Infestations of Olive Fruit Fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), in Different Olive Cultivars in Çanakkale, Turkey

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Abstract-The olive fruit fly, Bactrocera oleae (Rossi), is an economically important and endemic pest in olive (Oleae europae) orchards in Turkey. The aim of this study was to determine olive fruit fly infestation in different olive cultivars in the laboratory. Olive fly infested fruits were collected in Çanakkale province to establish wild fly population. After having reproductive olive fly colonies, 14 olive cultivars were tested in the controlled laboratory conditions, at 23±2 °C, 65% RH and 16:8 h (light: dark) photoperiod. The olive samples from 14 different olive cultivars were collected in October 2015, in Campus of Dardanos, Canakkale Onsekiz Mart University. Observations were carried out detecting some biological parameters such as the number of oviposition stings, active infestation, total infestation, the number of pupae and the adult emergence. The results indicated that oviposition stings were not associated with pupal yield. A few pupae were found within olive fruits which were not able to exit. Screening of the varieties suggested that less susceptible cultivar to olive fruit fly attacks was Arbequin while Gemlik-2M 2/3 showed significant susceptibility. Ovipositional preference of olive fly females and the success of larval development in different olive varieties are crucial for establishing new olive orchards to prevent high olive fruit fly infestation.

Keywords—Infestation, olive fruit fly, olive cultivars, oviposition sting.

I. INTRODUCTION

THE olive, *Oleae europaea* L. is an evergreen tree and common crop cultivated in Turkey, Greece, Spain, Italy and other Mediterranean countries [1]. The olive is known to be originated in the sub-Saharan Africa region and then distributed to the Mediterranean region [1], [2]. Olive is relatively a new crop cultivated in California, Argentina and Florida. Some studies indicate that olive domesticated in the Eastern Mediterranean region in 10,000 years ago. Olive has a great diversity of cultivars, currently known to be more than 1200 cultivated olive trees in worldwide and can be distinguished by their physical and chemical properties such as size, shape and oil content [2]-[5].

The olive fruit fly, *Bactrocera oleae* Rossi (Diptera: Tephritidae), is the one of the most important insect pests of the olive orchards [3], [6], [7]. It is considered as homodynamic pest [8], meaning that it reproduces and develops all year around in favorable climates. However,

adults of olive fruit fly are not active in hot summers above 35 °C; they probably hide themselves in cooler places.

The olive fruit fly is a monophagous pest on both cultivated and wild olive fruits [6], [8]. The females deposit their eggs into olive fruits [6], embryonic development takes about 3-4 days at 24-25 °C and then the newly hatched larvae start feeding on mesocarp of the olive fruit [6], [9]. The direct damage is caused by larval feeding. Microbial infections may occur in infested fruits resulting further rotting. Adult females also damage fruits during egg laying that make table olives unmarketable. Even though damage can be variable years to years, the estimated economic losses are caused by olive fruit fly are approximately \$800 million/year [6], [10].

There are several studies on different aspects of olive fruit fly such as its biology and laboratory rearing on natural host [6], [8], [11], domestication of olive fruit fly [3], [13] rearing on artificial diet [9], [12], residual pesticide effect on adults [14], embryonic development of olive fruit fly [15], organophosphate resistance in the acetylcholinesterase gene [16], [17] and germline transformation of olive fruit fly [18], [19].

Susceptibility of olive cultivars to olive fruit fly attack has also been studied in different countries previously [20]-[24]. It is known that visual and especially chemical clues are important for tephritid flies to find their proper hosts [24]. They use plant color, fruit shape and size, and some volatiles which help females to identify the host or cultivars. Olive fly female walks on the fruit and searches an appropriate spot with their tarsi then inserts the ovipositor into the fruit, sometimes she deposits an egg in that place or not [2], [6], [24]. Oviposition process takes about 6-13 minutes in the laboratory conditions [6]. The oviposition behavior results in a brown spot either with an egg or not causing decrease of market value of table olive fruits.

The aim of the present study is to determine the infestation of olive fruit fly in different olive cultivars in the laboratory conditions.

II. MATERIALS AND METHODS

Infested olive fruits were collected from the experimental olive orchard at Çanakkale Onsekiz Mart University, Dardanos Campus in October 2015 in Çanakkale. The olive cultivars were shown in Fig. 1. Infested olives were placed in paper bags and brought to the laboratory. Laboratory rearing of olive fruit fly procedures were followed to establish a colony [6], [15].

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International Journal of Biological, Life and Agricultural Sciences ISSN: 2415-6612 Vol:10, No:7, 2016



Fig. 1 Experimental olive orchard- Campus of Dardanos, Çanakkale Onsekiz Mart University



Fig. 2 Establishment of a laboratory colony using different olive cultivars (a) oviposition into different cultivars and (b) larva exits fruits



Fig. 3 Larval development inside the olive fruits (a) partially visible larva feeding inside the fruit, (b) mature larvae marked with red circle and red arrows indicate the exit holes (c) mature larvae and pupae

The healthy and untreated olive fruits from different olive varieties were collected from the experimental olive orchard (Fig. 1) to use for both colony maintenance and set up the experiments. Adult flies were placed in plastic and fine mesh screen cages ($30 \times 30 \times 30$ in dimension) provided with adult food and water supply [6]. Established laboratory colony of adults was shown in below. Females of the flies deposit eggs individually inside the olive fruits (Fig. 2 (a)) after three instars, the larva exits from the fruit (Fig. 2 (b)). Several olive

fly larvae may develop inside a single olive fruit [6] (Fig. 3 (a)), mature larva just before exiting the fruit can be visible with a hole (Fig. 3 (b)). Mature larvae (3^{rd} instar) and pharate pupae were shown in a petri dish (Fig. 3 (c)). They are used to maintain laboratory population of olive fruit fly.

After reproductive olive fruit fly colony established in the laboratory in November 2015, 14 olive cultivars, Manzanilla, Nergal-1, Nergal-2, Nergal-3, Luques, Ascolano. Tavşanyüreği, Arbequin, Verdial, Karamürsel Su, Eğriburun, Gemlik 2M 2/3, Gemlik 3G 12/2 and Gemlik 0-12 were tested in this study. Several olive fruit samples (20-30 fruits) from each cultivar were taken randomly and kept in the labeled paper bags at 4°C until used. The experimental adult cages had 200 individuals (100 , 100 ,) and were about a week old. Fifteen olive fruit samples from each olive cultivar were placed inside the adult cage and kept for 2 days for oviposition. Then each tested fruit was weighted and oviposition stings were counted and examined under Olympus SZX9 stereozoom microscope and photographed. The fruit infestation and injury were monitored from the beginning of egg laying to the adult emergence. All experiments were taken in the controlled laboratory conditions, at 23±2 °C, 65% RH and 16:8 h (light: dark) photoperiod. Observations were carried out to detect some biological parameters such as, the number of oviposition stings, the number of pupae and the adult emergence

III. RESULTS AND DISCUSSIONS

The olive fruit fly oviposition and infestation of fourteen cultivars, Manzanilla, Nergal-1, Nergal-2, Nergal-3, Luques, Ascolano, Tavşanyüreği, Arbequin, Verdial, Karamürsel Su, Eğriburun, Gemlik 2M 2/3, Gemlik 3G 12/2 and Gemlik 0-12, were investigated in this study. The observed olive cultivars showed different level of susceptibility to olive fruit fly attack in the laboratory conditions (Fig. 4). Oviposition (depositing eggs) and oviposition behaviour were shown in the cultivars Lucques (Figs. 4 (a) and (b)) and Tavsanyüreği (Figs. 4 (c) and (d)). Several females were ready to lay eggs and searched for appropriate places on a single fruit (Fig. 4). Ovipositions usually occurred in the late afternoon and most of the eggs were laid about in the first 48-hour after mating. Several oviposition stings on each olive fruit cultivars were shown in Fig. 5. It was obvious that some parts of the olive fruit were more preferable for egg laying than others (Fig. 5).

All olive fruit samples representing each cultivar were collected in October, 2015. Tested olive cultivars and the mean values of olive fruit fly infestation for each cultivar were indicated in Table I. The mean weight of olive fruits varied from 2.34 g to 6.69 g. The results showed that olive fruit fly attack was not correlated with fruit weight. Although, both fertile and sterile eggs were determined, sterile oviposition stings were abundant for several tested cultivars (Table I); therefore, the numbers of oviposition stings were not representing the amount of pupae or the number of harvested pupae. After larval development was completed inside the fruits, the infested fruits were dissected to check any presence of mature larvae and/or pupae. Totally, there were 31 dead

International Journal of Biological, Life and Agricultural Sciences ISSN: 2415-6612 Vol:10, No:7, 2016

mature larvae/pupae within olive fruits which were not able to exit, 2 individuals from Nergal-III, 1 from Luques, 7 from Ascolano, 5 from Tavşan yüreği, 8 from Arbequin, 3 from Verdial, 2 from Karamürsel Su, 2 from Gemlik 3-G 12/2, and 1 from Gemlik O-12. As a result, screening of the different olive varieties to olive fruit fly attack showed that less susceptible cultivar to olive fruit fly attacks as Arbequin while Gemlik-2M 2/3 showed significant susceptibility (Table I). The females of olive fruit fly preferred to lay eggs in cultivar Gemlik 2M-2/3 (83.21±6.14) which had the highest number of oviposition stings per olive fruit (Table I). The lowest number of oviposition stings was found in cultivar Arbequin (11.58±2.69). Pupal yield was related with the weight of the cultivars. Eğriburun, a local cultivar, weighted about 6.69±0.79 g and the resulting as better larval performance and the number of pupae was 10.28±4.51 and 8 adults (3 $^{\bigcirc}_{+}$ and 5⁽³⁾) emerged. Manzanilla cultivar had 30.84±2.58 oviposition stings and 6.02±3.10 pupal yield with 5 adults (4^{\bigcirc}_{+} and 1^{\triangleleft}_{-}). The lowest pupal yield was found in cultivar Gemlik 0-12 as 1.66±1.54 and only one female survived (Table I).

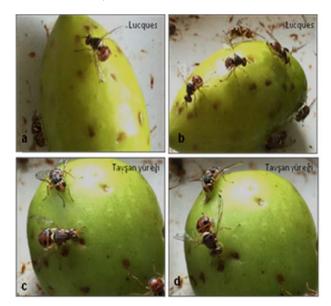


Fig. 4 Oviposition of olive fruit fly female (a) oviposition into Lucques cultivar, (b) several females lay eggs into Lucques, (c) oviposition into Tavşan yüreği cultivar and (d) female search a place to oviposit into Tavşan yüreği cultivar

Several studies showed that there was a correlation between oviposition preference, larval performance and cultivars [2], [5], [7], [20], [21], [23]. It is also known that olive fruit fly females have a critical role in the selection of the olive varieties, in olive infestation and the phenological stage of the olive fruit [2]. Previous studies referred that the colour of the fruit was also important to choose the oviposition sites among the cultivar in the same olive orchards [2], [5], [7]. Olive fly females prefer varieties having bigger fruits than smaller ones; however; some studies showed that size of the fruit is not a key factor determining ovipositional preferences [2].



Fig. 5 Oviposition stings of olive fruit fly on different olive varieties

TABLE I
OLIVE CULTIVARS AND MEAN VALUES OF OLIVE FRUIT FLY INFESTATION TO
FACE CHITIMAR IN THE LAROPATORY CONDITIONS (MEAN+SD)

Entern Cobin	EACH CULTIVAR IN THE LABORATORY CONDITIONS (MEAN±				. of
Cultivar	Weight (g)/olive*	oviposition stings/olive**	No. of Pupae	Ad ♀	ults ð
Manzanilla	4.42 ± 0.88	30.84±2.58	6.02±3.10	4	1
Nergal -I	3.74 ± 0.58	21.86±2.65	4.93±2.21	2	2
Nergal -II	3.21±0.61	18.14±1.27	5.13±2.68	2	2
Nergal -III	3.24±0.41	20.38±2.29	2.76 ± 2.20	1	1
Luques	3.45±1.01	27.77±2.35	3.88±1.53	2	1
Ascolano	4.18±0.67	25.73±1.03	3.66 ± 2.02	1	1
Tavşan yüreği	$3.40{\pm}0.27$	18.13±3.75	3.60 ± 2.06	1	1
Arbequin	4.01±0.27	11.58±2.69	3.16±2.28	1	0
Verdial	3.54±0.51	26.40±3.39	2.93±1.43	1	1
Karamürsel Su	3.83±0.45	24.90±2.97	3.81±2.23	2	1
Eğriburun	6.69±0.79	36.92±4.13	10.28 ± 4.51	3	5
Gemlik 2M-2/3	4.99±1.07	83.21±6.14	5.02±3.24	4	1
Gemlik 3G-12/2	$2.34{\pm}0.42$	26.25±3.55	2.83±1.89	1	1
Gemlik 0-12	2.78 ± 0.27	22.04±1.28	1.66±1.54	1	0

* The mean weight of infested olive fruit samples ** The mean number of oviposition stings on an infested olive fruit

This work showed that each oviposition sting does not have to be related with olive fly eggs, larvae or pupae. A few mature larvae were not able to exit the olive fruits to pupate. Different olive varieties were screened in this study. Arbequin cultivar was found to be less susceptible to olive fly infestation however, Gemlik-2M 2/3 cultivar was reported as highly susceptible to olive fly effects. Therefore, ovipositional preference and the larval development in different olive varieties are very important for establishing new olive orchards to prevent olive fruit fly infestation.

IV. CONCLUSION

In this study, infestations of olive fruit fly, *Bactrocera oleae* (Rossi), in different olive cultivars were determined in the laboratory. The conclusions obtained in the present study showed that the olive fly females prefer to choose Gemlik–2M

2/3 cultivar among others for oviposition and cultivar Eğriburun which is another local cultivar is chosen for larval development.

ACKNOWLEDMENTS

The author is grateful to Turkish Academy of Sciences (TÜBA-GEBIP 2009) for the travel funding. The author also thanks to Şeyhmus Güçlü and Seda Yücel for their technical help during the study.

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