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Presence of mature eggs in olive fruit fly, *Bactrocera oleae* (Diptera Tephritidae), at different constant photoperiods and at two temperatures

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Abstract

The effect of the constant photoperiod on presence of mature eggs in olive fruit fly was investigated. Adults of *B. oleae* were submitted to different photoperiodic treatments (LL:DD), at temperature of 20 °C: 9:15, 10:14, 12:12, 15:9, 16:8, continuous light (LL) and continuous dark (DD). Light was obtained from neon tubes and the light intensity, estimated inside the plexiglas cage, was approximately 1000 lux. In order to evaluate the effect of temperature on the production of mature eggs the treatments 15:9, 12:12 and 9:15 were also conducted at temperature of 26 °C. Moreover, to evaluate a possible effect of light intensity, the treatments 16:8, 15:9 and 12:12 were also performed by using lights producing an estimated light intensity of approximately 3000 lux. Treatment duration was a fixed term of 15 days after emergence.

Results showed that all the photoperiodic treatments induced egg ripening in almost the totality of females (from 86.7% to 100%) and the mean number of eggs per female was relatively high (from 21.95 to 52.8), while in the DD treatment it was evident that this photoperiod induced egg maturation only in 10% of the treated populations and the mean number of eggs/female was the lowest. With regard to ovarian maturity, the treatments with a 16:8, 12:12, 10:14 and LL photoperiod induced a significantly higher response than the other treatments. Moreover, with the treatments including two different light intensities, it was evident that the light intensity can positively influence only the number of eggs/female and not the percentage of treated specimens with mature eggs. Lastly, no significant differences were found when comparing ovarian maturity at the temperatures of 20 °C and 26 °C. Overall, it was concluded that olive fruit fly can be reared in laboratory by using a constant photoperiod and, indifferently, a temperature of 20 °C or 26 °C. The evidence that the amount of mature eggs is influenced by light intensity suggests it is more effective to use a number of neon tubes producing relatively high light intensities.

Key words: constant photoperiod, reproductive diapause, seasonal development, insect ecology, mass rearing.

Introduction

The olive fruit fly *Bactrocera oleae* (Rossi) was for a long time considered as a homodynamic insect, able to reproduce and develop throughout the year provided that the temperature and humidity are favourable and host fruit is available (Tzanakakis, 2003 and references therein). However, there is clear evidence that adult females, in many areas, manifest ovarian immaturity during late spring-early summer and that, in the same period of the year, most females had no sperms in their spermathecae and males did not respond to sex pheromone traps (Tzanakakis, 2003 and references therein). Earlier authors considered the effect of different abiotic factors on egg ripening in olive fruit fly (Fletcher *et al*., 1978; Fletcher and Kapatos, 1983; Kapatos and Fletcher, 1984; Tzanakakis and Koveos, 1986; Tzanakakis, 1987; Koveos and Tzanakakis, 1990, 1993). Among these factors, the role of the photoperiod was not fully clarified. An experimentally-induced ovarian immaturity in *B. oleae* is reported for the first time by Tzanakakis and Koveos (1986), who demonstrated that a high percentage of females did not mature their oocytes when the preimaginal stages developed under 18-20 °C and a short day; the adults were then kept under a higher temperature and a long day. Raspi *et al.* (1997) published a survey of field data relative to captures of *B. oleae* in central Italy, showing that this phytophage exhibits two annual reproductive peaks, in March-April and one in September-October, and a lack of mature eggs in the ovaries during late spring and early summer. This evidence is confirmed by other experimental data in different geographic areas (Baranov, 1937; Ayoutantis *et al*., 1954; Stavrakis, 1973; Delrio and Prota, 1975-76; Delrio and Cavalloro, 1977; Economopoulos *et al*., 1977; McFadden *et al*., 1977; Fletcher *et al*., 1978; Ballatori *et al*., 1981; Neuenschwander *et al*., 1986; Tzanakakis and Koveos, 1986; Raspi *et al*., 1996). More recently, Raspi *et al*. (2002) conducted a laboratory research aiming to verify the role of variable photoperiod on eggs maturation in olive fruit fly, showing markedly different responses (some treatments induced reproductive diapause) as a function of treatments administered and providing an explanation of the findings observed in nature. However, as far as we know, the relationship between the constant photoperiod and the presence of mature eggs in *B. oleae* has not been fully clarified previously. In successful *B. oleae* adult mass rearing, individuation of the optimal constant photoperiod for maximum egg production is of great importance, since it simplifies the general management of the rearing. Therefore, in the present work we conducted a laboratory study to investigate the effect of different constant photoperiods on the presence of mature eggs in *B. oleae*.

Materials and methods

Adults of *B. oleae* were submitted to the following photoperiodic treatments (LL:DD): 9:15, 10:14, 12:12,

15:9, 16:8, continuous light (LL) and continuous dark (DD). The adults of *B. oleae* to be subjected to treatment derived from fully-grown larvae originated from field infested olives, that were homogeneous both by sampling period (October-November) and locality (coastal Tuscany). The collected pupae were placed in cylindrical plexiglas cages inside climatic chambers at temperatures of 20°C, with relative humidity ranging from 55% to 60% and photoperiod as defined above. Emerged adults were fed on a diet consisting of a dried mix of sugar and yeast extract (Sigma® yeast extract - Y 4000) at ratio 10:1, respectively. Water was provided separately. Artificial light was obtained from typical neon tubes (Philips 30W/33); light intensity, measured inside the plexiglas cage, was approximately 1000 lux. The different photoperiodic treatments were carried out on 30 females that emerged during the photoperiodic treatment within a 24-h period of time and were consequently of the same age. Treatment duration was a fixed term of 15 days after emergence. For each photoperiodic treatment, the 30 females were randomly subdivided into 3 groups of 10 each. Each group (10 females and 10 males) was placed in a separate cage and subjected to treatment; therefore, there were 3 replicates for each treatment. After 15 days, the females, anaesthetized in CO_2 and embedded in 30% alcohol, were dissected and examined under a stereomicroscope in order to check for the possible presence (and number) of mature eggs.

To evaluate a possible effect of different types of light source, the treatment 15:9 was also conducted by using a new generation neon tube (Philips 18W/965 - high frequency). Moreover, in order to evaluate the effect of different light intensities the treatments 16:8 and 12:12 were also conducted using neon tubes producing a light intensity of approximately 3000 lux (Osram 18W/865 - high frequency). Finally, to evaluate the effect of temperature on the production of mature eggs the treatments 15:9, 12:12 and 9:15 were also conducted at a temperature of 26 °C.

The spectral quality (and light intensity) of the artificial lights used was estimated over the 300-1100 nm waveband (figure 1) using a LI-1800 spectroradiometer (LI-COR Inc., Lincoln, NE, USA) equipped with a remote cosine receptor.

The data on the effect of different photoperiodic treatments at 20 °C were subjected to one-way analysis of variance (ANOVA), while a two-way ANOVA was used to compare data obtained by using neon tubes producing different light intensities; percentage data were converted into angular values and means were separated with the least significant difference method (LSD) (Sokal and Rholf, 1981). The χ^2 test and the Student's *t* test were used to evaluate the effect of different types of light source and different temperatures (Sokal and Rholf, 1981).

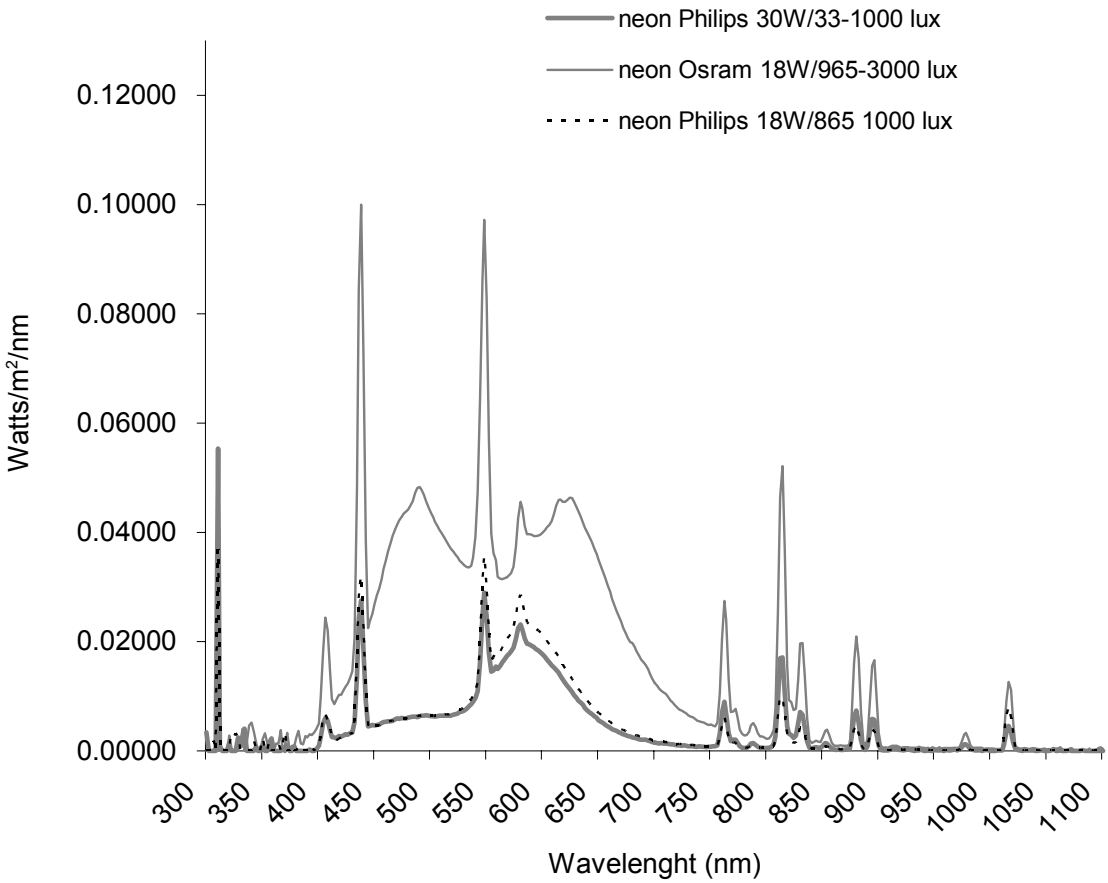

Figure 1. Comparison of the spectral quality, estimated inside the plexiglas cage, of the light sources used in the photoperiodic treatments. For each wavelength, the light intensity is given in Watt/m^2/nm. The values in lux are calculated in the wavelength 370-790 nm.

Results

Analysis of the results showed that all the photoperiodic treatments induced egg ripening in almost the totality of females (from 86.7% to 100%) and the mean number of eggs per female, at 15 days after the emergence, was high (from 21.95 to 35.4) (table 1). In contrast, in the case of DD treatment it was evident that this photoperiod induced egg maturation only in 10% of the treated population and the mean number of eggs/female was the lowest (table 1). The percentage of females with mature eggs was significantly higher in the treatments with a 16:8, 12:12, 10:14 and LL photoperiod than the remaining treatments (table 1). With regard to the treatments including two different neon typologies, no differences were found either in the amount of females with eggs or in the number of eggs/females (table 2). In contrast, when neon light producing different light intensities was used, a positive relationship between light intensity and number of eggs/female was found, while the percentage of specimens with mature eggs was not significantly different (table 3). Finally, it was evident that an increment in temperature (26 °C) did not influence either the percentage of females with mature eggs or the mean number of eggs/females (table 4).

Table 1. Ovarian maturity of olive fruit flies maintained for 15 days at various constant photoperiods, at 20 °C. Data followed by different letters within a column are significantly different (One-way ANOVA, P<0.05) using the Least Significant Difference (LSD) test. (S.D.= standard deviation; n=30).

Photoperiod (LL:DD)	Light intensity (lux)	Mean % females with eggs	Mean number eggs/female (S.D.)
16:8	1000 (Philips 30W/33)	100 a	28.4 (7.8) ab
15:9	1000 (Philips 30W/33)	90 bc	21.95 (15.9) b
12:12	1000 (Philips 30W/33)	100 a	35.4 (14.3) a
10:14	1000 (Philips 30W/33)	96.7 ab	32.9(16.45) a
9:15	1000 (Philips 30W/33)	86.7 c	31.9 (14.8) a
LL	1000 (Philips 30W/33)	100 a	34.8 (19) a
DD	-	10 d	12 (4) c

Table 2. The effect of light source. Ovarian maturity of olive fruit flies maintained for 15 days at constant 15:9 photoperiod obtained by a typical neon tube (30W/33) and a high frequency neon tube (18W/865), at 20 °C. Data followed by different letters within a column are significantly different (P<0.05) using the χ^2 test for the percentage of females with eggs and Student's *t* test for the mean number of eggs/females (S.D.= standard deviation; n=30).

Photoperiod (LL:DD)	Light type (lux)	Mean % females with eggs	Mean number eggs/female (S.D.)
15:9	Philips 30W/33 (1000)	90 a	21.95 (15.9) a
15:9	Philips 18W/865 (1000)	93.3 a	28.5 (13.7) a

Table 3. The effect of light intensity. Ovarian maturity of olive fruit flies maintained for 15 days at two constant photoperiods and two light intensities (1000 and 3000 lux), at 20 °C. Data followed by different letters within a column are significantly different (Two-way ANOVA, P<0.05) using the Least Significant Difference (LSD) test. (S.D.= standard deviation; n=30).

Photoperiod (LL:DD)	Light intensity (lux)	Mean % females with eggs	Mean number eggs/female (S.D.)
16:8	1000 (Philips 30W/33)	100 a	28.4 (7.8) b
16:8	3000 (Osram 18W/865)	100 a	52.8 (15.9) a
12:12	1000 (Philips 30W/33)	100 a	35.4 (14.3) b
12:12	3000 (Osram 18W/865)	100 a	50 (18) a

Table 4. The effect of temperature. Ovarian maturity of olive fruit flies maintained for 15 days at various constant photoperiods and at two temperatures, 20 °C and 26 °C. For each treatment, light intensity was 1000 lux (Philips 30W/33). For the same photoperiodic treatment, data followed by different letters within a line are significantly different (P<0.05), using the χ^2 test for the percentage of females with eggs and Student's *t* test for the mean number of eggs/females (S.D.= standard deviation; n=30).

Photoperiod (LL:DD)	Mean % females with eggs		Mean number eggs/female (S.D.)	
	20 °C	26 °C	20 °C	26 °C
15:9	90 a	96.7 a	21.95 (15.9) a	22.3 (13.4) a
12:12	100 a	100 a	35.4 (14.3) a	32.5 (11.6) a
9:15	86.7 a	100 a	31.9 (14.8) a	30.3 (13.65) a

Discussion and conclusions

Our results clearly showed that all the photoperiodic treatments induced egg ripening in almost the totality of females. It was also evident that the light intensity can positively influence only the number of eggs/female and not the amount of specimens with mature eggs, while an increment in temperature did not translate into significant differences as regards either the percentage of females with eggs or the mean number of eggs/female. Moreover, it was observed that the typical neon tubes and the high frequency neon tubes did not produce different responses. This latter result was expected, because the spectral quality of these two artificial lights proved to be very similar (figure 1). On the other hand, in photoperiod-controlled experiments it is of primary significance to define the source of light used (Philogène, 1982).

Our results are at variance with the data obtained by Raspi *et al*. (2002) using variable photoperiods, in where markedly different responses were found as a function of treatments administered. This latter evidence is of difficult interpretation and raises the question of why the olive fruit fly, in laboratory conditions, responds only to variable photoperiods. Obviously, the conditions of constant photoperiod do not exist in nature, because natural photoperiods change day by day as a function of latitude and time of the year. However, most experimental work on the induction of insect diapause has been carried out using constant photoperiod and the proportion of population entering diapause is plotted as a function of daylength (Saunders, 1982). Since the majority of insects are summer active, the most frequent photoperiodic response curve is the long-day type (the insects develop or reproduce in long days but become dormant in short days), while the short-day type of photoperiodic response characterizes a small number of insect species that are spring-autumn or winter-active, and pass the summer in an aestival diapause (Saunders, 1982). Moreover, a number of species living in latitudes where the summers are hot and dry and the winters cold may show both long and short-day responses (intermediate response), entering into diapause twice a year, namely in summer and winter, and becoming active at two seasons, spring and autumn (Danilevskii, 1965; Saunders, 1982). In a recent review on seasonal development and dormancy of insects feeding on olive Tzanakakis (2003) pointed out that the olive fruit fly is adapted to develop best in autumn, when its larval food is at its optimal condition for larval growth. In Tzanakakis' opinion, the lack of ovarian maturation during late spring-early summer, and the laboratory induction of reproductive diapause under conditions resembling those of that season (Tzanakakis and Koveos, 1986), shows that *B. oleae* is a short-day species (Tzanakakis, 2003). In our opinion, it is not possible to classify *B. oleae* in any of the above reported categories (long, short or intermediate response), because it is acknowledged that in various climatically distinct Mediterranean areas *B. oleae* shows a presence of two reproductive peaks (presence of females with mature eggs) in late winter and late summer, and a lack of mature eggs in the ovaries during late spring and early summer, both in the presence or absence of host fruits (see Raspi *et al*., 1997 and references therein). This field evidence is well in line with results of variable photoperiod treatments published by Raspi *et al*. (2002). In this respect, *B. oleae* may be considered as an intermediate response species but with two different active seasons, winter and summer (Raspi *et al*., 2002). The reproductive peak of *B. oleae* in late winter for the Mediterranean area may be justified considering that *B. oleae* is present, like most of the other *Bactrocera*, in Asia, in the Indian subcontinent at the level of the 34th parallel (Silvestri, 1916), i.e. a geographic area that lies within the isotherm 20 °C - 30 ºC (Pinna, 1977) and is also rich in wild Oleaceae.

Further investigations are necessary in order to fully clarify the different role of the constant and variable photoperiod on ovarian maturation in *B. oleae*. Overall, with regard to the rearing of this species, the results of this work confirmed that the olive fruit fly can be uninterruptedly reared in laboratory by using a constant photoperiod and temperatures ranging from 20 °C to 26 °C (Tzanakakis, 1989 and references therein). However, the use of a long photophase may be useful, because it makes it possible to go beyond the natural photoperiod. The evidence that the amount of mature eggs is influenced by light intensity suggests it may be effective to use a number of neon tubes producing high light intensities.

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