On the Biology of *Dirhinus giffardii* (Silvestri) (Hymenoptera: Chalcididae) Parasitizing Pupae of the Peach Fruit Fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) in Egypt

El-Husseini[*], M. M.; E. A. Agamy[*]; M. H. Saafan[**] and Walaa M. Abd El-Khalek[**]

[*]Center of Biological Control & IPM, Faculty of Agriculture, Cairo University,Giza,Egypt

[**]Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

(Received: November 23 and Accepted: December 21, 2008)

ABSTRACT

Biology of the pupal parasitoid, *Dirhinus giffardii* (Silvestri) was studied in its host, the peach fruit fly, *Bactrocera zonata* (Saunders) under laboratory conditions of 25-27 C and 50-60% relative humidity. All immature stages were described. The egg incubation period lasted only 24 hrs; it measured 14.8±3.05μ in width and 42.1± 3.07 μ in length. The first larval instar measured 30.2±7.1 and 77.5±7.84 μ in width and length with a stable duration of 2 days. The 2nd larval instar measured 98±24.97 and 197±26.58 μ in width and length, respectively. Its duration lasted between 1-2 days with an average of 1.2±0.42 days. Third larval instar measured 129±13.7 and 374±53.79 μ in width and length, respectively. It showed longer duration period compared to the previous instars ranging between 3-4 days with an average of 3.3±0.48 days. Prepual stage measured 196±13.5 and 371±18.53 μ in width and length, respectively and lasted always one day before turning slowly into pupa. The well developed pupa measured 175±8.5 and 416.5±27.9 μ in width and length, respectively. The pupal period averaged 9.5±0.71 days. Longevity of adult male reached 19.4±5.29 and 3.2±0.96 days under feeding and starvation conditions, respectively. Meanwhile, females lived for 19.73 ±9.80 and 4.14 ±0.95 days under such respective conditions. Fecundity recorded 43.07±18.50 eggs /female, while no eggs were laid under starvation.

Key Words: *Bactrocera zonata, Dirhinus giffardii*, parasitoid, biology.

INTRODUCTION

In the last two decades, the peach fruit fly, *Bactrocera zonata* (Saunders) has become a destructive pest on different fruits in Egypt (Al-Eryan *et al.*,2005 and Afia *et al.*,2005). It was first recorded by Efflaton (1924) and El-Ghawabi (1928).Its potentiality to develop into a serious economic pest in Egypt was stated by El-Menshawy *et al.* (1999). Recent strategies of IPM for controlling tephritid fruit flies in Egypt support the use of classical biological control including augmentation and preservation of their natural enemies, beside male sterile technique and mating disruption by sex pheromones.

Biological control of fruit flies in Egypt dated back to introduction of the braconid *Diachaseva tryorii* in 1934 and 1936 followed by the eulophid *Tetrastichus giffardianus* in 1938; both from Hawaii (Sarhan.1981 and Tawfik, 2002). The pteromalid pupal parasitoid *Spalangia afra* was recorded by Fetoh (2003) and an unidentified chalcidid was recorded by Fetoh *et al.*(2004).

The chalcidid pupal parasitoid, *Dirhinus giffardii* (Silvestri) recorded in the present study was originally described from Nigeria by Silvestri (1914) after introduction in Hawaii from West Africa (Silvestri 1914; Clausen *et al.* 1965, Wharton 1989 and Purcell 1998).This parasitoid is a native species in West Africa where its original host is *C.capitata* (Silvestri, 1914 and Dresner, 1954). It has been introduced in many countries, mainly in the Pacific and Central American regions (Noyes, 2002). Its introduction in Hawaii 1912 targeted the control of *Ceratitis capitata,* and in Italy 1913 targeted the olive fruit fly, *Bactrocera oleae* (Gmelin) (Silvestri, 1914; Clausen *et al.,* 1965 and Wharton, 1989). In 1968, it was introduced from Hawaii into Israel for controlling *C.capitata* (Rivany, 1968). Also, the parasitoid was introduced to control other tephritid fruit fly pests belong to the genera of *Anastrepha, Bactrocera, Ceratitis* and *Dacus* in Australia, Central America, Pakistan, and Samoa, respectively (Rivany, 1989). Rather than tephritid fruit fly species, *D.giffardii* successfully parasitized pupae of the house flies of family Muscidae (Noyes, 2002). Purcel (1998) reported that this parasitoid has become established in Hawaii, but its importance has never been documented.

Recording *D.giffardii* in the present study in Egypt emerging from pupae of *C. capitata* and *B. zonata* collected from soil of different orchards, is most probably due to its introduction from Hawaii to Israel earlier than 1968 (Rivany, 1968), where it was established there and crossed the border through Sinai; the same way parasitoids of the citrus leaf miner that were recently recorded in Egypt.

Although the use of classical biological control against endemic and exotic agricultural insect pests still of increasing interest, the biology of certain

biocontrol agents from the parasitoids is not fully studied. *D.giffardii* was recorded as a generalist pupal ectoparasitod inside puparia of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) and other tephritid species (Podoler and Mazor, 1981a & 1981b). Also, *D.giffardii* acted as a hyperparastoid on the primary braconid larval-pupal parasitoids; *Diacasmimorpha craussii* Fullaway and *Psyttalia concolor* (Szépligeti) in *C.capitata* and *Bactrocera latifrons* (Hendel), respectively (Wang and Messing, 2004b).

Although *D.giffardii* gained such an important role in control programs of fruit flies world wide, only few of its biological aspects have been documented (Dresner, 1954; Podoler and Mazor, 1981a, and Wang and Messing, 2004a). Thus, the present study aims to shed more light on the biology of this parasitoid when parasitizing pupae of *B.zonata* in the laboratory.

MATERIALS AND METHODES

Rearing of *Bactrocera zonata*

Larvae of the peach fruit fly were reared in the laboratory on the semi-synthetic diet described by Afia *et al.*(2005) with the slight modification using molasses instead of sugar by the same amount. Full grown larvae were separated and placed in trays furnished with a thin layer of sand (2-3 cm) for pupation. The fly culture was maintained according to Afia *et al.*(2005).

Rearing of *Dirhinus giffardii*

Adult parasitoids were collected by an aspirator from soil surface under infested peach trees from orchards at the Horticulture Research Institute in El-Kanater area, Qualubia governorate. Adult parasitoids (males and females) were introduced to large numbers of newly formed pupae of *B.zonata* placed in a half-liter glass jar covered by cloth secured with a rubber band. Jars were provided each with a piece of cotton wool soaked with bee honey in water (1:1), as food for the parasitoid adults. Host pupae were renewed every other day with fresh formed ones. Pupae previously exposed to parasitism were kept in glass jars under the same laboratory conditions of room temperature (25-27 °C) and relative humidity (50-60%) until emergence of parasitoid adults.

Biological parameters of *D.giffardii*

A large number of newly formed *B.zanata* pupae were once exposed for 24 hours to large number of mated *D.giffardii* females for oviposition. At least, 30 pupae were sampled daily for dissection in saline solution under stereobinocular microscope to locate, measure, describe and calculate durations of egg stage, different larval instars, prepupal and pupal stages of the parasitoid each in one replicate of 10 individuals. Thus, a variable number of sampled pupae were dissected each time; the rest was returned back to the parasitized stock of pupae.

Emerged parasitoid adults were left for mating; thereafter each couple was confined in a half-liter glass jar and provided with 25 newly formed pupae of *G.giffardii.* Each jar was provided with a piece of cotton wool soaked with diluted bee honey as previously described for adult feeding. The host pupae were replaced daily with new ones until death of the adult parasitoid female. From the daily exposed host pupae in relation to the emerged parasitoid adults from them, preoviposition, oviposition, and postoviposition periods as well as fecundity were calculated. Also, adult longevity was recorded under feeding on diluted bee honey in water (1:1) and under starvation.

RESULTS AND DISCUSSION

Egg stage:

Eggs of *D.giffardii* are minute, elongated in shape and slightly narrower at one end (Fig.1). They were inserted by female ovipositor through the puparial wall and placed externally on the inside pupa. When dissecting freshly parasitized pupae of *B.zonata,* the developed parasitoid embryo could be easily seen through the translucent thin egg shell with its head at the broader egg end. The chorion bears no surface sculpturing. The egg measurements averaged 14.8±3.05μ in width and 42.1± 3.07 μ in length (Table 1). Eggs hatched always after 24 hours from deposition on the host pupae and thus showed a stable incubation period of only one day (Table 2). The parasitoid female inserts only one egg in each attacked host pupa. Thus, the parasitoid female is able to differentiate between parasitized and non parasitized pupae of *B.zonata.*

Larval stage:

First-instar larva (L_1)

It is of the hymenopteran mandibulate type, with a large triangular head to support the mandibular muscles. The larva shows 13 body segments, which usually protruded laterally to both sides (Fig.2a). By mounting L_1 in Hoyer's medium, the hooked mandibles became visible, and also the main lateral tracheae appeared with branches reaching the lateral protruded segments of the body (Fig.2 b). It seems that these protrusions resemble the locations of the

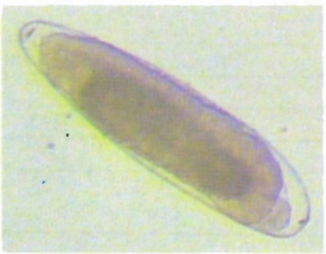

Fig. (1): Newly laid egg with well developed embryo

Fig. (2a): First larval instar with filled gut

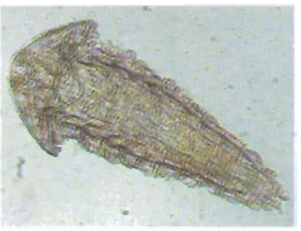

Fig. (2b): Mounted L_1 in Hoyer's medium

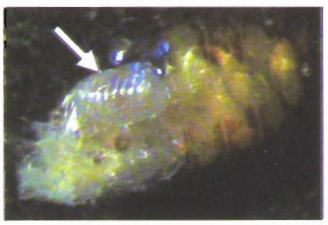

Fig. (3a): L_2 feeding on pupa inside the puparium.

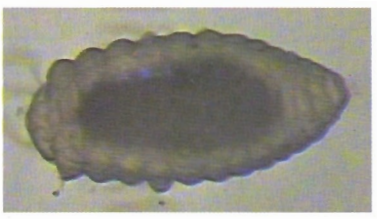

Fig. (3b): Second instar larva.

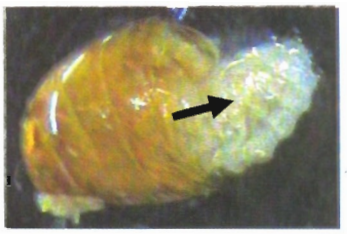

Fig. (4a): L_3 filling the puparium.

Fig. (4b): Full-grown L_3.

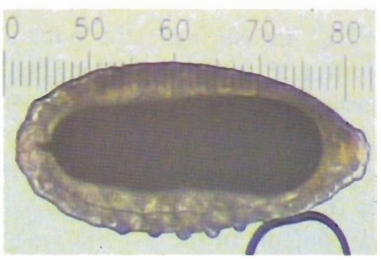

Fig. (4c): L3 in Hoyer's medium with filled closed gut.

Fig. (5a): Meconium ball and early pupal formation.

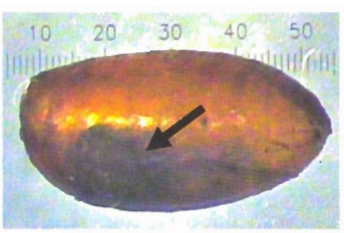

Fig. (5b): Meconium seen through puparium.

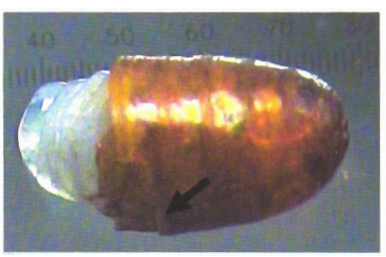

Fig. (5c): Prepupa and the spilled dark meconium.

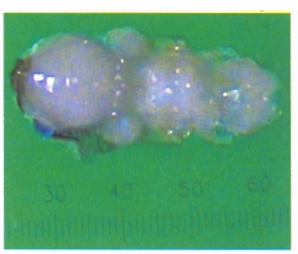

Fig. (6a): Incomplete pupa with characteristic head.

Fig. (6b): Parasitoid pupa inside puparium of the host.

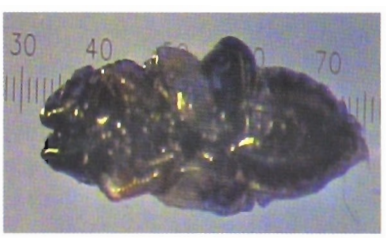

Fig. (6c): Complete developed pupa of *D.giffardii* with its characteristic black color of the adults.

Table (1): Width (W) and length (L) measurements (/μ) for immature stages of *G.giffardii* developed in pupae of *B.zonata.*

Meas.	Egg		L1		L2		L3		Prepupa		Pupa	
	W	L	W	L	W	L	W	L	W	L	W	L
Min.	12	40	24	70	70	180	100	300	180	360	160	360
Max.	20	48	40	88	135	235	140	450	230	310	180	350
Mean ±S.D.	14.8 ±3.05	42.1 ±3.07	33.8 ±7.1	77.5 ±7.84	98 ±24.97	197 ±26.58	129 ±13.7	374 ±53.79	196 ±13.5	371 ±18.53	175 ±8.5	416.5 ±27.9

Table (2): Durations (/days) for immatures of *G.giffardii* developed in pupae of *B.zanata.*

Values	Incubation period	L1	L2	L3	Prepupa	Pupa	Total
Min.	1	2	1	3	1	9	17
Max.	1	2	2	4	1	11	21
Average	1±0.0	2±0.0	1.2±0.42	3.3±0.48	1±0.0	9.5±0.71	18±0.94

vestigial spiracles of the larva. On the average, L_1 measured 30.2±7.1 and 77.5±7.84 μ in width and length, respectively (Table 1) showing a stable duration of 2 days (Table 2).

Second-instar larva (L_2)

It is obviously larger than L_1, measuring 98±24.97 and 197±26.58 μ in width and length, respectively (Table 1). Its duration lasted between 1-2 days, with an average of 1.2±0.42 days (Table 2). It shows a shiny white color due to color of inside tissues especially the adipose tissues (Fig.3a); but it is somewhat translucent that the dark contents of the closed gut could be easily discerned (Fig.3b). The duration of L_2 ranged between 1-2 days, with an average of 1.2±0.42 days (Table 2).

Third-instar larva (L_3)

Developed L_3 occupies nearly the entire volume inside the puparium replacing the host pupa (Fig.4a). The body is shiny with clear white opacity in color which allow pinpointing the dorsal heart chambers (Fig.4b). The dark content of the gut is difficult to be seen through the heavy formed white colored fat bodies; but mounting in Hoyer's medium facilitate observation of the gut content (Fig.4c). As long as the gut is still closed and the larva did not reach the phase of opening the hind-gut spilling the meconium out, no other sign appears through the puparial cuticle. The 3rd larval instar measured 129±13.7 and 374±53.79 μ in width and length, respectively (Table1). It showed longer duration period compared to the previous instars ranging between 3-4 days, with an average of 3.3±0.48 days (Table2).

Prepupal stage:

Completing the larval development, the hind-gut of 3rd instar larva becomes opened and the remaining gut contents are spilled out forming the meconium. Freshly spilled meconium is shaped spherical (Fig. 5a) prior to pressing towards the inside puparial wall by the movement of the 3rd instar during this process to give the required space for subsequent stages. This phase was easily identified with the formation of a dark black area that appears inside the puparium and can easily be seen through the puparial wall (Fig.5b). The prepua is white in color (Fig.5c) allowing no inside organs to be seen and measured 196±13.5 and 371±18.53 μ in width and length, respectively (Table1). This stage lasted always one day before turning slowly into pupa (Table2).

Pupal stage:

At the early beginning of this stage, only the outlines of a white colored pupal body can be recognized (Fig.5a). By the time, details of the body started to appear with the appendages still fused to the body; and the characteristic deep antennal concavity formed by two ridges extending out from the face became visible (Fig.6a) bearing the same white color. Further developed, the body appendages became well defined separated from the body turning slowly shiny black in color (Figs. 6b & c). The full developed pupa measured 175±8.5 and 416.5±27.9 μ in width and length, respectively (Table 1). The pupal period ranged between 9-11 days, with an average of 9.5±0.71 days (Table2).

Total developmental period of immature stages:

The present study revealed that the total developmental period of the immature stages of *D. giffardii* under the previously described laboratory conditions ranged between 17-21 days, with an average of 18±0.94 days. Longer developmental period was recorded when parasitizing pupae of *C. capitata* ranging between 23-35 days as reported by Wang and Messing (2004a) under laboratory conditions of 23±1°C and 65±10% R.H.

Table (3): Longevity (/days) of *G. giffardii* adults under feeding and starvation conditions.

Values	Fed adults		Starved adults	
	♀	♂	♀	♂
Min.	2	7	3	2
Max.	32	24	6	5
Mean ±S.D.	19.73 ± 9.80	19.40 ±5.29	4.14 ±0.95	3.20 ±0.96

Table (4): Number of eggs/female *D.giffardii* under feeding and starvation conditions

Values	Fed females	Starved females
Min.	13	0
Max.	58	1
Mean ± S.D.	43.07±18.50	0.57±0.51

Adult longevity:

Female longevity ranged between 2 to 32 with an average of 19.73±9.80 days under feeding condition, compared to 3-6 days that averaged 4.14 ±0.95 days when starved (Table 3). Meanwhile, longevity of males averaged 19.4±5.29 and 3.2±0.96 days under conditions of feeding and starvation, respectively.

Fecundity

Under feeding conditions, fecundity of *D.giffardii* female ranged between 13-58 eggs with an average of 43.07±18.50 eggs/female (Table 4). The variability in numbers of eggs laid by the females may be attributed to the variability in their size that is related to size of the parasitized host pupa. It was observed that large sized pupae of *B. zonata* produced larger adults of *D. giffardii.* Such observation agreed with that of Wang and Messing (2004b) who added that the females prefer to attack larger host pupae when given the choice, resulting in larger parasitoid offspring without affecting developmental time or survival. Also, this low fecundity level was mentioned by Podoler and Mazor (1981a) and Wang and Messing (2004a).

On the other hand, almost no eggs were laid by starved (Table 4). Thus, the availability of nectar at the surrounding natural environment is essential for *G. giffardii* adults to precede oogenesis and succeed in parasitizing its host in soil (dipteran pupae).

Previous descriptions and durations concerning the immature stages of *D. giffardii* parasitizing pupae of the peach fruit fly, *B. zonata* were not available in the literature. Few authors (Dresner, 1954; Podoler and Mazor, 1981a, and Wang and Messing, 2004a&b) reported some biological aspects of *D. giffardii* in pupae of other tephritid hosts, *e.g., B.dorsalis* and *C. apitata.*

REFERENCES

Al-Eryan, M. A. S.; Ramadan, H. M. and Salem, M. H. 2005. Pest status of peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera:Tiphritidae) at northern coast of Egypt. Proceeding of the 3rd Conference of Applied Entomology, Cairo, Egypt, pp 55-68.

Afia, Y. I; M. A.El-Sheikh; A. G.Hashem and S. El-Nagar. 2005. Influence of different constant temperatures on the biological aspects of two fruit flies (Diptera:Tiphritidae) in Egtpt. I. On survival and developmental rates of immature stages. Egypt. J. Appl. Sci., 20(7):257-276.

Clausen, C. P. D. W. Clancy and Q.C.Chock. 1965. Biological control of the oriental fruit fly *dacus dorsalis* (Hendel) and other fruit flies in Hawaii. Tech.Bulletin 1322.U.S.Dept.Agric., Wachington DC.

Dresner, E. 1954. Observations on the biology and habits of pupal parasites of the oriental fruit fly *Dacus dorsalis* (Hendel) and other fruit flies in Hawaii. Tech. Bulletin 1322, USDA, Washington, DC.

Efflaton, H. C. 1924. A monograph of Egyptian Tryponeidae. Mem. Soc. Roy. Entomol. Egypte, 2:1-132.

El-Ghawabi, A. 1928. The Mediterranean fruit fly. Agric.J.Egypt: 111-136, Series, Ministr,Agric., G. Press, Cairo, Egypt.

El-Menshawy, A. M.; Al-Eryan, M. A. S. and Awad, A. I. 1999.Biological and morphological studies on the guava fruit fly, *Bactrocera zonata* (Diptera:Tephritidae) found recently in Egypt. 8th Nat.Conf.of Pests & Dis. of Vegetables & Fruits, Ismalia, Egypt,71-82.

Fetoh, B. A. and Y. I. Afia.2004. New recrds for parasitoidspecies on the curcurbit fruit fly, *Dacus ciliatus* (Loew) and the peach fruit fly, *Bactrocer zonata* (Saunders), Diptera: Tephritidae) in Egypt. Egypt. J. Biol. Pest Control, 14(2): 425.

Fetoh, B. A. 2003. Recent record of parasitoid species of cucurbit fly, *Dacus ciliatus* (Loew) (Diptera: Tephritidae) in Egypt. Egypt.J.Biol.Pest Control, 13(1&2): 127.

Noyes, J. S. 2002. Interactive catalogue of world Chalcidoidea 2001. Compact disc., Taxapad, Vancouver, Canada.

Podoler, H. and M. Mazor. 1981a. *Dirhinus giffardii* Silvestri (Hym.: Chalcididae) as a parasite of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera:Tephritidae). I. Some biological studies. Acta Oecol., 2:255-265.

Podoler, H. and M. Mazor. 1981b. *Dirhinus giffardii* Silvestri (Hym.: Chalcididae) as a parasite of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera:Tephritidae). II. Analysis of parasite response. Acta Oecol., 2: 299-309.

Purcel, M. F. 1998.Contribution of the biological control to integrated pest management of tephritid fruit flies in the tropic and subtropics. Integr. Pest Manag. Rev. 3: 63-83.

Rivany, E. 1968. Biological control of pests in Israel (a review 1905-1965). Isr. J. Entomol., 3:1-156.

Sarhan, A. A. A. 1981. Studies on the natural enemies of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.). Ph.D. thesis, Faculty of Agriculture, Cairo University.

Silvestri, F. 1914. Reports of an expedition to Africa in search of natural enemies of fruit flies (Trypaneidae). Territory of Hawaii Board of Agriculture and Forestry Bulletin 3:1-146.

Tawfik, M. F. S. 2002. Biological control of insect pests in Egypt. Egyptian Society for Biological Control of Pests, pp.45.

Wang X. G. and R. H. Messing. 2004a. Potential interaction between pupal and egg- or larval-pupal parasitoids of tephritid fruit flies. Environ. Entomol, 33(5):1313-1320.

Wang X. G. and R. H. Messing. 2004b.Two different life-history strategies determine the competitive outcome between *Dirhinus giffardii* (Chalcididae) and *Pachycreboideus vindemmiae* (Pteromalidae), ectoparasitoids of cyclorrhaphous Diptera. Bull. Ent. Res. 94:473-480.

Wharton, R. A. 1989. Classical biological control of fruit Tephritidae, pp.303-313. In A.Robinson and G.Harper (eds.), World crop pests, fruit flies: their biology, natural enemies, and control, vol.3b. Elsevier, Amsterdam.