. ingenuus* :

The obtained data represented in Table (1) indicate that the total developmental time was 9.48 days. The larval mortality was the highest among all stages; it was 0.299, 0.230 and 0.216 for larval, egg and pupal stages, respectively.

Egg incubation period is relatively short (1.59±0.42 days) whereas larval and pupal development takes about 3.85±0.57 and 4.04±0.57 days, respectively. The developmental period of *C. ingenuus* is relatively long. *C. ingenuus* completes its entire development from oviposition to emergence in 9.48 days (Table, 1). The percentage of adult emergence was 78.40±3.52% from pupa (Table, 3).

Pre-oviposition, oviposition and post-ovipostion periods of *C. ingenuus* lasted 1.80±0.92, 8.90±0.88 and 1.90±0.74 days, respectively. As shown in Table (6), *C. ingenuus* adult female longevity was twice (12.60±0.95 days) that of males (6.90±0.99 days). While, life span of parasitoid female and male was 22.08 and 16.38 days.

1-2- Pnigalio sp. :

The obtained data represented in Table (2) indicate that the total developmental time was 8.35 days. The mortality (Q_X) was highest among egg stage (0.293) followed by the pupal stage (0.288) while the larval mortality was the lowest one (0.278).

Egg incubation period is very short (1.00±0.25 days) whereas larval and pupal development takes about 2.87±0.38 and 4.48±0.29 days, respectively. The developmental period of *Pnigalio* sp. is relatively short. *Pnigalio* sp. completes its entire development from oviposition to emergence in 8.35 days. The percentage of adult emergence from the pupal stage was 71.20±3.27% (Table, 3).

Pre-oviposition, oviposition and post-oviposition periods of *Pnigalio* sp. lasted 1.20±0.79, 5.60±0.84 and 1.50±0.53 days, respectively. *Pnigalio* sp. adult female longevity was once and half approximately (8.30±1.06 days) that of males

(5.40±1.07 days). While, life span of parasitoid female and male was 16.65 and 13.75 days.

2- Egg laying behavior of the parasitoids :

In respect to egg laying behavior of *C. ingenuus* female, as shown in Fig. (1), the pre-oviposition period is short in *C. ingenuus* and the daily ovipostion rate is approximately the same throughout the oviposition period (2.58 / female / day). So, the *C. ingenuus* female is synovigenic eggs developing and maturing in the ovaries continuously through much of the life span. The average number of deposited eggs was 30.90±3.81 per female (Table, 6).

With respect to *Pnigalio* sp. female (Fig. 1), the pre-oviposition period is short and the daily ovipostion rate was 5.80 / female / day. The average number of deposited eggs was 52.30±13.13 per female (Table, 6).

3- The age-specific fecundity tables of the parasitoids (*C. ingenuus* and *Pnigalio* sp.) and their CLM host :

The net reproductive rate (Ro), intrinsic rate of natural increase (r_m) and generation time (T) of the parasitoids, *C. ingenuus* and *Pnigalio* sp. as well as their host (CLM) were calculated and recorded in Tables 4, 5 and 6, respectively.

3-1- C. ingenuus :

As shown in Tables (4 and 6), the estimated values of the biological parameters, Ro, T and r_m were 14.64, 0.18 and 15.19 for the parasitoid, *C. ingenuus* and 15.67, 18.84 and 0.15 for CLM, respectively.

The obtained data indicated that the parasitoid, *C. ingenuus* have one and quarter generation to one of the host (Tables, 4 and 6). These results are very important for the potential rate of parasitoid increase. However, the parasitoid in nature may produce 14.64 female progeny per female parent per generation (Ro). Whereas, the host CLM will approximately produce 15.67 females progeny per female parent per generation. Consequently, the parasitoid can potentially produce 29 female progeny during the period of one generation of the host.

The intrinsic rate of natural increase (r_m) of the parasitoid was 1.2 times that of the host.

3-2- *Pnigalio* sp. :

The biological characters, Ro, T and r_m of the parasitoid, *Pnigalio* sp. were 22.45, 11.80 and 0.26 days. The obtained data obviously indicated that the parasitoid, *Pnigalio* sp. have one and half generation to one of the host (Tables, 5 and 6). These results are very important for the potential rate of parasitoid increase.

However, the parasitoid in nature may produce 22.45 female progeny per female parent per generation (Ro). Whereas, the host CLM will approximately produce 15.67 females progeny per female parent per generation. Consequently, the parasitoid can potentially produce 149 female progeny during the period of one generation of the host.

The intrinsic rate of natural increase (r_m) of the parasitoid was 1.7 times that of the host.

DISCUSSION

Biological studies under laboratory conditions indicates that the average duration of all developmental stages of the parasitoid, *Pnigalio* sp. was (8.35 days) lower than *C. ingenuus* (9.48 days). At 30°C, the total developmental time of *Pnigalio* sp from egg to adult was 8.6 days (Pena, 1996). While, it was 14.0 days (Hoy & Nguyen, 1994) and 12.43 days (Ateyyat & Mustafa, 2002) for *C. ingenuus.*

Egg-laying behavior of the parasitoid females approved that both parasitoid species are synovigenic especially *C. ingenuus* females. This means that egg developing and maturing in the ovaries continuously during the female life span. Therefore, the parasitoids feed on a number of CLM larvae by predatory host-feed (Hoy &Nguyen, 1994; Browning & Pena, 1995 and Lo Pinto & Salerno, 1998).

In the present study, the average fecundity of *C. ingenuus* was 30.90±3.81 eggs / female. Similar results (29.75 eggs / female) were obtained by Ateyyat & Mustafa (2002). On the contrary, the obtained results disagreed with Ding *et al.* (1989), they recorded high fecundity of *C. ingenuus* females (47.5 eggs).

Pena (1996) mentioned that *Pnigalio* females are able to lay about 186 eggs; while, the average fecundity in the present investigation was only 52.3±13.13 eggs / female.

The present results indicated that the number of emerged parasitoids females outnumbered that of males especially *C. ingenuus.* Similar results were obtained on *C. ingenuus* (Argov & Rossler, 1996; Moustafa 1999 and Ateyyat & Mustafa, 2002) and

Pnigalio sp. (Moustafa 1999). On contrary, the number of males was relatively higher than females of *C. ingenuus* (Ding *et al.*, 1989) and *Pnigalio* sp. (Vercher *et al.*, 1997).

According to Ateyyat & Mustafa (2002), differences in the biological aspects of parasitoids with previous studies could be attributed to one or more of the following; the strain of CLM, the used host plants and may be there was a difference in the comparison of honey that was fed to parasitoid adults.

Life and fecundity tables data cleared that the generation time (T) of both parasitoids are lower than the CLM. The net reproductive rate (Ro) and the intrinsic rate of natural increase (r_m) of the parasitoids are higher than that recorded for the CLM. Similar conclusion were obtained by Radke & Kandalkar (1987) and Batra & Sandhu (1989).

Generally, it could be concluded that the eulophid parasitoids have played a major role in eminently successful biological control projects directed against CLM populations. They have some of the most important attributes of effective natural enemies {i.e. life cycle is considerably shorter than the host, usually capable of inflicting high mortality in host population, have an excellent net reproductive rate (Ro) and intrinsic rate of increase (r_m)}. Therefore, *Pnigalio* sp. appears to offer good prospects for biological control of CLM on citrus orchards, in comparison with *C. ingenuus*.

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Table (1): Life table of *C. ingenuus* immature stages reared on navel orange seedlings under laboratory conditions.

Stage	**X**	I_x	d_x	Q_x
Eggs	1.59±0.42	200	46	0.230
Larvae	3.85±0.57	154	46	0.299
Pupae	4.04±0.57	108	23	0.216

Table (2): Life table of *Pnigalio* sp. immature stages reared on navel orange seedlings under laboratory conditions.

Stage	X	I_x	d_x	Q_x
Eggs	1.00±0.25	200	58	0.293
Larvae	2.87±0.38	142	40	0.278
Pupae	4.48±0.29	102	10	0.288

Table (3): Some biological aspects of the parasitoids, *C. ingenuus* and *Pnigalio* sp. reared under laboratory conditions.

Biological aspects	*C. ingenuus*	*Pnigalio* sp.
Adult male longevity (days)	6.90±0.99	5.40±1.07
Adult female longevity (days):	12.60±0.95	8.30±1.06
- Pre-ovipostion period	1.80±0.92	1.20±0.79
- Ovipostion period	8.90±0.88	5.60±0.84
- Post-ovipostion period	1.90±0.74	1.50±0.53
Life span of female (days)	22.08	16.65
Life span of male (days)	16.38	13.75
No. of progeny / female	30.90±3.81	52.30±13.13
Adult emergence (%)	78.40±3.52	71.20±3.27
Sex ratio% (F : M)	1.94 : 1	1.44 : 1

Table (4): Fertility table for *C. ingenuus* reared on navel orange seedlings under laboratory conditions (29±3.5°C and 66±5.5 R.H.%).

X	L_x	m_x	$L_x\, m_x$
9.5	0.744	0.066	0.049
10.5	0.744	0.462	0.344
11.5	0.744	1.518	1.129
12.5	0.744	2.574	1.915
13.5	0.744	2.047	1.523
14.5	0.744	2.244	1.669
15.5	0.744	2.376	1.768
16.5	0.744	2.244	1.669
17.5	0.744	2.574	1.915
18.5	0.744	2.706	2.013
19.5	0.670	0.924	0.619
20.5	0.447	0.066	0.030
21.5	0.149	0.000	0.000
22.5	0.000	0.000	0.000

calculated parameters were as follow :
Ro = 14.64 T = 15.19 r_m = 0.18

Table (5): Fertility table for *Pnigalio* sp. reared on navel orange seedlings under laboratory conditions (29±3.5°C and 66±5.5 R.H.%).

X	L_x	m_x	$L_x\, m_x$
8.4	0.709	0.335	0.252
9.4	0.709	2.056	1.458
10.4	0.709	5.105	3.619
11.4	0.709	6.594	4.675
12.4	0.709	8.012	5.681
13.4	0.709	6.239	4.423
14.4	0.496	3.869	1.919
15.4	0.354	1.168	0.413
16.4	0.071	0.078	0.006
17.4	0.000	0.000	0.000

The calculated parameters were as follow : Ro = 22.45
T = 11.80 r_m = 0.26

Table (6): Life and fertility tables for *P. citrella* reared on navel orange seedlings under laboratory conditions (29±3.5°C and 66±5.5 R.H.%).

X	L_x	m_x	$L_x\, m_x$
0	Immature stages		
-			
-			
-			
16.5	0.602	0.0	0.0
17.5	0.550	10.25	5.64
18.5	0.550	8.30	4.57
19.5	0.445	8.40	3.74
20.5	0.445	2.45	1.09
21.5	0.356	1.80	0.64
22.5	0.150	0.0	0.0

The calculated parameters were as follow : Ro = 15.67
T = 18.84 r_m = 0.15

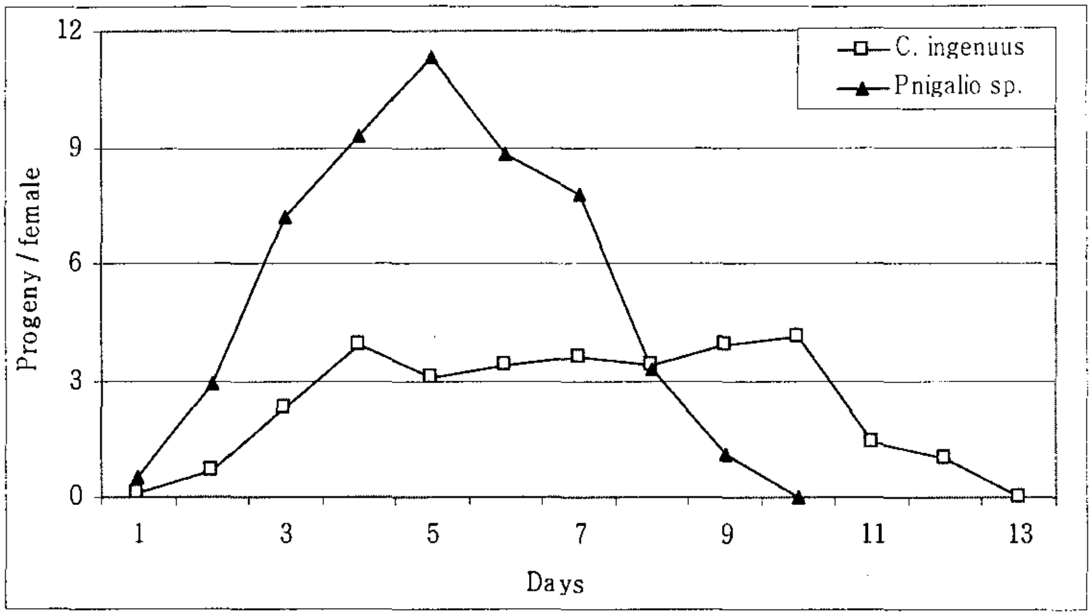

Fig. (1). Daily oviposition rates (No. of eggs / female / day) in *C. ingenuus* and *Pnigalio* sp. females under laboratory conditions.

جداول الحياة الخاصة بالعمر والخصوبة لنوعين من طفيليات صانعة أنفاق أوراق الموالح *Cirrospilus ingenuus* Gahan و *Pnigalio* sp.

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تم دراسة جداول الحياة الخاصة بالعمر والخصوبة لنوعين من طفيليات صانعة انفاق أوراق الموالح *Phyllocnistis citrella* Stanton هما *Cirrospilus ingenuus* Gahan و *Pnigalio* sp. وذلك لمعرفة بعض الخصائص البيولوجية الهامة لهما تحت الظروف المعملية. حيث أوضحت النتائج أن فترة حضانة البيض وطول فترة حياة كل من العمر اليرقي والعذراء لطفيل *C. ingenuus* بلغت ١,٥٩±٢,٤٠ ، ٣,٨٥±٠,٥٧ و ٤,٠٤±٠,٥٧ يوم على التوالي في حين كانت ١,٠±٠,٢٥ ، ٢,٨٧±٠,٣٨ و ٤,٤٨±٠,٢٩ يوم على التوالي لطفيل *Pnigalio* sp. . فترة ما قبل وضع البيض ، وضع البيض وما بعد وضع البيض للطفيل *C. ingenuus* بلغت ١,٨٠±٠,٩٢ ، ٨,٩٠±٠,٨٨ و ١,٩٠±٠,٧٤ وللطفيل *Pnigalio* sp. كانت ١,٢٠±٠,٧٩ ، ٥,٦٠±٠,٨٤ و ١,٥٠±٠,٥٣ يوم على التوالي . كما أوضحت النتائج أن طول فترة حياة أنثى طفيل *C. ingenuus* كانت ضعف (١٢,٦٠±٠,٩٥ يوم) طول فترة حياة الذكر (٦,٩٠±٠,٩٩) بينما كان طول فترة حياة أنثى طفيل *Pnigalio* sp. (٨,٣٠±١,٠٦) يعادل بمقدار مرة ونصف طول فترة حياة الذكر (٥,٤٠±١,٠٧) .كما أوضح سلوك وضع البيض أن إناث كلا الطفيليين من النوع Synovigenic . كما بلغ معدل الخصوبة ٣٠,٩٠±٣,٨١ و ٥٢,٣٠±١٣,١٣ بيضة / أنثى للطفيل *C. ingenuus* و *Pnigalio* sp. . بلغ صافي معدل خصوبة الإناث (Ro) ومعدل التزايد الطبيعي (r_m) ومدة الجيل (T) لكلا الطفيليين وعائلهما نحو (١٤,٦٤ ، ٢٢,٤٥ و ١٥,٦٧) ، (٠,١٨ ، ٠,٢٦ و ٠,١٥) و (١٥,١٩ ، ١١,٨٠ و ١٨,٨٤) على التوالي .