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Full Length Research Paper

Survival and development of *Bactrocera oleae* Gmelin (Diptera:Tephritidae) immature stages at four temperatures in the laboratory

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***Bactrocera oleae* Gmelin (Diptera:Tephritidae) is the most important and widespread pest in the olive growing countries in the Mediterranean basin. The development and survival of olive fruit fly, *B. oleae* from egg to adult stage was studied in the laboratory at 16, 22, 27 and 35°C. The objective of the study was to get information on the influence of temperature on immature stages as a prerequisite to optimize rearing procedures and to understand geographical pattern of fruit fly occurrence. Embryonic development was fastest at 35°C but there was no pupal development and, of course, no adults at 35°C. The slowest development of immature stages was at 16°C. The highest percentage of adults obtained from an initial set of 100 eggs was 74% at 27°C. The lower development thresholds for the egg, larval and pupal stages were 9.19, 13.94 and 12.36°C, respectively. The optimum temperature for development and survival of immature stages was 27°C.**

Key words: *Bactrocera oleae*, olive fruit fly, rearing, temperature, Tephritidae.

INTRODUCTION

The olive fruit fly, *Bactrocera oleae* Gmelin, (Diptera : Tephritidae) is a monophagous pest that infests olive fruits. This major pest of olives is widespread throughout the Mediterranean Basin. The female lays eggs in green olive fruits, and larval development is completed within the fruits. Despite the economic importance of the olive fruit fly in the region, little is known about its main biological features. Temperature is the one of the important factor determining the development of the immature stages and the adult maturation of all insects, including olive fruit fly (Fletcher, 1989).

The olive fruit fly was considered as homodynamic pest (Tzanakakis, 2003). It can reproduce and develop throughout the year as long as temperature and humidity are favorable and host fruit are available (Tzanakakis, 2003). In hot summers, olive fruit fly populations are low and it is hard to find them in nature in any stages. Also, adults captured from the field in hot summers do not lay eggs or lay few eggs in the laboratory. It takes about 3 - 5 generations for them to adapt to laboratory conditions, provided that they lay enough eggs to keep the colony going (Dr. Nikos Cosmidis personal communication). However, the precise effect of temperature on development and reproduction needs to be clarified. Our laboratory study was conducted to investigate the influence of temperature on developmental time and survivorship of the immature stages of different population of olive fruit flies in order to optimize laboratory rearing procedures and to understand and predict geographical distribution of olive fruit fly for ecological and pest management purposes.

MATERIALS AND METHODS

Adult cultures

The laboratory culture of olive fruit flies originated from the Agriculture University of Athens, Greece, and was kindly donated by Dr. Nikos Cosmidis. A wild olive fruit fly colony was established from

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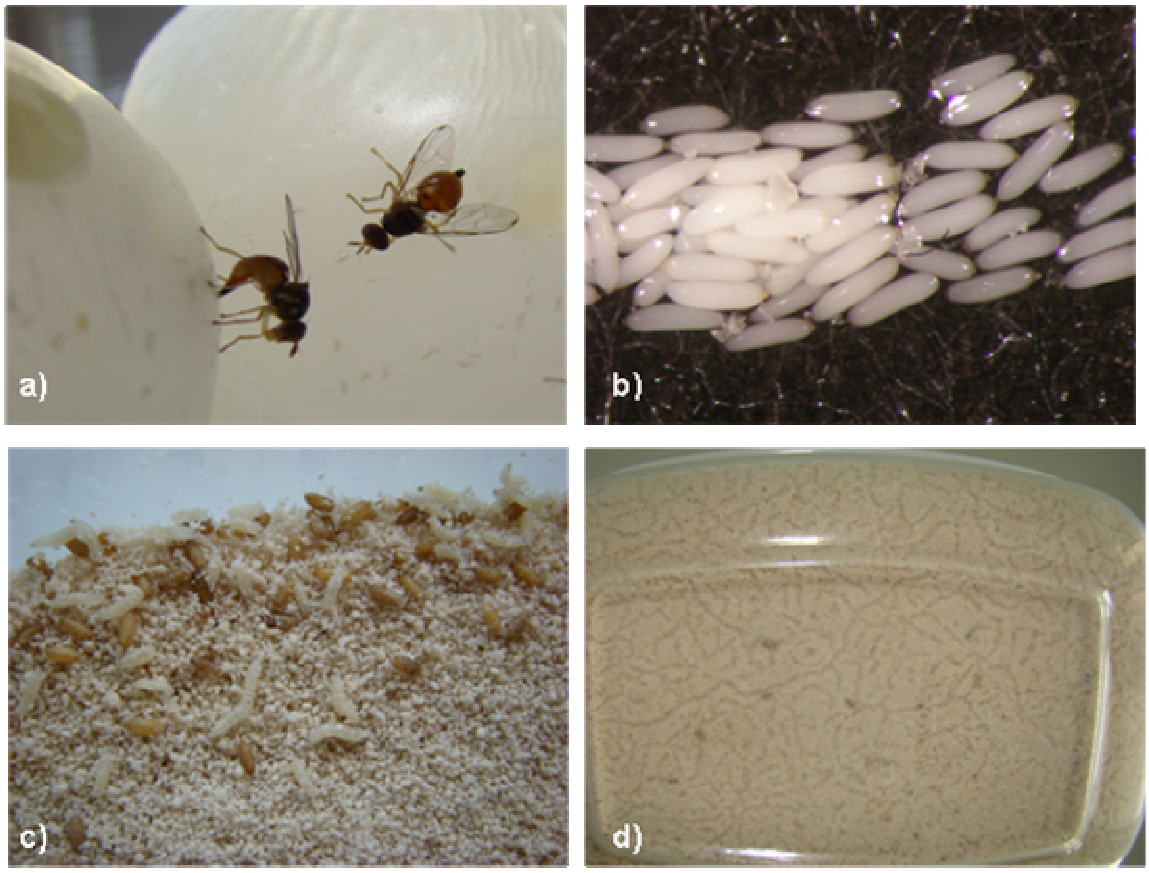

Figure 1. Laboratory rearing of olive fruit fly on cellulose diet; **a**) females laying eggs into paraffin domes, **b**) collected eggs before transferring diet, **c**) mature larvae and pupae on diet, and **d**) larvae tunnels at the bottom of the rearing container.

infested olive fruits in Kemalli, Canakkale, Turkey. Adult flies were kept at 26 ± 1°C with 18:6 (L: D) photoperiod and 65% RH. The larval diet used in this study consisting of 825 mL distilled water, 45 g soy hydrolysate, 112 g unhydrolysated brewer's yeast, 30 g sugar, 30 mL olive oil, 11.25 mL Tween, 3 g Nipagin, 0.75 g potassium sorbate, 45 mL HCl and 462 g cellulose powder (Tsitsipis and Kontos, 1983; Tzanakakis, 1989). The experimental cages set up for this work was as follows: Cage I contained only wild flies, Cage II contained only laboratory reared flies donated by Dr. Nikos Cosmidis and Cage III contained wild and laboratory flies in equal numbers [(50 ♀: 30 ♂) wild and (50 ♀: 30 ♂) laboratory flies]. All experimental cages were plastic, 20 X 20 X 20 cm, and contained (100 ♀: 60 ♂) individuals in total. Water, solid and liquid diets were provided in the cages (Tzanakakis, 1989). The time required for olive fruit fly individuals to achieve a particular development stages was determined at 16, 22, 27 and 35°C (±1°C).

Rearing procedures

Paraffin cones were used as artificial oviposition devices (Tzanakakis, 1989) in the cages (Figure 1a). Eggs were collected daily at 24-h intervals from paraffin cones with a 0.3% propionic acid solution. The duration of the egg stage was determined by transferring 100 randomly selected eggs from each experimental cages on Petri dishes lined with moistened filter paper (N=5) and kept at 16, 22, 27 and 35°C (±1°C) (Figure 1b). The eggs were observed three times a day under the microscope to determine the development time and percentage of hatching. The mean development time of the egg stage was determined. The artificial diet developed by Tsitsipis and Kontos (1983) was used to rear olive fruit fly larvae in the laboratory (Figure 1c and 1d). Newly hatched 100 larvae were gently collected on a moist black filter paper under the microscope and transferred to Petri dishes (9 cm diameter) containing 17 g larval diet and kept at 16, 22, 27 and 35°C (±1°C). The larvae moved to the artificial diet. When the larvae became in the third instar, the Petri dishes were moved to larger boxes containing a layer of moist sterile sand on the bottom. The larvae were observed three times a day to determine the end of larval development stage, which was defined by larvae crawl out of the Petri dish into the sterile sand. The mean development time of the larval stage was determined. To determine pupal development time, pupae were sieved out of the sand three times a day, and kept in a small Petri dish and the numbers of pupae were counted at tested temperatures. Pupae were transferred into a plastic screen cages following 3-4 days of pupation. The mean pupal duration was determined. The number of newly emerged adults was recorded three times a day.

Temperature summation model

The model of Brévault and Quilici (2000) was used to predict the development rate of individual life stages of olive fruit flies. The assumption were made that above a certain lower threshold for development, the temperature-development rate is linear (Flecther,

Table 1. Mean developmental time of *Bactrocera oleae* immature stages at four constant temperatures (n=5 replicates).

Temp (℃)	Stage duration (days)								
	Egg development			Larval development			Pupal development		
	Cage I	Cage II	Cage III	Cage I	Cage II	Cage III	Cage I	Cage II	Cage III
16	11.0±0.31	11.8±0.35	11.4±0.50	32.8±0.73	33.4±0.39	33.8±0.37	29.2±0.85	28.0±1.56	28.0±1.11
22	4.4±0.54	4.4±0.54	4.2±0.37	14.0±0.44	14.2±0.37	14.2±0.19	12.4±0.50	12.4±0.50	13.2±0.58
27	2.6±0.24	2.8±0.19	2.6±0.24	10.8±0.37	10.8±0.37	11.0±0.44	8.2±0.44	8.2±0.19	8.4±0.24
35	2.2±0.19	2.2±0.19	2.0±0.00	5.0±0.31	5.0±0.31	5.2±0.37	*	*	*

Values are mean ± SE.
*No development occurred.

1989). Thus, a constant number of heat units which is usually expressed by day-degrees above the threshold are crucial to complete development of each life stages (Flecther, 1989; Wagner et al., 1984; Brévault and Quilici, 2000). In order to establish this relationship, the developmental time of required for 50% of individuals to reach each life stage was determined at a series of constant temperatures. Development rate was calculated as 100/development time and plotted against temperature (Brévault and Quilici, 2000). The lower development threshold which is shown as *t* (the temperature at which the development rate is zero) was then determined by extrapolation of the line back to the *x-axis*.

Survival rate

It was determined by dividing the number of individuals alive at the end of each specific stage by the initial number. Thus, the number of emerged adults per 100 eggs was calculated as the survival rate of each stage (Rijn et al., 1995; Brévault and Quilici, 2000).

Statistical analysis

All developmental and survivorship tests were replicated five times. Multiple factorial design and analyses of variance (ANOVA) were used to test the effects of treatment on developmental time or survival rate. When a significant F value was obtained in the ANOVA, means were then separated by Duncan's Multiple Range Test (P<0.01) (SPSS 15).

RESULTS

Relationship between developmental time and temperature

The developmental time of each life stage significantly decreased with increasing temperature from 16 up to 27℃ (Table 1). There was no pupal development at 35℃ and no adults. The longest developmental time was observed for the larval stage, followed by the pupal and egg stages at all temperatures. There were no significant differences between the wild, laboratory and mixed cages at tested temperatures (P<0.88) (Table 1). Neither interaction between the cages and stages (P<0.590), nor cages and temperatures (P<0.998) was significantly different (Table 1). However, comparison of mean development times at the different temperatures and duration of immature stages of olive fruit fly was significantly different (P >0.001) (Table 2). The egg development time at 22 and 27℃ was not significantly different. At 35℃, egg and larval development times were the shortest but no pupation occurred and not adults were produced (Table 2). The best laboratory temperature to rear olive fruit fly was shown to be 27℃. Total development of immature stages at 27℃ takes about 22 days (Table 2).

A linear regression model between temperature and development rate was established for each immature stages within the range of 16 - 35℃ (Figure 2). For egg (Figure 2a), larval (Figure 2b), and pupal stages (Figure 2c), a strong positive linear relationship was observed between temperature and development rate (R^2 = 0.99, 0.98, and 0.99 at 16, 22, and 27℃, respectively). The temperature at which the development rate is theoretically zero was calculated by linear regression analysis and found to be 9.19, 13.94 and 12.36 ℃, respectively, for egg, larval, and pupal development.

Survival rate

The survival of immature stages varied significantly relative to the wild, laboratory and mixed cages and temperature (P >0.001) (Table 3). Survivorship of egg, larva and pupal stages was highest at 27℃ and decreased significantly at higher and lower temperatures (Table 3). The percentage of adults emerging from 100 eggs peaked at 74% at 27℃ compared to 47% at 16℃. The number of adults obtained from 100 eggs was maximum at 27℃ (Table 3). The highest degree of survival and shorter development time were observed for immature stages at 27℃. This rearing temperature can be considered favorable for laboratory rearing. Even though, the development times of egg and larva were the shortest at 35℃, survivorships were lowest and not adults could be produced, so this temperature is lethal.

DISCUSSION

As expected and shown in many other insects, development and survival of each olive fruit fly life stage are influenced by temperature. When rearing temperature increased, development rates increased predictably at

Table 2. The comparison of mean development time of *Bactrocera oleae* immature stages by (temperature*stage).

Temp (°C)	Stage duration (days)		
	Egg development	Larva development	Pupal development
16	11.40 ± 0.23 C a	33.40 ± 0.30 A c	28.40 ± 0.67 B a
22	4.30 ± 0.15 C b	14.13 ± 0.19 A a	12.67 ± 0.30 B b
27	2.67 ± 0.12 C bc	10.86 ± 0.16 A b	8.26 ± 0.11 B c
35	2.13 ± 0.09 B c	5.06 ± 0.18 A d	*

Values are mean ± SE.
*No development occurred.
[1]For a particular temperature, means followed by different uppercase letters in the same row are significantly different.
[2]For a particular stage, means followed by different lowercase letters in the same column are significantly different.

Table 3. Mean survival percentage of *Bactrocera oleae* immature stages at four constant temperatures (n=5 replicates).

Cages	Temp (°C)	Stage viability (%)			
		Eggs	Larvae	Pupae	Adults
1	**16**	49.2 ± 1.2 A**c**I	37.0 ± 2.0 B**c**II	30.4 ±2.3 C**c**II	24.4±2.7 D**c**II
	22	66.8 ± 1.0 A**b**II	53.0 ± 1.3 B**b**II	44.0±1.8 C**b**II	36.4±2.2 D**b**II
	27	74.4 ± 1.3 A**a**II	69.4 ± 0.8 A**a**II	62.6±1.4 B**a**II	50.0±1.6 C**a**II
	35	1.6 ± 0.2 B**d**I	7.6 ± 1.1 A**d**I	*	*
2	**16**	55.0 ± 3.5 AB**c**I	59.8 ± 2.2 A**c**I	52.2±1.1BC**c**I	47.4±1.3 C**b** I
	22	72.0 ± 1.1 B**b**III	82.0 ± 1.4 A**b**I	74.2±1.6 B**b**I	69.6±1.7 B**a**I
	27	85.2 ± 1.2 AB**a**I	89.6 ± 0.8 A**a**I	82.2±0.9 B**a**I	74.4±0.5 C**a**I
	35	2.2 ± 0.2 B**d**I	6.2 ± 0.73 A**d**I	*	*
3	**16**	54.4 ± 1.6 B**c**I	61.8 ± 1.5 A**c**I	56.0±1.9 B**c**I	47.4±1.3 C**c**I
	22	73.4 ± 1.3 B**b**I	80.4 ± 1.3 A**b**I	73.0±1.1 B**b**I	66.2±1.5 C**b**I
	27	87.0 ±1.6 AB**a**I	89.2 ± 0.7 A**a**I	82.0±0.8 B**a**I	74.8±1.1 C**a**I
	35	2.0 ± 0.1 A**d**I	6.8 ± 0.19 A**d**I	*	*

Values are mean ± SE.
* Survival is not observed.
[1]For the same cage and temperature, means followed by different uppercase letters in the same column are significantly different
[2]For the same cage and stage, means followed by different bold lowercase letters in the same column are significantly different
[3]For same temperature and stage, means followed by Roman letters in the same column are significantly different.

16, 22, 27, and 35°C, but 35°C is lethal to pupae, so no adults were produced at 35°C. The higher developmental thresholds and the low survivorship of the immature stages of olive fruit fly at 16°C should contribute to limit its spread. Our results may help improve laboratory rearing of olive fruit flies and may useful to understand the geographical distribution of olive fruit flies as related to environmental low and high temperatures. It will be possible to make simulation models to predict the presence of olive fruit fly in different areas.

Several studies have shown linear relationships between temperature and development times of the life stages of tephritids (Vargas et al., 1996; Brévault and Quilici, 2000; Duyck and Quilici, 2002; Duyck et al., 2004). As in our experiments, Duyck and Quilici (2002) found similar effects of 35°C on development of the immature stages of *Ceratitis rosae* Karsch. Previous studies of olive fruit fly showed an increase of the duration of ovarian maturation at high temperatures. The development time for ovarian maturation was found to be different for *Ceratitis capitata*, *Ceratitis rosa* and *Ceratitis catoirii* within the range of 15 - 30, 20 - 30 and 25 - 30°C, respectively. Ovarian maturation time was the shortest for *C. capitata* and the longest for *C. catoirii* between 25 - 30°C (Duyck and Quilici, 2002; Flether and Kapatos, 1983; Tzanakakis and Koveos, 1986). This phenomenon is similar with other studies in which linear regression models fit when the temperature approaches the threshold (Duyck et al., 2004). The estimates of the lower temperature thresholds for *C. capitata* (Wiedemann) and *Bactrocera cucurbitae* (Coquillett) immature stages were reported as 9.9, 5.2, 9.1°C and 10.1, 6.6 and 9.4°C,

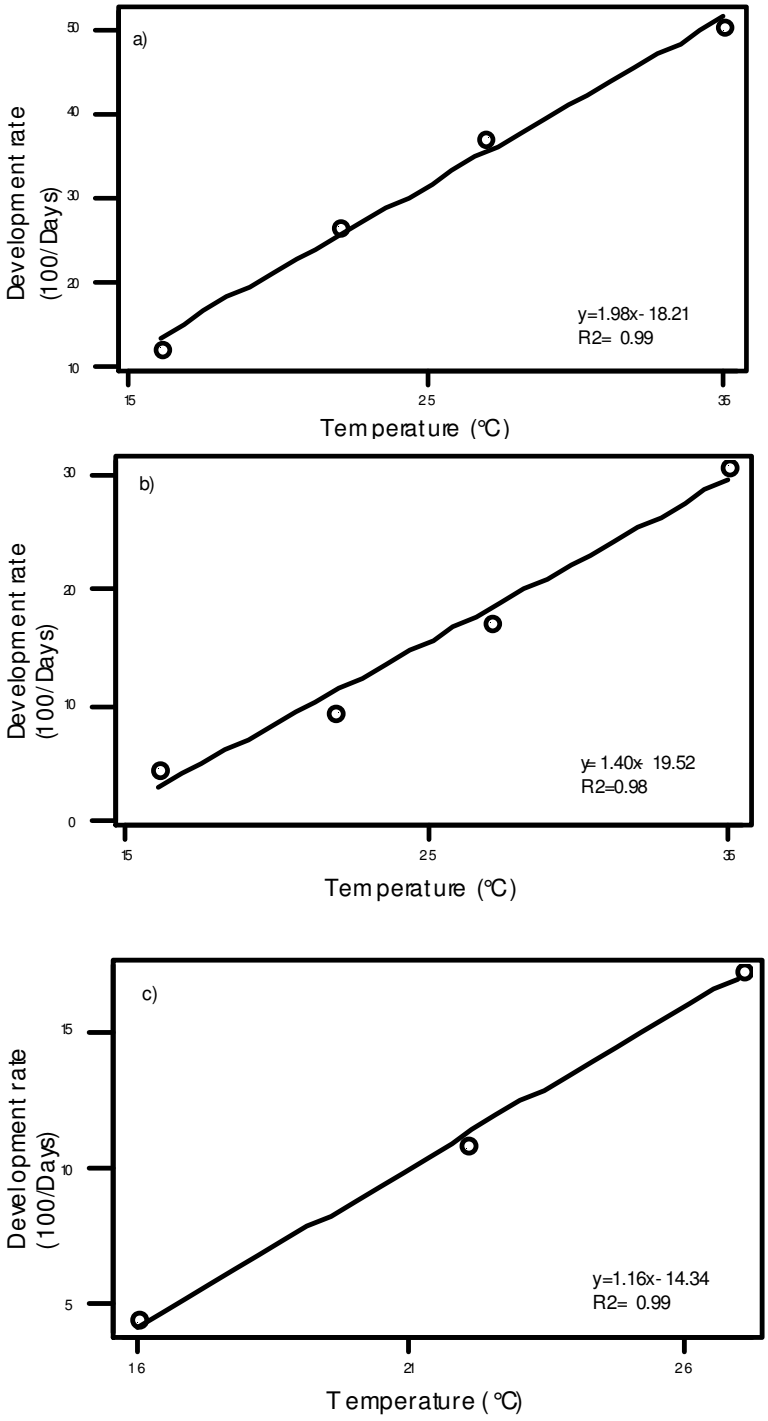

Figure 2. Effect of constant temperatures on development rates (100/Days) of immature stages of *Bactrocera oleae*; **a**) egg, **b**) larva, and **c**) pupa.

respectively (Vargas et al., 1996). Brévault and Quilici (2000) calculated lower temperature thresholds for *Neoceratitis cyanescens* (Bezzi) as 11.4, 11.9, 10 and 11.1°C for egg, larval, pupal and ovarian maturation, respectively. The lower development thresholds for the egg, larval and pupal stages were 9.19, 13.94 and 12.36°C, respectively, in this study. No previous work has been published on the development and survivorship of olive fruit fly at different constant temperatures. However the results presented in this study were similar to the earlier

data on concerning the development time of other tephritids (Brévault and Quilici, 2000; Orian and Moutia, 1960; Duyck et al., 2004).

It is known that temperature plays an important role in the laboratory rearing of insects. This study of the relationship of development time and survival at different constant temperatures may contribute towards improving small scale laboratory rearing of the olive fruit fly. The results should also be useful in optimizing the rearing conditions in the laboratory that are necessary for biological studies and control methods such as releases of parasitoids for biocontrol or releases of sterile flies for eradication programmes. In biological control of olive fruit fly for parasitoid rearing, the control of these parameters is essential to pool the production of larvae at a suitable stage for parasitism. In other words, larval development time and survival are important in rearing parasitoids for biological control of olive fruit fly. The presented study has provided information on the influence of temperature on the life parameters of *B. oleae*, including development rate and survival of different life stages. As we know temperature is the main factor influencing development, but the further studies should be on the effect of humidity which is known to be very important for pupal development of some tephritid species (Teruya, 1990).

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