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DETERMINATION OF THE LIFE CYCLE OF THE OLIVE FRUIT LEAF MOTH, *PALPITA UNIONALIS (LEPIDOPTERA:PYRALIDAE) IN THE LABORATORY*

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Abstract

Palpita unionalis (Hübner), the olive leaf moth, is one of the most important pests of olive nurseries and olive orchards in Turkey and also in the Mediterrean Basin generally. In this study, *P. unionalis* was reared at 24 ± 1 °C, 65% RH and 16:8 h L:D in the laboratory on leaves of its natural olive host plant, *Oleae europeae* cv. 'Ayvalık'. Eggs were laid individually or in clusters on the underside of the olive leaves, but at random in the adult cages. There were 6 instars based on molts and head capsule measurements. Larval development was completed in an average of 23.35 ± 2.13 d and the survival rate was 60%. Adult longevity averaged 16.0 ± 1.57 d for females and 16.3 ± 1.21 d for males. The mean fecundity was 352 ± 42.9. Developmental period of immature stages, adults' life span, fecundity and sex ratios were determined in the laboratory.

Key Words: *Palpita unionalis*, olive leaf moth, biology, life cycle, olive, laboratory rearing

Resumen

Palpita unionalis (Hübner), la polilla de la hoja del olivo, es una de las plagas más importantes en los huertos y viveros de olivos. En este estudio, *P. unionalis* fue criado a 24 ± 1 °C, 65% HR y con un fotoperíodo L:O (luz y oscuridad) de 16:08 horas en el laboratorio sobre hojas de olivo, *Olea europea* L, en su planta hospedera natural. Los huevos fueron puestos por los adultos en las jaulas individualmente o en grupos en el envés de las hojas de olivo. Hubo 6 estadios basado sobre las mudas y las medidas de la cápsula cefálica. El desarrollo de la larvas se completó en un promedio de 23.35 ± 2.13 días y la tasa de supervivencia fue del 60%. El promedio para la longevidad de los adultos fue 16.0 ± 1.57 dias para las hembras y 16.3 ± 1.21 días para los machos. El promedio del número de huevos puestos por las hembras fue de 352 ± 42.9. Las polillas han sido criadas en el laboratorio continuamente durante 2 años en las hojas de olivo.

Olives (*Olea europea* L.) are one of the economically important crops in the Mediterranean Basin, where 98% of the world's olive production is located (Herz et al. 2005). Turkey is the 5th largest olive growing country in the world, having a 600.000 ha olive production area. On the other hand, several insect pests attack olive trees and cause considerable yield losses. These pests belong to Diptera, Lepidoptera, Hemiptera, Orthoptera, Coleoptera, and Thysanoptera (Spooner-Hart et al. 2007). Many studies have been done on the biology of insect pests of olive trees. (Ramos et al. 1998; Broumas et al. 2002; Shehata et al. 2003). There are 2 well known lepidopteran pest species of olives, namely, the olive moth (*Prays oleae)* (Lepidoptera: Yponomeutidae), the and Jasmine moth, also known as the olive leaf moth (*Palpita unionalis)* (Lepidoptera: Pyralidae). The olive leaf moth, *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae: Pyraustinae) was a secondary pest of olive (*Oleae europea* L.; Lamiales: Oleaceae) cultivars but now it is considered as a primary pest in olive nurseries or irrigated young olive plantations. Also its importance has increased in mature olive trees. *Palpita unionalis* has been known as *Margaronia unionalis* (Hübner) (Santorini & Vessiliana-Alexopoulou 1976) or *Palpita vitrealis* (Anonymous 2011), but Kitri & Rose (1992) suggested from morphological studies based on the internal and external differences in the reproductive system of males that this species belongs to the genus *Palpita*.

The olive leaf moth is a migratory pest that originated in the Mediterranean region and is now distributed in Italy, Spain, Greece, North Africa, Portugal, the tropical regions of the Americas, and Japan (Balachowsky 1972). It has been reported in Sweden and Poland (Santorini & Vessiliana-Alexopoulou 1976; Sevensson 1988), and is a quarantine pest in Iran (Saieb 1999). The adults of the olive leaf moth are characterized mainly by white wings with a brown

leading edge of the forewing and two black spots in the middle. The wings appear triangular at rest (Alford 1998; Khaghaninia & Pourabad 2009). Adult males and females look alike. It is active from July to late autumn, and has 2 to 3 generations per yr. The larvae are also very active, and are mainly greenish to yellowish green in color. They feed on young shoots and ripening olive fruits. Damage to the new shoots is extensive in nurseries (Athanassiou et al. 2004). Pupation occurs within a cocoon inside to spun of several leaves. The eggs are laid singly or in small groups on undersides of host plant leaves. It is reported in northern Europe on Jasmine (*Jasmimum* spp. Oleacea), and infestations are sometimes found on decorative, container-grown olive trees, imported into northern Europe from Italy and other countries where the pest is endemic (Alford 1998). The olive leaf moth is a serious pest of the Oleaceae family, especially the genera of *Jasmine*, *Ligustrum*, *Oleae*, *Fraxinus*, and *Phyllyrea* (Tzanakakis 2003; Athanassiou et al. 2004), and it is now an important pest in olive orchards and nurseries in Turkey. Strawberry (*Fragaria* × *ananassa*; Rosales: Rosaceae) and Viburnum (*Viburnum* spp.; Dipsacales: Adoxaceae) are also reported as alternative hosts for this pest (Khaghaninia & Pourabad 2009).

Various biological aspects of the olive leaf moth have been reported. It has been studied with respect to population fluctuations in Bursa, Turkey (Kovancı et al. 2006), life tables reported on different hosts (Kumral et al. 2007), rearing on *Ligustrum vulgare* (Azimizade et al. 2003), its pest biology in Egypt (Shehata et al. 2003; Khaghaninia & Pourabad 2009; Alavi 2010), and the influence of temperature on embryonic development (Loi 1990). The moth has 4 or 5 generations in Greece, Iran and Italy, 6 in Israel (Avidov & Harpaz 1969; Zervas et al. 1989; Fodale & Mule 1990; Tzanakakis 2003; Nouri et al. 2007), 5 in Spain (Fodale & Mule 1990), 10 in Eygypt (El-Kifl et al. 1974), 1 or 2 in France (Balachowsky 1972) and 2 complete and 1 partial generation in Turkey every year (Kovancı et al. 2006). The pest biology was studied in the laboratory at 25 ± 1 °C showed that there were 5 instars (Grossley 2000; Khaghaninia & Pouraba 2009; Noori & Shirazi, 2012). On the other hand, other investigators (El-Khifl et al. 1974; Badawi et al. 1976; Tzanakakis 2003 and Alavi 2010) have reported 6 instars, but none of the these studies included head capsule measurements to determine the exact number of the instars.

When outbreaks occur of *P. unionalis* occur, it causes severe damage to olive orchards and especially to nursery plants (Kovancı & Kumral 2004; Kovancı et al. 2006). Recently there have been increasing incidences of olive leaf moth outbreaks in Turkey, but there is still no insecticide has been registered for use against this pest. Farmers usually use organophosphate and systemic insecticides registered for olive moth (*Prays oleae*) to control olive leaf moth. Precise knowledge of the developmental periods of the immature stages, adult survival and fecundity are very important in planning an IPM programme to control olive leaf moth. Maintenance of the laboratory colony on it's primary host is also crucial to the development of new and alternative management techniques, such as sterile insect technique (SIT).

The objectives of the present study are to characterize the immature stages of *P. unionalis* feeding on olive leaves under laboratory conditions, to elucidate some aspects of the biology of the pest including the number of larval stages based on larval head capsule measurements. This information will support development of strategies for management of this pest.

Materials and Methods

The initial population of the olive leaf moth was started with field collected larvae from infested olive orchards in Çanakkale province, Turkey. The larvae were kept in Tupperware® containers (30 × 18 × 7 cm) with fresh olive shoots coiled with moist cotton (Fig. 1A). Pupae were transferred to plexiglass cages (30 × 17 cm). Fresh olive shoots were provided in the adult rearing cages along with 10% honey solution dispensed on cotton balls (Fig. 1B). Eggs were laid on olive shoots as well as on the plexiglass cage. Eggs were removed daily and kept in petri dishes with moist filter paper until hatching (Fig. 1C). The colony was maintained at 24 ± 1 °C, 65% RH and 16:8 h L:D in the growth chamber.

To determine the larval development stages, newly enclosed larvae were individually transferred into plexiglass containers (16 × 10 × 5 cm) with 3-4 tender fresh olive leaves (*Oleae europeae* cv. 'Ayvalık') obtained from nurseries (Fig. 1D). Larval food was changed every other d by inserting new plants. The number of instars was determined from data collected from 35 indivually kept larvae (Fig. 1D), examined daily, and whose molts were recorded. Shed larval head capsules were obtained, measured at the widest point by micrometer attached on the ocular of the stereozoom microscobe and preserved in 70% ethyl alcohol. Each day 35 larvae were weighed individually and their lengths were measured. Embryonic development (d from oviposition to hatching), larval period (d from egg hatch to prepupation), prepupal period (d from prepupation to pupation) and pupal period (d from pupation to adult emergence) were examined and photographed by an Olympus C7070 wide zoom zamera attached to an Olympus SZX9 stereo zoom microscope. To determine the oviposition time, fecundity and adults' survival, a number of pu-

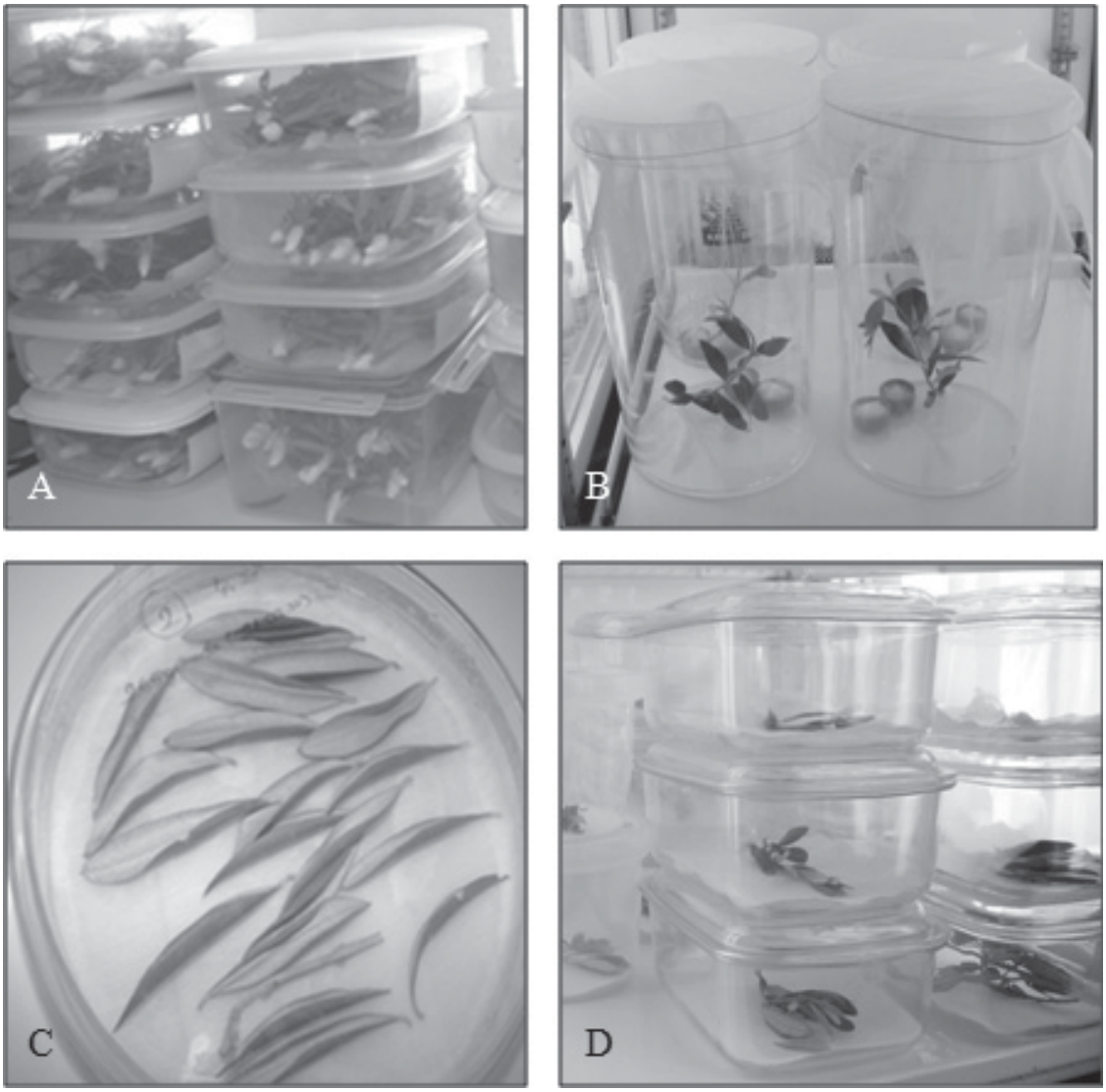

Fig. 1. Laboratory maintenance of the olive leaf moth, *Palpita unionalis*. (A) Rearing of the larval colony; (B) Adult cages; (C) Eggs harvested from the colony; (D) Rearing of individual larvae.

pae were collected from laboratory colony. Newly emerged adults (1♀:1♂, per container with 13 replicates) were placed in plexiglass cages containing 3-4 tender fresh olive shoots coiled with moist cotton and were provided with 10% honey solution. After mating, adult mortality and the number of eggs laid were recorded daily. The sex ratio of adults was determined by careful examination and recorded. Data analysis were carried out through ANOVA (SAS 1999). When necessary data were normalized through proper conversion. The LSD test at 0.05 level of significance was used to determine seperation and significance of means (SAS 1999).

Results

Eggs

Olive leaf moths deposit their eggs individually or in small masses (2-11 eggs) on the undersides of olive leaves, as well as on the walls of the adult cages (Fig. 2A). They usually deposit egg masses of 6-36 eggs, and the biggest egg mass observed in the laboratory had 120 eggs. Eggs are elongated, flattened, and about 0.80 ± 0.10 mm in length, 0.5 1± 0.07 mm in diam, weigh about 0.0001 g (n = 35). Newly laid eggs were yellowish green, and as the embryo developed,

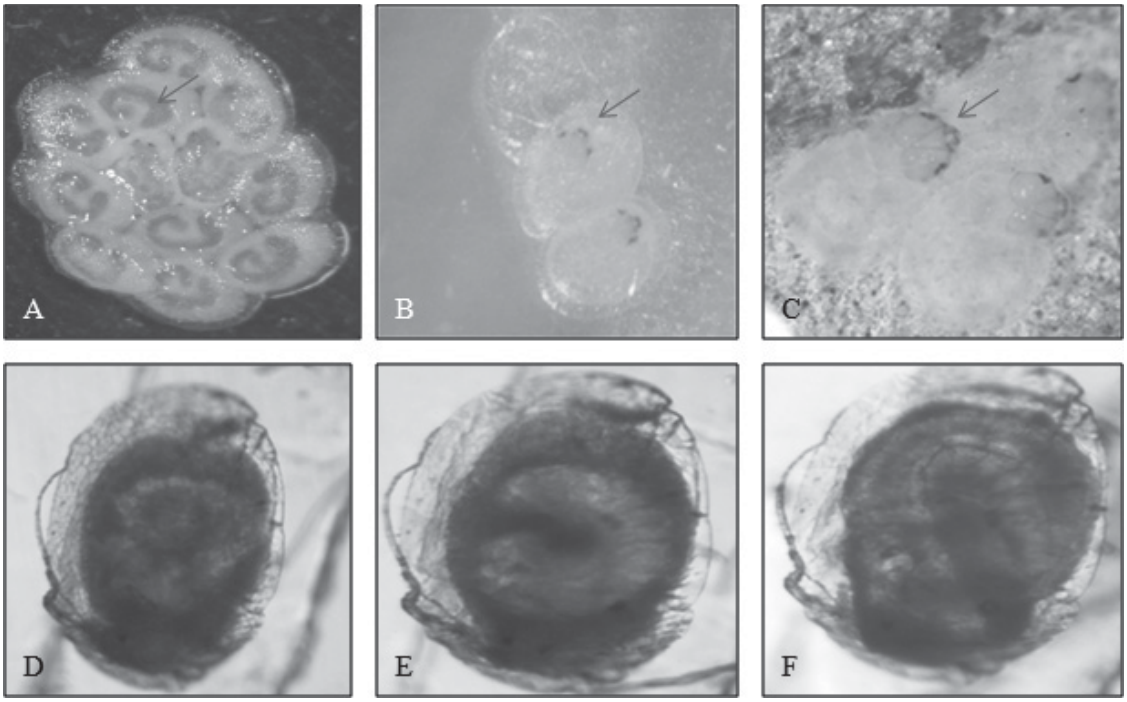

Fig. 2. Egg masses and embryonic development of the olive leaf moth. (A) Newly laid egg mass; (B) Developing eggs; (C) Hatching eggs; (D) One day old first instar; (E) Two day-old larva; (F) Four day-old larva. Arrow shows the visible larva developing inside the egg shell.

brownish red spots appeared on the dorsal side of the eggs (Fig. 2B and C), and subsequently the head of the developing larva became visible through the chorion (Fig. 2C). The mean period for egg incubation was 4.16 ± 0.09 d at 24 ± 1 °C, with 82.6% viability. The chorion was clear and had a honeycombed appearance. A day-old embryo was visible inside the chorion (Fig. 2D). When the embryo was 2 d-old, there were a few red-brown spots on the dorsal side of the egg (Fig. 2E). The mandibles, head of the larva and larval segments became visible in 3 d-old embryos. On the d 4 of embryonic development, the larva was active, and the movement of head was very apparent. The mandibles of the larva were brown and black spots of the eyes could be seen through the chorion (Fig. 2F).

Table 1. Measurements (mean ± SD, N = 14) of lengths and widths of head capsules of the six larval instars of the olive leaf moth, *Palpita unionalis*.

Instar	Head capsule length (mm)	Head capsule width (mm)
First	0.21 ± 0.02 A	0.18 ± 0.03 A
Second	0.30 ± 0.11 B	0.27 ± 0.01 B
Third	0.45 ± 0.02 C	0.45 ± 0.02 C
Fourth	0.68 ± 0.02 D	0.68 ± 0.02 D
Fifth	1.01 ± 0.13 E	1.03 ± 0.01 E
Sixth	1.96 ± 0.44 F	1.53 ± 0.04 F
LSD*	0.0356	0.0271

*LSD = Fisher's Least Significant Difference between any two means. The means within the column followed by a different letter are significantly different from each other ($P \leq 0.05$).

Larvae

We observed 6 instars based on direct quantification of molts and measurements of the head capsule length and width (Table 1). The size of the head capsule was 0.18 ± 0.03 mm for the first instar and up to 1.53 ± 0.04 mm for sixth instar. The first instar was pale light yellow or translucent yellow, with long setae on the body (Fig. 3A). The head was pale light brown and the width of the head was wider than the first larval thoracic segment. The legs and prolegs were translucent yellow. First instars aggregated, and usually fed on the parenchyma of the olive leaves and on the tender buds. The length and width of each of the 6 instars, and their corresponding weight and duration are shown in Table 2. The first instar was 2.15 ± 0.02 mm long, 0.23 ± 0.05 mm wide, and weighed 0.1 ± 0.01 mg (Table 2). The duration of the first stadium was 2.93 ± 0.73 d in the laboratory. The second instar was light yellow in color, but the head

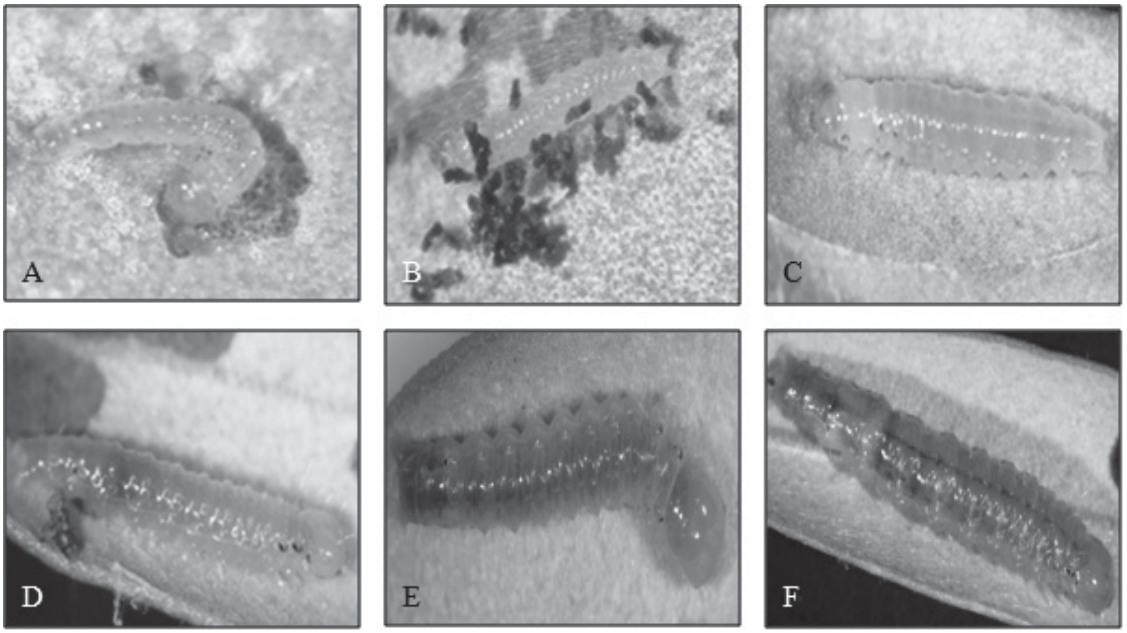

Fig. 3. Larval stages of the olive leaf moth. (A) First instar larva; (B) Second instar larva; (C) Third instar larva, (D) Fourth instar larva; (E) Fifth instar larva; and (F) Sixth instar larva.

was light brown and the mandibles were dark brown (Fig 3B). The thoracic legs were brown. This instar was 2.90 ± 0.05 mm long, 0.33 ± 0.01 mm wide, and weighed 0.3 ± 0.01 mg (Table 2). The duration of the second stadium was 3.42 ± 1.55 d in the laboratory. The third instar was light green in color and each thoracic segment had a lateral black spot (Fig. 3C). The head was dark yellow brown and the first thoracic segment was light yellow in color. The third instar was 4.38 ± 0.27 mm long, 0.51 ± 0.03 mm wide, and weighed 0.93 ± 0.1 mg (Table 2). The duration was 3.42 ± 1.15 d in the laboratory. Fourth and fifth instars were similar in appearance to each other (Fig. 3D and E). The fourth instar was 6.98 ± 0.33 mm long, 0.86 ± 0.06 mm wide, and weighed 3.1 ± 0.6 mg (Table 2). The duration of the fourth stadium was 3.00 ± 0.96 d in the laboratory. The fifth instar was 9.93 ± 0.33 mm long, 1.39 ± 0.07 mm wide, and weighed 8.6 ± 1.8 mg (Table 2) and the duration was 3.57 ± 1.28 d in the laboratory; and its thoracic legs were brown. The sixth instar was green to dark green in appearance with a dark brown head. The sixth instar was 14.80 ± 0.37 mm long, 1.88 ± 0.07 mm wide, and weighed 32.6 ± 2.1 mg (Table 2). The duration of the sixth stadium was 7.00 ± 1.56 d in the laboratory. The total period of larval development of the olive leaf moth was 23.35 ± 2.13 d. The survival of larval stages was 61.6% on olive leaves.

Prepupae

Mature larvae stopped feeding and attached to a few olive leaves or any supportive surface in the larval feeding containers with silken filaments and remained there about 1.81 ± 0.40 d. They changed within 4-5 minutes into the characteristic pupal appearance (Fig. 4A)

Table 2. Measurements of the lengths, widths and weights of olive leaf moth larvae in each instar (mean ± sd, N = 14), and duration of the corresponding stadia.

Instar	Length (mm)	Width (mm)	Weight (mg)	Duration (Day)
First	2.15 ± 0.02 A	0.23 ± 0.05 A	0.01 ± 0.01 A	2.93 ± 0.73
Second	2.90 ± 0.05 B	0.33 ± 0.01 B	0.03 ± 0.01 B	3.42 ± 1.55
Third	4.38 ± 0.27 C	0.51 ± 0.03 C	0.09 ± 0.01 CD	3.52 ± 1.15
Fourth	6.98 ± 0.33 D	0.86 ± 0.06 D	0.31 ± 0.06 D	3.00 ± 0.96
Fifth	9.93 ± 0.33 E	1.39 ± 0.07 E	0.86 ± 0.01 E	3.57 ± 1.28
Sixth	14.80 ± 0.37 F	1.88 ± 0.07 F	3.26 ± 0.02 F	7.00 ± 1.56

The means within the column followed by a different letter are significantly different from each other ($P \leq 0.05$)

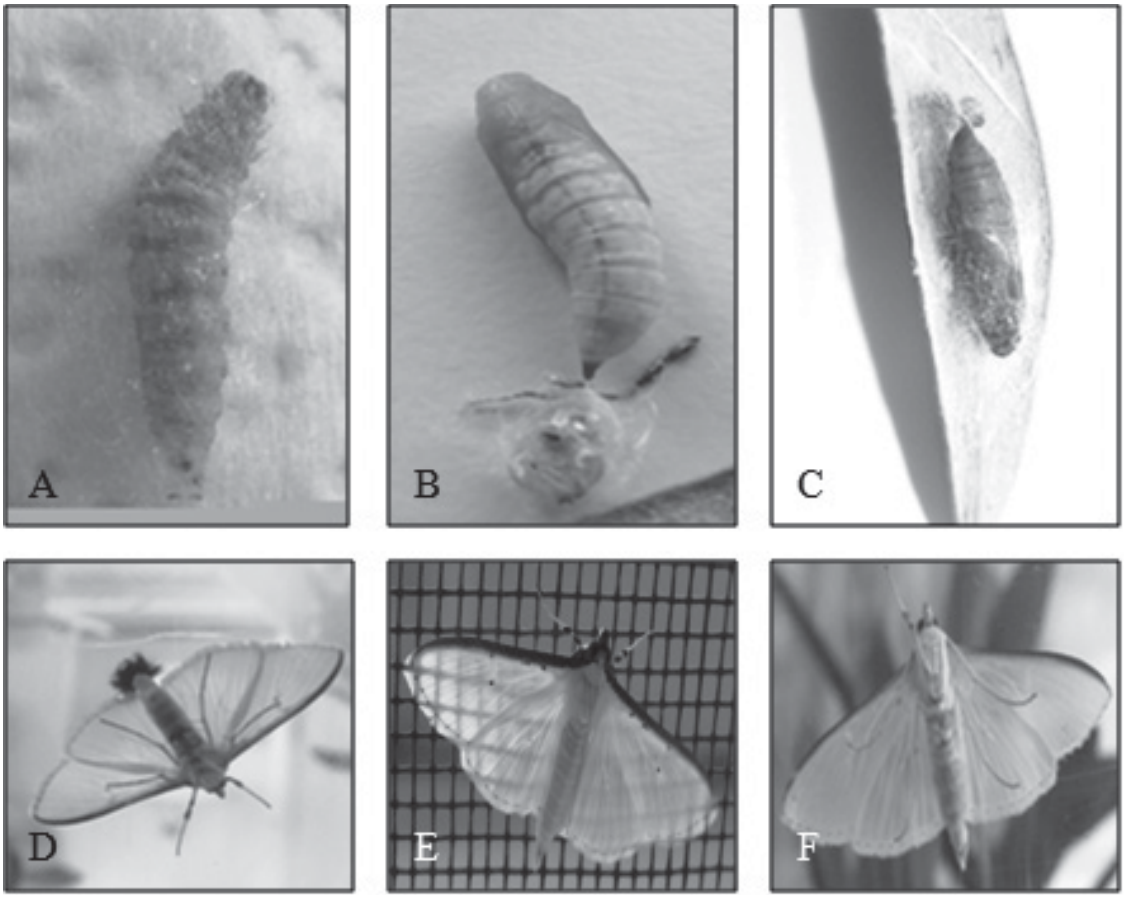

Fig. 4. Olive leaf moth. (A) Prepupa; (B) Puparium; (C) Pupa about to emerge; (D) Male, (E) Female dorsal view; (F) Female ventral view.

Pupae

Initially the pupae were soft, and light green in color (Fig. 4B). They had light green to brown areas over the abdomen and green over the wings and head (Fig. 4B). The pupal color turned to brown on the following day (Fig. 4C). Female pupae measured about 14.01 ± 0.90 mm long, 3.07 ± 0.23 mm wide, and weighed 73.61 ± 11.77 mg. Male pupae were about 13.38 ± 0.80 mm long, 2.98 ± 0.21 mm wide and weighed 70.6 ± 12.78 mg. The duration of pupal stadium was 9.87 ± 1.03 d. Developing wings were apparent on the dorsal side of the pupa. Pupal emergence was 82.8% . The observed sex ratio was (male:female) 1.2:1 (Fig 4).

Adults

Adult males and females were similar in apperance. They were pearl or white, wings were semitransparent, and triangular at rest. The costal margin was brown and there were 2 black spots in the middle on the forewings. The wingspan was about 25.0 ± 3.3 mm in males and 22.6 ± 5.1 mm in females (n = 25) (Fig. 4D, E and F) . Mating occurred within a d after emergence. Table 3 shows adult longevity, duration of oviposition and the number of eggs laid per female. The female preoviposition period lasted about 2.3 ± 0.3 d. The duration of the oviposition and postoviposition periods were 8.0 ± 0.7 d and 2.4 ± 0.4 d, respectively. Adult longevity was similar in males and females. The mean longevity was 16.3 ± 1.21 d for males and 16.0 ± 1.57 d for females. Moreover, most of the eggs were deposited a few days after oviposition. Females laid more eggs in early ages and then fecundity decreased towards the end of their lives (Fig. 5).The mean fecundity was 352 ± 42.9 eggs. The survival rate of eclosed adults was 86.9 % in males and 82.8% in females.

Discussion

Detailed description of the immature stages and the life cycle of *Palpita unionalis* Hübner were de-

TABLE 3. ADULT LONGEVITY, DURATION OF OVIPOSITION AND FECUNDITY OF THE OLIVE LEAF MOTH (MEAN ± SD, N = 13).

Replication	Adult longevity (days)		Duration of oviposition (days)			No. of eggs laid per female
	♀	♂	Preoviposition	Oviposition	Postoviposition	
1	13	8	3	5	4	432
2	14	10	3	5	2	148
3	17	12	4	7	1	447
4	13	13	5	6	1	145
5	11	13	2	5	3	168
6	17	17	1	14	1	385
7	26	26	2	10	1	169
8	22	25	2	11	5	611
9	13	17	2	8	2	496
10	15	12	1	9	4	454
11	12	22	1	9	1	465
12	20	20	2	6	5	388
13	15	17	2	9	2	268
Mean ± SD	16.0 ± 1.57	16.3 ± 1.21	2.3 ± 0.3	8.0 ± 0.7	2.4 ± 0.4	352 ± 42.9

termined in this study. The mean total developmental period for *P. unionalis* reared on Ayvacık olive cultivar at 24 ± 1 °C, 65 % RH and with 16:8 (L:D) was found to be 24 d. Our data from laboratory rearing of the moths are similar to data obtained by Kumral at al. (2007) as 27.52 to 30.00 d on different host plants, 27 d reported on olive by Shehata et al. (2003), and 26 d reported by Vassilaina-Alexopoulou & Santorini (1973). However, El-Khawas (2000) reported that duration of pre-imaginal development varied from 21 to 30 days at 27 °C. Badawi et al (1976) and Arambourg (1986) stated that post-embryonic development ranged from 29.2 to 32 days on different host plants. The differences on larval developmental time could depend on larval host, olive varieties, and the biotype of the pest.

The first detailed embryonic development of *P. unionalis* eggs were described in this study. The embryo was clearly evident through the chorion during development, with head, mouth parts, and legs visible. Mean duration of the embriyonic development was found as 4.16 ± 0.09 d at 24 ± 1 °C, while Noori & Shirazi (2012) have reported 5.8 ± 1 d and El-Kifl et al (1974) stated 3-9 d under suitable conditions.

There have been different reports on the larval development and instars. We determined 6 instars based on molts and width of head capsules in the laboratory. Similarly, El-Kifl et al. (1974), Badawi et al. (1976), and Tzanakakis (2003) was also stated that it has 6 instars. Khaghaninia & Pourabad (2009), however, reported 5 instars on olives.

Mean duration of pre-pupal and pupal stages was found as 1.81 ± 0.40 d and 9.87 ± 1.03 d under laboratory conditions. Similarly, El Kifl et al.

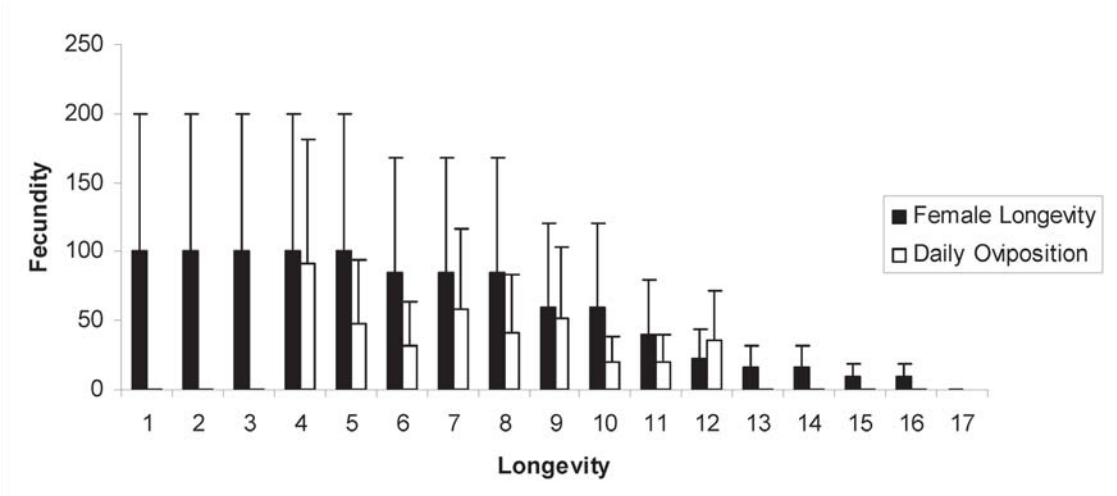

Fig. 5. Longevity (days) and daily oviposition curve of *Palpita unionalis* females.

(1974) reported pre-pupatin and pupation period as 1-2.5 d and 9 d respectively.

Fecundity of *P. unionalis* determined about 352 ± 42.9 eggs at 24 ± 1 °C. However, it was different from results obtained by Vassilaina-Alexopoulou & Santorini (1973) who reported the number of eggs laid was varied from 86 and 515 with a mean of 209. Kumral et al. (2007) reported that the average number of eggs laid by per female ranged from 194 to 390 under similar laboratory conditions.

Adult longevity of *P. unionalis* reared on olives was 16.3 ± 1.21 d for males and 16.0 ± 1.57 d for females. However, Kumral et al. (2007) and Shehata et al. (2003) reported that adult longevity varied 9.92 d for females and 9.00 d for males and 11.4 d for females and 11.9 d for males on olive under similar laboratory conditions. The differences on adult longevity could be due to adult diet.

In our study, we reared *P. unionalis* on fresh olive leaves and olive shoots in the laboratory for 2 years. Immature stages of *P. unionalis* were studied in details and photographed. Adults were mated readily and laid eggs anywhere in the cages. Availability of the olives in the adult cages were not critical for oviposition and the moths could be reared all year around. We found the moths easy to rear in the laboratory, and during our 2 years of rearing, we did not find any evidence of disease or difficulties with rearing. Meanwhile, the olive leaf moth can be a model lepidopteran species for further research in physiology, molecular biology, behavior and pest management.

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