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Biological Aspects of the Peach Fruit Fly, *Bactrocera zonata* (Saund.) (Diptera: Tephritidae) and its Parasitoid Species, *Aganaspis daci* Weld. (Hymenoptera: Eucoilidae)

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ABSTRACT

The peach fruit fly (PFF), *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is one of the serious tephritid insect pests attacking tropical and subtropical fruits. In Egypt, the pest is present and widespread over most of the Egyptian governorates causing serious problems to many fruit crops. The present study aimed to shed light on some biological aspects of different stages of the PFF and its exotic parasitoid species, *Aganaspis daci* Weld. (Hymenoptera: Eucoilidae) under laboratory conditions. Biological studies included; durations of different immature stages, fecundity, longevity, survival rate and sex ratio. All studies were carried out under the laboratory conditions of 25±2ºC, 54-65% R.H. and 14:10 L: D photoperiod. Total developmental period of PFF (from egg deposition to adult emergence) averaged 18.7 days, while that of the parasitoid was 23.3 days. The PFF female required 13-21days post emergence to start laying eggs and the total number of eggs/ female reached 235.72 eggs. In case of *A. daci*, its fecundity averaged 39.57 eggs/ female (32-55 eggs). Sex ratio (female: male) was 1.2: 1 in PFF and 1: 1 in *A. daci.* Fed female and male longevity attained 50.6 and 47.3 days in PFF and 18.8 and 17.3 days in *A. daci*, respectively.

Key words: Biological aspects, Peach fruit fly, *Bactrocera zonata* (Saund.), Parasitoids, *Aganaspis daci* Weld.

INTRODUCTION

The dipteran family Tephritidae consists of over 4000 species, of which nearly 700 species belong to Dacine fruit flies (Fletcher, 1987). Nearly 250 species are of economic importance, and distributed widely in temperate, sub-tropical and tropical regions of the world (Christenson and Foote, 1960).

The peach fruit fly (PFF), *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is one of the serious insect pests attacking tropical and subtropical fruits (Fletcher, 1987). This pest is originally an Indian species, firstly reported from Bengal. It is now widespread over India, Pakistan, Nepal, Bangladesh, Srilanka, Burma and evidently over southeast Asia. In Egypt, the pest has widespread over most of the Egyptian governorates causing serious damages to many fruit crops. *B. zonata* has caused an estimation of 190 million EUR damage a year in Egypt (OEPP/EPPO, 2005).

B. zonata has been recorded on over 50 cultivated and wild plant species, mainly those with fleshy fruits. The main host plants of *B. zonata* are guava, mango and peach. Secondary hosts include apricot, fig and citrus. It has been frequently recovered from peach, hence, it is called the peach fruit fly and also due to its serious damage to guava, it is called "guava fruit fly" (Hussain, 1995).

In the last decade, many ecological and biological aspects of the pest have been studied to reach proper methods for its control. In Egypt, most researchers initially reared PFF on natural hosts specially, guava fruits to study its biology (Mohamed, 2000). Then, artificial diet for its mass rearing was used to obtain large production form eggs and larvae. In 2003, El-Gendy reared larvae of *B. zonata* on wheat bran artificial diet and different host plants and compared durations of different stages and larval mortality. He reported that the shortest larval duration was recorded on guava, while the longest occurred on sour orange in comparison to artificial diet. Amin (2003) evaluated two larval diets, the first one which was reported by Awadallah (1978) and the second was mentioned by Qureshi *et al.,* (1974) depending upon durations and survival percentage of immature stages. El–Naggar (2004) recorded longest incubation period and hatchability percentages on apple and the shortest was on peach. He mentioned that the longest pupal duration and pupal mortality percentages of *B. zonata* were on apple, peach, guava and apricot, respectively. The PFF was reared at different constant temperatures (15-40 ºC and 60-70% R.H) by Afia (2007) who studied the incubation period, larval duration, lower threshold of larval development and average thermal units required under different temperatures. Farag (2009) studied some biological aspects of PFF different stages under three constant temperatures (20, 25 and 30±1ºC) to estimate heat-unit requirements necessary for the development of different stages to complete one generation.

The pest problem of *B. zonata* in Egypt is a classical example of an invasive species that is accidentally moved from Asia to the African continent without its specific natural enemies. Therefore, the fly increased without check and became a pest. Parasitoids are one of the most potential and effective biocontrol agents of

controlling the fruit flies everywhere. Thus, introduction of Asian parasitoids from the native region of the pest to be evaluated in Egypt was considered. Therefore, the parasitoid species, *Aganaspis daci* Weld. (Hymenoptera: Eucoilidae) was introduced to Egypt, as a native species of the pest from South-East Asia.

The present study was carried out to shed light on some biological aspects of different stages of *B. zonata* as well its parasitoid species, *A. daci* under laboratory conditions.

MATERIALS AND METHODS

Rearing of *B. zonata*

Flies of *B. zonata* used in the present study were obtained from a laboratory stock culture at the Biological Control Dept., Plant Protection Research Institute, Agricultural Research Center at Giza, Egypt. Rearing technique of *B. zonata* was more or less similar to that of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), but with some variations in the ingredients of the artificial larval diet. The rearing was carried out under laboratory conditions of 25±2 ºC., 54-65% R.H. and 14: 10 L: D photoperiod. Eggs of the PFF were collected daily from special egg-laying collector and placed on an artificial diet in plastic trays covered with cloth lids, placed in an incubator at 25±1ºC and left for hatching and larval development. Third larval instars were transferred to trays furnished with a thin layer of sand for pupation. Pupae were sieved from the sand and transferred to adult cages till emergence. Adult flies were reared in cages (35 x 30 x 30 cm).

Flies were encouraged to lay eggs by providing them with egg collectors of plastic mandarins, perforated from the upper part and contained water inside to keep moisture for eggs. Eggs were collected daily and reared on larval artificial diet, the same for the Medfly, (described by Boller, 1985), but with some modifications as follows: 1000 g short wheat , 250 g molasses or (300 g sugar), 250 g yeast , 10 g sodium benzoate, 1ml Conc. HCL (or 20 ml HCL 2N), and 300 ml water.

Adult flies were fed mainly on sources of sugar, protein and water. Cotton wools soaked with nutritional material served as food source. For protein requirement, mixture of sugar and protein hydrolyzed enzymatic (3: 1, respectively) and besides an artificial diet was provided. This diet was described by Masood *et al*., (2006), as follows: 2 pieces Bananas, 6 pieces Egg yolk, 4 table spoon Honey, 2 table spoon Vit. B. Complex, 1table spoon Yeast, and 8 table spoon Sugar. Ingredients were mixed in a blender to make a thick syrup solution. For further use, the diet was kept in the refrigerator.

Rearing of the parasitoid *A. daci*

The parasitoid was reared successfully for several generations on *B. zonata* under the laboratory conditions of 26±2ºC, 60±5% RH and photoperiod 14: 10 light and dark. Second and third instars (3-7 d old) were exposed to the parasitoid in ovipositional units. Parasitization units were introduced to the cages (35 x30 x 30 cm) and provided with undiluted bee honey and water, as source of food.

The parasitoid was allowed to parasitize *B. zonata* larvae in two different types of parasitization units according to the host age. Small host larvae, 2-3 day old, were provided in larval diet in plastic trays. After parasitization, these units were transferred to transparent jars and covered for maturation. Large larvae, 4-7 day old, were exposed in modified Petri-dishes, tightly fitting with organdy lids. After exposure of larvae to the parasitoid for 24-48 h, pupae were collected into a plastic jar contained in its bottom a thin sand layer and covered with tightly fitting organdy or closes lid until adult emergence. The jars were maintained under the laboratory conditions (25±2˚C, 60±5% RH and photoperiod 14:10 light and dark).

Biological Studies

1- B. zonata

1.1. Durations of immature stages

Thirty newly deposited eggs (as replicates) were collected from the rearing colony and used as starters for the biological studies. Each individual egg was placed on artificial medium in a small Petri dish and covered with transparent organdy cloth. Petri dishes were incubated at 25±1ºC. The eggs were observed daily until hatching. Larvae were left to complete their development inside the medium until they occur on the surface of the medium and jump out from the Petri dishes to pupate in the sand.

1.2. Fecundity

Twenty mated females of PFF were used as replicates until their death. Plastic mandarins (have holes in their sides and provided with water) were placed in small cages containing small pieces of the mixture of sugar and protein hydrolyzate and a source of water served as oviposition site. Daily number of eggs laid by each female was counted. Pre–ovipositional, ovipositional and post–ovipositional periods was estimated.

1.3. Survival and Sex ratio

Hundred newly formed pupae were collected and placed into a plastic jar covered with organdy until adult emergence. Survival rate was estimated. Numbers of emerged adults were counted. The emerged adults were sexed to estimate the sex ratio.

1.4. Longevity

Newly formed pupae were placed individually into small glass vials until adult emergence. Two

groups (30 pairs; males and females each) were left to complete their life span; the 1st group was left unfed while the 2nd group was fed by a mixture of sugar and protein hydrolyzate and a source of water. Longevity of each sex was estimated.

All biological studies were carried out under the above mentioned laboratory conditions of 25 ± 2ºC, 54- 65% R.H. and 14 : 10 L : D photoperiod.

2- *A. daci*

2.1. Life cycle

Second instar of *B. zonata* larvae, 3 day old, was used to study the life cycle and development of immature stages of *A. daci*. The host larvae were exposed once for 24 hours to a large number of mated *A. daci* females for oviposition. Small host larvae were presented to the parasitoid in a small thin layer of larval diet in a glass Petri dish (9 cm diameter), contained 100 host larvae exposed to 3 parasitoid females for 6 hr. This number of host and time of exposure was used to avoid super-parasitism as reported by Nunez-Bueno (1982). The exposure was carried out for several times to get large numbers of parasitized host to use them in the dissection also, as replicates. Obtained data were recorded.

In order to observe the parasitoid's development, parasitized hosts were dissected every 24 hr. using drops of physiological saline and were examined under a microscope or a binocular. The parasitoid's egg, 1st and 2nd instars larvae were observed on the same microscopic slide using a phase microscope. Older parasitoids larvae 3rd, 4th instars, pre-pupa and pupa were observed using the binocular due to their sizes.

2.2. Fecundity

Newly emerged parasitoid adults were left for mating; thereafter each couple was confined in a plastic jar and provided with 25 newly 3rd larval instar of *B. zonata*. Each jar was provided with drops of bee honey and cotton wet with water for adult's feeding. The newly 3rd instar larvae were replaced daily with new ones until death of the adult parasitoid female. From the daily exposed host larvae in relation to the number of emerged parasitoid adults from them, pre-ovipositional, ovipositional and post ovipositional periods, as well as the fecundity were estimated. Also, adult longevity, sex ratio and survival rate were also recorded.

2.3. Survival and Sex Ratio

Survival and sex ratio of the parasitoid were estimated. Mated females of the parasitoid were provided daily with 100 larvae on artificial diet in small transparent jars until their death. Parasitized larvae were collected daily and allowed to complete their development on the larval artificial diet until pupation and the parasitoids to complete their life cycle. Parasitized pupae were kept in small jars until adults' emergence.

2.4. Longevity

Newly emerged adults of the parasitoid were placed individually into small glass vials and divided into two groups. 30 pairs; (males and females each) were left to complete their longevity; the 1st group was left unfed, while the 2nd was fed on small drops of undiluted honey. Longevity of each sex was estimated.

3. Statistical analysis

Obtained data were statistically analyzed using Microsoft Excel 2003.

RESULTS AND DISCUSSION

Biological Studies

1- *B. zonata*

1.1. Durations of immature stages

Durations of various immature stages of *B. zonata* are presented in table (1).

1.1.1. Incubation period of egg stage

Incubation period of the eggs ranged between 1-3 days, with an average of 2.7 days. The present results agree with those of FAO/ IAEA (2000), which reported that under favorable conditions, the eggs hatched to larvae within 2 days. This incubation period may be delayed when temperatures is below normal. Hussain (1995) recorded that the incubation of the egg stage of *B. zonata* ranged 24 – 43 hrs. Nearly similar results for incubation period had been reported by many authors such as; Mohamed (2002), Duyck *et al*., (2004), Afia (2007) and Farag (2009). They reported 3.0, 2.0 as well as 2.3 and 2.2 days at 25ºC, respectively.

1.1.2. Developmental period of larval stage

Larvae passed through three larval instars. They occupied 5-7 days, with an average of 5.8 days. This result is in accordance with the finding of Qureshi *et al*., (1993), Mohamed (2002), Afia (2007), and Farag (2009) who reported 5.8-12.2, 6.1-13.1, 5.4-14.8 and 5.3-12.8 days at 20–30 ºC, respectively as the ranges of total developmental larval period.

1.1.3. Duration of pupal stage

As shown in table (1), the duration of pupal stage averaged 10.2 days, and ranged between 9-11 days. This result was similar to that reported by Qureshi *et al*., (1993), El-Gendy (2003), Afia (2007) and Farag (2009) at 25ºC as they recorded 10.3, 10.8, 10.0, 11.2 and 10.8 days, respectively. Mohamed (2000) reported shorter pupal duration (7.7 days) at the same temperature on guava fruits.

Table (1): Some biological aspects of *Bactrocera zonata* under the laboratory conditions of 25± 1Cº and 54%-60 R.H.

Duration (days)	Means ± SE	Range
Egg	2.7 ± 0.15	1-3
Larva	5.8 ± 0.28	5-7
Pupa	10.2 ± 0.13	9-11
Total developmental period	18.7 ± 0.25	15-21
Survival rate %	58% ± 0.44	40 - 80%
Longevity (days) Fed:		
Female	50.6 ± 1.44	49 - 54
Male	47.3 ± 1.4	44 - 49
Un-fed:		
Female	2.8 ± 0.38	2 - 6
Male	3.3 ± 0.46	2 - 6
Fecundity:		
Pre –oviposition (days)	16.6 ± 1.97	13 - 21
Oviposition period	29.33 ± 1.15	28 - 32
Post-oviposition	4.6 ± 0.28	4 - 6
Daily mean no. (Eggs/ female)	10.5 ± 1.13	5 -16
Total No. eggs/ female	235.72 ± 6.2	227 - 248
Sex ratio % (female: male)	1.2 : 1.0	2 : 1

1.1.4. Total developmental period

Newly emerged adults are not sexually matured, thus the flies attained their sexual maturity within a mean of 16.6 days. The total developmental period of *B. zonata* (from egg deposition to adult emergence) averaged 18.7 days (Table 1).

1.2. Ovipositional periods and Fecundity

Data summarized in table (1) showed that the female required 13-21 days after emergence to start laying eggs. Pre-ovipositional period averaged 16.6±1.97 days at 25ºC. The mean daily number of eggs reached 10.5 eggs/ female. The total number of eggs/ female was 235.72±6.24. In agreement with the obtained results, Qureshi *et al*. (1974), in Pakistan reported that the pre-ovipositional period of *B. zonata* was 14.2 days at 27±2 ºC., and the female deposited 279 eggs at 25ºC. In (1995), Hussain recorded 13 days prior to female oviposition after emergence. FAO/ IAEA action plan (2000), reported that the pre-ovipositional period, which also included sexual maturation was 8-16 days and therefore, 10 to 23 days to the first egg when the time for sexual maturity was included. The female laid an average of 137 eggs in batches.

The results are nearly in accordance with those recorded by El-Gendy (2003) who found that re-ovipositional period was 14.6 and 26.6 days for females reared on peach and artificial diets, respectively. Female's fecundity varied from 372 to 734 eggs when reared on banana and mandarin, respectively. The same trend was recorded by Amin (2003), El-Naggar (2004), Afia (2007), Shehata *et al*. (2008), and Farag (2009). On the other hand, El- Minshawy *et al*., (1999) recorded a pre–ovipositional period of 45-60 days at 25ºC.

Most of the female flies ceased egg deposition for 4-6 days before death. These result is in agreement with that recorded by El-Gendy (2003), Afia (2007), and Farag (2009) who reported this period as 8.0 days at 24±3ºC, 5.8 and 6.2 days at 25±1ºC, respectively, Amin (2008) recorded 16.4 and 17.2 days at 25ºC when females were fed on solid and liquid protein hydrolysate, respectively.

1.3. Survival rate

Average percentage of adult emergence was 58% at 25±1Cº and 54%-60 R.H.

1.4. Sex ratio:

Sex ratio (female: male) was 1.2: 1 under the laboratory conditions of 25±1Cº and 54%-60 R.H.

1.5. Longevity

When adults were provided by water and a mixture of sugar and protein hydrolysate, they lived longer compared to those of unfed individuals. The female and male longevities were 50.6 and 47.3 days for the fed individuals, respectively and 2.8 and 3.3 for unfed ones at 25±1ºC. These results are nearly similar with that recorded by El-Gendy (2003), Afia (2007), and Farag (2009) who reported this period for male and female, as 59.6 and 82.5, 64.2 and 76.8 as well as 62.1 and 76.2 days at 25±1ºC, respectively. However, the obtained results are not in agreement with those reported by El-Minshawy *et al.,* (1999) who recorded 100.0 and 145.0 for male and female at 25ºC, respectively.

2- *A, daci*

2.1. Life cycle

2.1.1. Durations of immature stages

The durations of various immature stages of *A. daci* are presented in table (2).

2.1.1.1. Incubation period of egg stage

Female of *A. daci* deposited a single egg in the body cavity of the host larva and one parasitoid adult emerged from each host puparium. The incubation period of the eggs ranged between 2-3 days, with an average of 2.7 ±0.15 days. The present results agree

Table (2): Some biological data of *A. daci* when reared under the laboratory conditions of 25± 1Cº and 54%-60 R.H.

Developmental time (Duration)	Means (days)	Range
Egg	2.7 ±0.15	2-3
Larva/ 1st instar	2.5 ± 0.16	2-3
2nd instar	1.3 ±0.15	1-2
3rd instar	1.8 ±0.2	1-3
4th instar	4.2 ±0.24	3-5
Pre –pupa	1.6 ± 0.52	1-3
Pupa	11.2 ± 0.29	10-12
Total mean period of immature stages	25.3 ± 1.3	20-31

with those of Nunez-Bueno (1982) who reported that the eclosion of the first 30% of the eggs of *A. daci* on the 2nd instar larva of *A. suspense* was observed at 73 h. after parasitization and was completed in the following 12 h.

2.1.1.2. Developmental period of larval stage

Larvae of *A. daci* passed through four larval instars. The four larval instars occupied 8-16 days. After hatching, the 1st instar larva actively fed on the internal tissues of the host for 2-3 days. The 2nd instar larva fed inside the host pupa for 1-2 days, then developed to 3rd instar larva, which lasted 1- 3 days. In this stage, the transformation from endoparasitic to ectoparasitic behavior was observed. The 4th instar larva fed on the remains of the host tissues for 3-5 days. By the end of 16th day from parasitism, the parasitoid had totally consumed the host and the larval meconia were released in the posterior end of the host puparium. The voidance of the meconium was considered as the end of larval development. The pre-pupa lasted from 1-3 days.

2.1.1.3. Duration of pupal stage

As shown in table (2), the duration of the pupal stage averaged 11.2 days, and ranged between 10-12 days.

2.1.1.4. Total Developmental period

Total developmental period of *A. daci* from oviposition in the host larva to emergence of adult varied from 26-28 days in males and from 28-30 days in females. Thus, males tended to emerge somewhat earlier than females. The time of development of a parasitoid is influenced by the host instar, parasitoid species, age and nutritional suitability of the host and by environmental conditions (Salt, 1941 and Vinson and Iwantsch, 1980).

Information on the rate of development of *A. daci* in other hosts is scanty. Nunez-Bueno (1982) who studied the development of *A. daci* on the 2nd instar of *A. suspense,* reported that not all parasitoids from the same period of egg deposition developed and emerged as adults at the same time. Most of the males emerged at 26-27 days of age, and the majority of females did so at 28 days at 27.5±2ºC, 50-70% RH. In disagreement with the obtained results, Saiqa *et al.,* (2010) reported that the duration of the whole life cycles of male and female of *A. daci* on *B. zonata* larva were 16-18 days and 18-20 days, respectively. These days were distributed as 10-12 days for egg to adult's development period; 4-5 days of life of male *A. daci*; 8-9 days of female *A. daci*.

2.2. **Longevity**

When adults were provided by water and small drops of honey, they lived longer compared to those the unfed individuals. The female and male longevities were 18.8±0.1527 and 17.3±0.21 days for the fed individuals, respectively and 4.2±0.35 and 3.5±0.54 for unfed ones at 25±1ºC. Nunez-Bueno (1982) studied longevity of *A. daci* when reared in 1-6 days old *A. suspense* and recorded 8.27 and 11.78 days, and 6.57 and 16.80 days for male and female, respectively on *A. daci* larvae of 1 and 6 days old. Saiqa *et al.,* (2010) mentioned that the longevity of male *A. daci* was 5-6 days after the period of mating, while longevity of female was 8-9 days.

2.3. **Survival rate**

Data revealed that the average percentage of adult emergence was 44.7 % at 25± 1ºC and 54-60% R.H. In agreement with this result, Nunez-Bueno (1982) reported 47.58, 44.18 and 47.11% for *A. daci* survival rate on 1, 2 and 3 day–age of *A. suspense* larva, and 19.58% for 6 days-age larvae.

2.4. **Sex ratio**

Sex ratio (female: male) was 1: 1 under the laboratory conditions of 25±1Cº and 54%-60 R.H. Clausen (1978), Salt (1934) and, Vinson and Iwantsch (1980) mentioned that differences in sex ratio are related to the host's condition, sex, size, super-parasitism and environmental conditions, or the age of the parasitoid itself.

2.5. **Fecundity**

Examination of the reproductive system of gravid females revealed the presence of hundred eggs that might indicate a relatively high capacity. Each ovarian had near 80 eggs. The total number of eggs/female averaged 39.57±1.3 (32-55 eggs). It was observed that large sized pupae of *B. zonata* produced larger adults of *A. daci*. Non-mated females showed an ability to lay infertile eggs which result in male only. Nunez-Bueno (1982) reported that total numbers of mature eggs in nonoviposting female of *A. daci* were ranged from 239.44-273.69 eggs, when reared on 1 and 6 day-old *A. suspensa* larvae.

2.6. **Superparasitism**

Females of *A. daci* could discriminate between parasitized and unparasitized hosts, but when the parasitoid females were provided with small numbers of hosts, the phenomenon of super-parasitism occurred. This result agrees with Nunez-Bueno (1982) who reported that at high parasitoid: host ratios, reduced number of eggs laid resulted from the capacity of parasitoids to restrain oviposition, as well as from interference from other females. The number of parasitoid eggs per host increased with increasing parasitoid density, but the yield of parasitoid progeny decreased as a result of competition between supernumeraries and/or effect of host defense reaction.

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