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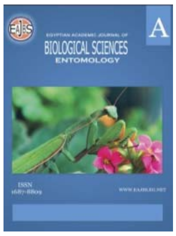

Thermal Requirements of the Peach Fruit Fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), and its Exotic Parasitoid Species *Aganaspis daci* (Weld) (Hymenoptera: Eucoilidae)

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ABSTRACT

The peach fruit fly, *Bactrocera zonata* (Saunders),is one of the serious invasive insect pests attacking tropical and subtropical fruits. The exotic parasitoid *Aganaspis daci* (Weld) is a solitary larval-pupal parasitoid of several fruit fly species. The influence of temperature on developmental periods of *B. zonata* and *A. daci* were studied at five constant temperatures (15, 20, 25, 30 and 35°C), 60± 5% R.H. and 14L: 10D photoperiod. The developmental time of egg, larval, pupal stages and egg to adult were estimated for *B. zonata* and *A. daci*. The longest total developmental period was recorded 59.3±0.18 and 108.9±0.32day, for *B. zonata* and *A. daci*, respectively, at 15°C. *A. daci* could not complete its life cycleat 35°C because the eggs didn't hatch. All temperatures at total developmental time of the parasitoid were longer than that of the host. The developmental thresholds of the egg, larval, pupal stages and egg to adult were 12.17, 6.45, 9.79 and 9.19 °C for *B. zonata*, and 14.35, 8.78, 10.43 and 10.7 °C for *A. daci*, respectively. The thermal unit requirements (k) value of the parasitoid was higher than those of its host they were 434.78 and 384.6 degree-days, respectively. This parasitoid can be used as a part of a biological controlling program but it is not expected to be a key agent for control *B. zonata*.

INTRODUCTION

Fruit flies attack fruit and vegetable crops reducing yield and affecting their quality. The peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), is one of the most harmful species of Tephritidae. It is an invasive species in Egypt, native to South East Asia. It is a polyphagous insect attacks over 50 cultivated and wild plant species, mainly those with fleshy fruits. The main hosts of *B. zonata* are guava, mango and peach. Its secondary hosts include apricot, fig and citrus (White & Elson-Harris 1992). It has also been recorded from wild host plants of the families Euphorbiaceae, Lecythidaceae and Rhamnaceae (Kapoor & Agarwal 1983). In infested orchards, it has been reported that it could compete with other highly damaging tephritid fruit fly species such as the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (OEPP/EPPO 2005).

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's population increased aggressively without natural control and it became a serious pest threatening not only the fruits in Egypt but also in the surrounding countries (Hosni *et al.,* 2011).

South-East Asia is the native area of the parasitoid species *Aganaspis daci* (Weld) (originally described as *Trybliographa daci* (Hymenoptera: Eucoilidae). *A. daci* is a solitary larval-pupal parasitoid of several fruit fly species in Southern East Asia and Australia. It was first collected from Malaysia and Borneo, and introduced into Hawaii as a potential biological control agent for *Bactrocera dorsalis* (Hendel) (Clausen *et al.,* 1965). *A. daci* was introduced to Florida (USA) where it was established successfully on *Anastrepha suspensa* (Loew) (Baranowski *et al.,* 1993).

In classical biological control programs release of exotic parasitoids (or predators) from the native region of the pest may be one of the control methods that can reduce pest populations to manageable levels. Therefore, through an Egyptian-American collaborative project "Non toxic control of *B. zonata* in Egypt" which was carried out during the period 2008-2011, *A. daci* was one of several exotic parasitoid species introduced to Egypt for evaluating their potential in controlling *B. zonata*.

In case of biological control, the thermal requirements of natural enemies are among many attributes (*e.g.* fecundity, searching capacity, host preferences …etc.) that together with environmental factors will influence the outcome of attempts at biological control of a host. The thermal requirements of a natural enemy may determine its success, or failure, in the biological control of a given host population.

The objective of this study was to estimate thermal requirements of *B. zonata* and the parasitoid *A. daci* as a biological control candidate against *B. zonata* under laboratory conditions.

MATERIALS AND METHODS

Insect culture:

The study was conducted with laboratory cultures of fruit fly *B. zonata* and the parasitoid *A. daci*. The laboratory culture of *B. zonat* collected from infested guava fruits at Giza Governorat, and reared on artificial diet in the laboratory for six generations. *A. daci* was introduced to Egypt from Kenya through an Egyptian-American collaborative project "Non toxic control of *B. zonata* in Egypt" and rearing in Biological Control Research Department (BCRD), Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), Giza, Egypt. *A. daci* was supplied from the stock culture at (BCRD). Laboratory stock cultures of both were maintained at 26± 2 ^{o}C, 60± 5 % R.H. and 14 L: 10 D photoperiod. Developmental times of *B. zonata* and the parasitoid *A. daci* were determined at five constant temperatures (15, 20, 25, 30 and 35± 1 ^{o}C).

Egg stage of *B. zonata*:

To determine developmental time of egg stage of *B. zonata*, the eggs were collected from artificial egg-laying devices offered to a stock colony for two hours. Thirty eggs were transferred to Petri dish (3cm in diameter X 1cm deep) with moistened piece filter paper using fine brush, four replicates giving total of 120 eggs for each temperature treatment. Eggs were observed daily under a stereo-microscope to determine the incubation period.

Larval stage of *B. zonata*:

To determine developmental time of larval stage of *B. zonata*, artificial diet made from short wheat and molasses using a technique developed by (defined by

Hosni *et al.,* 2011) was used for rearing *B. zonata*. Thirty newly hatched larvae were carefully transferred to a small Petri dish (5cm in diameter X 1.5cm deep) and then placed inside a plastic container (15cm in diameter X 2cm deep), the bottom of which was covered by a layer of sand to allow pupation of mature larvae (four replicates).

Pupa stage of *B. zonata*:

To determine developmental time of pupa, 30 newly pupae were randomly chosen at the time when the majority of larvae had just pupated then transferred into a plastic box (15cm in diameter), containing sand (four replicates).

Developmental time of *A. daci*:

To determine developmental time of different stages of *A. daci*: After emergence of the parasitoid adults, the males and females were held in vials(10cm in diameter) for 5 h for mating. After mating, one hundred second larval instar of *B. zonata*, were placed on a small thin layer of artificial diet in a glass Petri dish (9cm in diameter X 2cm deep) presented to the parasitoid females. Number of host larvae and time of exposure was used to avoid super parasitism as reported by Nunez-Bueno (1982). Larvae were dissected daily by a very fine needle, in a drop of Ringer's solution using a stereo-microscope to determine the developmental time of the immature stages.

Thermal requirements:

The relationship between temperature (T) and developmental rate (1/D) in laboratory, can be described by a linear regression equation of the formula: y = a + bx, where (y) is the rate of development 1/D, (D being the time in days required for the competition of a particular developmental stage at the temperature (x), in degrees centigrade); and (a) and (b) are constants. The lower developmental threshold (t_o) is equal to (-a/b). It can be estimated from the regression equation by solving for (x) when (y) = 0. The thermal units (K) required for the time to adult is given by the reciprocal of the slope (b) of the regression line (Campbell *et al.,* 1974, Wagner *et al.,*1984, Liu and Ye 2009 and Jalali *et al.,* 2010).

Statistical analysis:

The experimental design was completely random with five temperature treatments and four replicates, each consisting of 30 eggs, larvae and pupae for the immature stages. All developmental times for immature stages were replicated three times. Standard analysis of variance (ANOVA) was used, means were compared by using Duncan's Multiple Range Test (*P* = 0.05) (SAS Institute 2002).

RESULTS

B. zonata successfully developed from egg to the adult stage at the tested temperatures (15, 20, 25, 30 and 35± 1 ^{o}C). The developmental time for eggs and pupae of *B. zonata* at 30 and 35 °C was nearly equal (1.5±0.13 and 1.3±0.12 days for egg and 8.5±0.13 and 8.1±0.01for pupae, respectively) (Table 1). The shortest total developmental period was recorded 14.7±0.19 day,at 35 ^{o}C and the longest was 59.3±0.18 day, at 15 ^{o}C

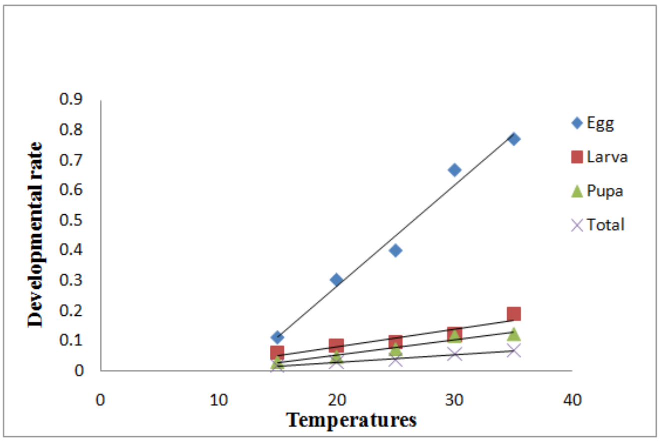
A. daci successfully developed from egg to the adult at 15, 20, 25 and 30 ± 1 ^{o}C, but could not complete its life cycleat 35 °C because the eggs didn't hatch. The shortest total developmental period was 23.3±0.1day, at 30^{o}C and the longest was 108.9±0.32day at 15^{o}C. All temperatures at total developmental time of the parasitoid were longer than that of the host (Table 1).

Table 1: Mean developmental time (Mean days ±SE) of immature stages of *B. zonata* and the parasitoid *A. daci* at five constant temperatures at 60±5% R.H. and L14:D10 photoperiod

	Insect stage	Temperatures (°C)				
		15	20	25	30	35
B. znata	Egg	8.9±0.16[a]	3.3±0.13[b]	2.5±0.13[c]	1.5±0.13[d]	1.3±0.12[d]
	Larva	16.5±0.16[a]	12.1±0.23 [b]	10.4±0.13 [c]	8.3±0.14 [d]	5.3±0.13 [e]
	Pupa	33.9±0.18[a]	21.1±0.2 [b]	13.7±0.11 [c]	8.5±0.13 [d]	8.1±0.01 [d]
	Egg-adult	59.3±0.18[a]	36.5±0.19 [b]	26.6±0.16 [c]	18.3±0.2 [d]	14.7±0.19 [e]
A. daci	Egg	9.3±0.23 [a]	5.7±0.18 [b]	2.3±0.16 [c]	1.4±0.13 [d]	Dead egg
	Larva	22.5±0.3 [a]	17.3±0.3 [b]	12.8±0.14 [c]	7.5±0.16 [d]	-
	Pupa	77±0.31 [a]	25.1±0.22 [b]	14.9±.17 [c]	14.4±.13 [c]	-
	Egg-adult	108.9±0.32 [a]	48.1±0.32 [b]	29.7±0.15 [c]	23.3±0.1 [d]	-

Means followed by different letters in the same row are significantly different at P<0.05.

Developmental rates for different stages (egg, larval instars, pre-pupa, and pupa) and total developmental timeto adult for the parasitoid *A. daci* and its host *B. zonata* are presented in Figures. (1&2). In general, the developmental rates of respective stages of *A. daci* and *B. zonata* were influenced by the temperature to which they were exposed. Developmental times were decreased and developmental rates were increased as temperature increased.



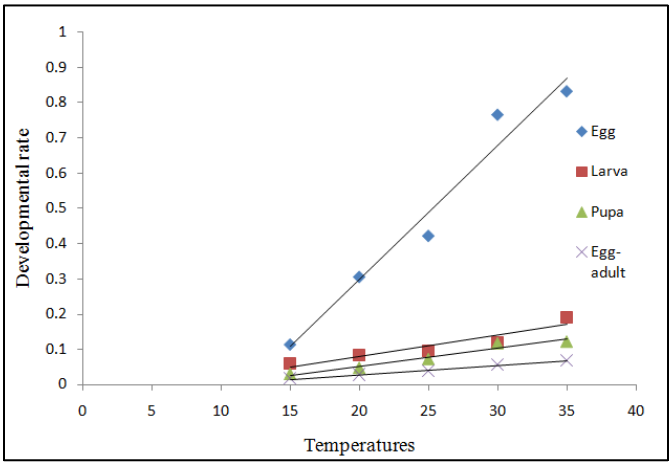


Fig. 1: Effect of constant temperatures on developmental rates of different immature stages of *B. zonata*

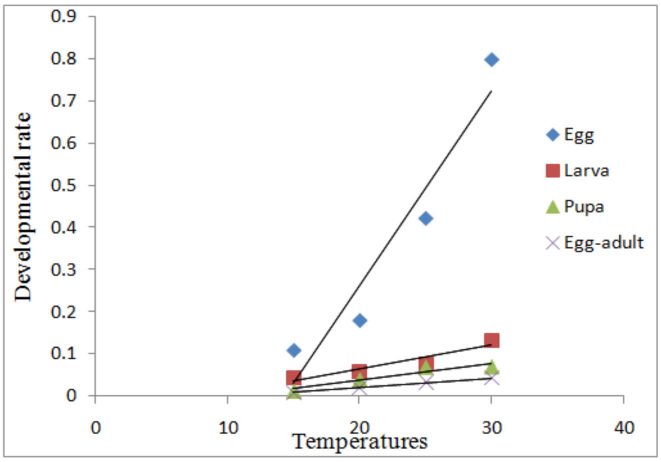


Fig. 2: Effect of constant temperatures on developmental rates of different immature stages of *A. daci*

The linear regression equations showed a positive relationship between the developmental rate and temperature at all immature stages of *B. zonata* and *A. daci* (Figs. 1& 2). Out of the regression equations, lower developmental threshold (t_o), and thermal units requirement (k) were presented in Table (2). The lower developmental threshold (t_o) of *A. daci* and *B. zonata* for total duration to adult were 9.19 and 10.7 °C, respectively. The thermal unit requirements (k) value of the parasitoid was higher than those of its host they were 384.6 and 434.78degree-days, respectively.

Table 2: Temperature thresholds (t_o), thermal constants (K) and coefficient of determination (R^2) of the different stages of *B. zonata* and the parasitoid *A. daci*

		Regression equation	t_o(°C)	K (DD)*	R^2
B. zonata	Egg	y = 0.038x - 0.4623	12.17	26.32	0.96
	Larva	y = 0.006x - 0.0387	6.45	166.66	0.89
	Pupa	y = 0.0052x - 0.0509	9.79	192.31	0.95
	Egg-adult	y = 0.0026x - 0.0239	9.19	384.6	0.99
A. daci	Egg	y =0.0464x - 0.6662	14.35	21.55	0.92
	Larva	y = 0.0058x - 0.0509	8.78	172.41	0.89
	Pupa	y = 0.004x - 0.0417	10.43	250	0.89
	Egg-adult	y = 0.0023x - 0.0246	10.7	434.78	0.99

* DD= Degree day

Coefficient of determination were high for both egg and pupal stages (R^2≥0.95) but lower for the larval stage (R^2= 0.89) of *B. zonata*, but for *A. daci* they were high for egg (R^2=0.92) and lower for both larval and pupal stages (R^2= 0.89) (Table 2).

DISCUSSION

The linearity of the relationship between temperature and developmental time from 15 and 40°C for *B. zonata* was consistent with previous studies (Mohamed, 2000; Duyck *et al.*, 2004 and Fetoh *et al.*, 2012).The larvae of *B. zonata* failed to complete their development at below 15°C and above 35°C (Mohamed, 2000), *B. zonata* developed to pupa but did not emerge as adult at 40°C (Fetoh *et al.*, 2012). Duyck *et al.*, (2004) mentioned that the larval and pupal stages were similar at 30 and 35°C (4±0.1 and 4±0.2 for larva and 8±0.1 and 8±0.1 days for pupa, respectively), which agreed with this study.

A. daci could not complete its life cycle at 35 °C and no results are available for this temperature in the literature. In this study; the total life cycle from egg to adult was 29.7± 0.15days at 25°C.The life cycle of *A. daci* form egg to adult was 10-12 days at (24-26 °C) and 54-56%R.H. when parasitized *B. zonata* (Andleeb *et al.,* 2010), and 25.3 days on the same host at 25 ± 2 °C and 54-65% R.H (Hosni *et al.,* 2011).

The estimation of lower temperature thresholds (t_o) of egg of *B. zonata* (12.17 °C) in this study trends with the literature that ranged 11.83-12.17°C, but for larva (6.45 °C) and pupa (9.79 °C) they were lower than in the literature, as it was 12.6, 12.13 and 17.3°C for larva and 12.8, 12.67 and 12.7°C for pupa, respectively. The thermal requirements ranged 25-43.2 for egg, 68-169 for larva and 117-141.21 for pupa (Duyck *et al.,* 2004; Sharaf El-Din *et al.,* 2007 and Fetoh *et al.,* 2012).

The discrepancies among the present results and other literatures for the estimated lower temperature thresholds and thermal requirement values may be due to the differences among the pest strains at different geographical areas (local climatic) or the type of food provided for the larvae (artificial diet or guava fruit) as suggested by Honek and Kocourek, (1990); Fernandes-Da-Silva and Zucoloto, (1993) and Duyck *et al*., (2004).

The evaluation of the thermal requirements of *A. daci*has not been studied before. The short developmental time was recorded at high temperature 35°C. The low temperature thresholds of *B. zonata* and *A. daci* confirmed that these species are well adapted to thermal wide climate and *B. zonata* should thrive during the summer. These results may explain the presence of *B. zonata* throughout the year round on different host plants; peach, mango, guava, citrus. These results consistent with that of El-Nagar *et al.,* (2010), who reported that the highest incidence of *B. zonata* was during summer season, followed by spring and autumn. Therefore, it represents a threat to mango, guava, and pear fruits during summer season much more than to citrus in winter. *B. zonata* appeared to prefer warm conditions and seemed well adaptable to hot climates.

In this study, the developmental threshold of the parasitoid *A. daci* was higher than that of the *B. zonata*, that means the host appears early in the field before the parasitoid. According to Campbell *et al*., (1974) the threshold of the parasitoid is higher than the host. Therefore, the buildup of the parasitoid population will be delayed in the field until average temperature increases and the host becomes well established in spring. For all practical purposes, the parasitoid season cannot start earlier than that of the host.

B. zonata is suitable for rearing the parasitoid in the laboratory and the parasitoid can be recommended as a part of a biological control program but it is not expected to be a key agent for control *B. zonata*. The releases of parasitoid adults to control *B. zonata* must be conducted during the morning or late afternoon. In addition, temperatures should be below 35°C because the parasitoid population might not be able to achieve adequate levels of control at high temperatures.

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RABIC SUMMERY

الاحتياجات الحرارية لذبابة الخوخ (.Saund) *Bactrocera zonata* والطفيل المستورد (Weld) *Aganaspis daci*

داليا عدلى
معهد وقاية النباتات – قسم ابحاث مكافحة الافات

تعبر ذبابة ثمار الخوخ *Bactrocera zonata* (Saunders) من اخطر الافات الحشرية الغازية التى تصيب الفاكهة الاستوائية وشبه الاستوائية. يتطفل الطفيل المستورد الفردى *Aganaspis daci* (Weld) على يرقات وعذارى العديد من أنواع ذباب الفاكهة. تم دراسة تأثير خمس درجات حرارة (١٥، ٢٠، ٢٥، ٣٠ و ٣٥ م°) على فترات نمو وتطور كل من *B. zonata* و*A. daci* تحت ظروف المعمل (الرطوبة ٦٠ ± ٥% وفترات إضاءة ١٤ إضاءة: ١٠ ظلام). تم دراسة فترات النمو للبيضة واليرقات والعذارى والفترة من البيضة – الحشرة الكاملة لكل من ذبابة الفاكهة والطفيل. أطول فترة نمو سجلت كانت 59.3±0.18 و108.9 ±0.32يوم على درجة حرارة 15 °C لكل من *B. zonata* و*A. daci* على التوالى. لم يستطيع الطفيل ان يكمل دورة حياته على 35 °C بسبب عدم فقس البيض.دورة حياة الطفيل كانت اطول من دورة حياة ذبابة اخوخ على جميع درجات الحرارة. قدرت أقل درجة حرارة يبدأ عندها نمو وتطور كلا من طور البيضة، اليرقة ، العذراء واجمالى دورة الحياة بـ 6.45,12.17 , 9.79 , 9.19 م° لذبابة ثمار الخوخ *B. zonata* و 14.35 ,8.78, 10.7,10.43م° للطفيل *A. daci* ، على التوالى. قيمة الاحتيجات الحرارية للطفيل كانت اعلى من ذبابة الافاكهة، 434.78 و 384.6درجة – يوم على التوالى. يستنتج من هذه الدراسة امكانية استخدام الطفيل كجزء من برنامج المكافحة الحيوية المستخدم لمكافحة ذبابة الخوخ ولكن لا يمكن الاعتماد عليه كعنصر اساسى فى البرنامج.