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Attractive response of sterile Mediterranean fruit fly males to olive fruits in laboratory conditions

Privlačenje sterilnih mužjaka mediteranske voćne muhe prema plodovima masline u laboratorijskim uvjetima

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ABSTRACT

Sterile insect technique (SIT) control method is integrated in many ongoing area-wide programs for *Ceratitis capitata* W. control management who largely depends on the sterile male competitiveness. The use of semiochemical supplements originated from host and non-host plant materials to better manage the physical conditions and handling of sterile *C. capitata* males in fly emergence and release facilities improve the cost-effectiveness of the SIT worldwide. Since *C. capitata* adults are regularly captured in groves of nonhost olive, *Olea europaea* L. in the Mediterranean region, the aims of this research were to asses: (1) attraction of sterile males by olive fruit of different maturity stages; (2) attraction to olive fruits surface (egzocarp) and mesocarp and (3) attraction to three different olive cultivars. The results provide clear evidence that sterile males show strong attraction to volatile compounds from the surface and mesocarp of immature and semi matured olive fruits with no significant difference between fruits treated and nontreated with artificial cuts in mesocarp. The response of sterile *C. capitata* males to fully matured fruits were significantly the weakest. Sexually mature sterile males were attracted to green immature olive fruits stronger than sexually immature sterile males, while both categories showed strong attraction to semi matured olive fruits without significant difference between both categories.

Keywords: attraction, α-copaene, *Ceratitis capitata*, *Olea europaea*, sterile males

SAŽETAK

Metoda suzbijanja tehnikom sterilnom insekata (SIT) integrirana je u mnoge tekuće programe za upravljanje suzbijanjem Mediteranske voćne muhe *Ceratitis capitata* W., a koji uvelike ovisi o konkurentnosti sterilnih mužjaka. Korištenje semiokemijskih dodataka koji potječu iz materijala biljki domaćina i onih koji nisu domaćini s ciljem poboljšanja fizičkim svojstvima i manipulaciji sterilnim mužjacima *C. capitata* u objektima za razvoj i oslobađanje sterilnih muha poboljšava isplativost SIT-a širom svijeta. Budući da se odrasle jedinke *C. capitata* redovito love u nasadima masline, *Olea europaea* L. u mediteranskoj regiji, ciljevi ovog istraživanja bili su ocijeniti: (1) atraktivnost sterilnih mužjaka od strane plodova masline u različitim fazama zrelosti; (2) atraktivnost prema površini ploda masline (egzokarpu) i mezokarpu te (3) atraktivnost sterilnih mužjaka prema trima ponuđenim različitim sortama maslina. Rezultati daju jasan dokaz da sterilni mužjaci pokazuju snažnu privlačnost prema hlapivim spojevima s površine i mezokarpa nezrelih i poluzrelih plodova masline bez značajne razlike između plodova tretiranih i netretiranih umjetnim rezovima u mezokarpu. Reakcija sterilnih mužjaka značajno je najslabija prema potpuno zrelim plodovima. Spolno zrele sterilne mužjake privlače zeleni (nezreli) plodovi masline jače od spolno nezrelih sterilnih mužjaka, dok su obje kategorije pokazale jaku privlačnost prema poluzrelim plodovima masline bez značajne razlike između obje kategorije.

Ključne riječi: privlačenje, α-copaene, *Ceratitis capitata*, *Olea europaea*, sterilni mužjaci

INTRODUCTION

The Mediterranean fruit fly, *Ceratitis capitata* Wiedemann 1824 (Diptera, Tephritidae), is considered one of the most important tephritid fruit fly pests of significant economic importance, which will continue to expand its geographic range (Carey, 1996; Deschepper et al., 2021; Lemić et al., 2021; Gilioli et al., 2021). *C. capitata* is one of the most important invasive phytophagous pests worldwide, affecting over 260 different plant species. Potential host plant species for the *C. capitata* include a broader range of genera that, due to the ripening period, allow the pest to potentially develop several generations from spring to fall and cause significant infestation of host plant fruits and economic damage (Liquido et al., 1991; Prokopy et al., 1996; De Meyer et al., 2022). Its particular ability to disperse and adapt, as well as its high reproductive rate, enable the establishment of its populations in new areas (Paini et al., 2016; Malcrida et al., 2007; Sardain et al., 2019; Bjeliš et al., 2022) followed by the implementation of different control measures and restriction of export of products to *C. capitata* free areas (Hulme, 2009).

In addition to conventional control methods, the sterile insect technique (SIT), a species-specific and selective control method based on the release of sterile, male-only insects, is nowadays integrated in many ongoing area-wide programs for eradication, prevention, and suppression of the *C. capitata* (Hendrichs et al., 2002; Bjeliš et al., 2016; Enkerlin et al., 2017; Asadi et al., 2020; Perez-Staples et al., 2013). The effectiveness and success of SIT programs largely depend on the ability of released sterile males to attract and mate with wild females, resulting in reproductive failure of wild males (Perez-Staples et al., 2013). However, due to the demands of settlement and laboratory mass rearing conditions, followed by the stress and damage caused by sterilization, shipping, and release, SIT programs usually produce sterile males that are of lower quality than the wild males, which is reflected in a decrease in the quality parameters of sterile males and decreases their mating competitiveness in the field (Liimatainen et al., 1997; Cayol, 2000; Lux et al., 2002; Bjeliš et al., 2013). In turn, the mating success of sterile males depends largely on their ability to compete with wild males to achieve copulations with wild females (Shelly et al., 1994). In such species, sterile males of inferior quality may not meet the mating criteria of females, and a small group of wild males may obtain the most mating with wild females despite a high overflooding ratio of sterile to wild males (Shelly et al., 1994; McInnis et al., 1996).

In nature, both sexes of *C. capitata* spend considerable time foraging and feeding on various sources of carbohydrates and proteins as energy source (Yuval et al., 2000; Yuval et al., 2022). Several articles have reported the effects of adult diet on the reproductive behaviour of males of *C. capitata*. This species exhibits a “lek” mating system in which aggregated group males defend individual leaves on host trees as mating territories and attract females to their perch by producing a sex pheromone (Prokopy and Hendrichs, 1979; Arita and Kaneshiro, 1989; Whittier et al., 1992). About twice as many sightings of females were recorded when males were fed sugar-protein diets than when they were fed sugar alone (Shelly et al., 2002). This strongly suggest that adult diet has an important effect on the ability of *C. capitata* males to meet the energy costs associated with both courtship behaviour and pheromone production and emission (Warburg and Yuval, 1997; Yuval et al., 1998; Blay and Yuval, 1997; Shelly et al., 2002).

It is documented that *C. capitata* males are attracted to foods containing various volatiles originating from both host (Wharten and McInnis, 1989; Prokopy et al., 1997; Katsoyannos et al., 1997) and non-host fruits (Katsoyannos, 1984; Wharten and McInnis, 1989; Prokopy et al., 1997; Nishida et al., 2000; Bjeliš, 2007). The sesquiterpene hydrocarbon α-copaene is a complex, highly volatile, widely distributed plant compound found in a variety of host plants of *C. capitata* including orange, *Citrus sinensis* L., lemon, *Citrus limon* L., mango, *Magnifera indica* L. papaya, *Carica papaya* L. and guava, *Psidium guajava* (Nishida et al., 2000). *C. capitata* males are attracted to plant material from both host and non-host plants containing α-copaene inhibiting short-range attraction/feeding stimulation of *C. capitata* sterile males (Wharten and McInnis, 1989; Flath et al., 1994). The α-copaene was shown to be highly attractive for *C. capitata* males arresting on the host plant *Litchi chinensis* Sonn. (Sapindaceae) and non-host plants, *Ficus retusa* L. and *F. benjamina* L. (Moraceae) followed by its isolation and identification (Wharten and McInnis, 1989). Several further researches confirm attractiveness of the *Persea americana* Mill. (Lauraceae), *Angelica archangelica* L. (Apiaceae) and *Zingiber officinale* Roscoe (Zingiberaceae) plant material (bark, seed oil, roots) containing α-copaene to *C. capitata* males (Flath et al., 1994; Shelly, 2001; Nigoret et al., 2011).

Even the mechanism underlying the heightened mating success is unknown, it has been observed that feeding on the α-copaene plant source enhances signaling activity of *C. capitata* males and hence their mating success (Shelly, 2001; Shelly and Villalobos, 2004). These findings suggest that signaling efforts affect female's choice and thus results in more frequent visit of females to calling males than to males that are present but inactive (Shelly, 2001; Shelly and Villalobos, 2003).

While *C. capitata* larvae can develop inside fruits of Wild olive, *Olea europaea* subsp. africana Miller, and Forest olive, *Olea woodiana* Knoblauch (Oleaceae) (DeMeyer et al., 2002), *C. capitata* adults are regularly captured in groves of nonhost olive in the Mediterranean region (Katsoyannos, 1984; Bjeliš, 2007)]. The reason for the presence of wild *C. capitata* adulst in non-host olive orchards, despite the presence of many preferred hosts bearing fruits in the same seasonal period (Katsoyannos, 1984; Bjeliš, 2007) may be clarified by the need of *C. capitata* males for α-copaene documented above. The α-copaene has been described as volatile and aroma - active compounds in European olive, *Olea europaea* L. (Oleaceae) fruit and olive oil extracts in different olive cultivars from Turkey (Kesen et al., 2014), Spain (Alfonso et al., 2014) and Portugal (Malheiro et al., 2015).

These findings confirm the importance of continuously developing simple and inexpensive means to increase the mating success of sterile *C. capitata* males by searching for sources of attractive semiochemicals in nature.

In this study, we aimed to evaluate the attractive response of sterile *C. capitata* males, with the accent to immature sterile males to three different maturation stages of olive fruits surface and mesocarp.

MATERIALS AND METHODS

Sterile male preparation and holding

Laboratory mass-reared sterile *C. capitata* males of the Vienna-8 tsl genetic strain, which is commonly used strain in *C. capitata* SIT programs worldwide were used. The sterile male medflies originated from Insect Pest Control Section of the FAO/IAEA Laboratory in Seibersdorf, Austria. Prior to the experiment, sterile pupae were placed in a Plexiglas cage (50 x 30 x 30 cm) covered with wire mesh at the top and with a 10 cm diameter window of nylon mesh on two opposite sides to allow access and handling of the flies in the cage. 10 olive branches with leaves, 15-20 cm long were fixed on the top and four sides of cages ensuring resting place for males after their introduction to the cage (Katsoyannos et al., 1997). Branches with leaves in each cage were appropriately thinned to allow easy access and observation inside the cage. Wet cotton balls were placed in 3–4 branches of each tree to provide water for the flies following methodology described by Katsoyannos et al. (1997). To obtain sterile males of uniform age for the experiment, adult males hatched from pupae were removed from the cage every two days and transferred by using of mouth aspirator to the additional Plexiglas cages described above, where they were kept for sexually maturation until the age defined for the experiments. The cages for sexual maturation contained groups of 1200-2000 individuals. Adults of sterile males were kept for maturation process in cages at 25 ± 2 °C and 65 ± 10% RH, with an L12 : D12 photoperiod and an artificial light intensity of 2300 ± 300 lux. Resting place (15 x 40 cm white zig-zag vertical paper), food plate (mixture of yeast hydrolysate (ICN Biomedicals Inc., Irvine, USA) and sugar in a 1:4 ratio) and cotton pads soaked with water (6 cm diameter, 4 mm thick) were provided. Experiments were performed with sterile males of different ages: a) sexually immature males (2-3 days old) and b) sexually mature males (8-10 days old). Earlier experiments showed that the majority of males are considered matured after 8 days. Thus, adults of 8-10 days old were considered mature in this study (Katsoyannos et al., 1997). During all three experiments, the male origin from the same groups of laboratory mass reared individuals. Experiment were conducted from 12 to 16 replicates. Temperature and humidity in the cages were controlled using a digital thermometer-hygrometer (TH668-Mini, Shenzhen Green May Tech. Co. Ltd, China). Light intensity was measured with a light meter (LUX Light Meter, CEM DT -1500, Shenzhen Everbest Machinery Industry Co. Ltd, China).

Olive fruits phenological stages tested

The olive fruits were picked from same trees in same orchard where no pesticides had been used in the last five years. Olive fruits were picked during season following their different phenology maturation stages. Three different olive fruit maturity stages were included based on the fruit coloration degree (Sanz-Cortes et al., 2002). Fruit coloration degree is defined according to the BBCH system for coding phenological growth/fruit maturity stages (Meier et al., 2009):

1) BBCH code 80: Fruit deep green color becomes light green, yellowish (in this study: "immature fruits");
2) BBCH code 85: Increasing of specific fruit coloring (in this study: "semi-mature fruits");
3) BBCH code 89: Harvest maturity: fruits get the typical variety color, remaining turgid, suitable for oil extraction (in this study: "fully mature fruits").

Sterile male attraction to olive fruit surface and pulp

The experimental method in this study follows a previously developed and used method for similar experiments on the response of *C. capitata* to chemicals in citrus, which has been proposed as a standard technique for evaluating fly quality in sterile insect technique programs (Katsoyannos et al., 1997). Olive fruits at different fruit maturity stages were picked from the olive grove on the day of attractiveness evaluation. The fruits were transferred to the laboratory and the most appropriate fruits were selected based on fruit color (Sanz-Cortes et al., 2002). To assess response to peel chemicals from the peel (flavedo), uninfected olive fruits were wounded by removing a ca. 0.5 mm deep disk of peel tissue at 5 different positions on each fruit with a sharp razor provoking the release of peel and endocarp components (Katsoyannos et al., 1997). Therefore, attractive response of sterile males to olive fruit with artificial cuts (T) and fruit without artificial cuts (NT) on the fruit surface attractiveness were compared.

Olive fruits were placed in the test cages on Petri dishes (10 cm diameter). Plexiglas cages (50 x 30 x 30 cm) as described above without interior supplement (resting place, food and water) were used as a test cage. Sterile males were transferred from the cage to the newly prepared test cages for holding and maturation using a mouth aspirator. The cages with insects were observed for 15 minutes and the number of landings of sterile males on a fruit surface and remained there displaying contact of the male mouthparts with the fruit surface or the juice from the cuts on mesocarp for at least 30 seconds were counted. With the fact that same sterile males can be attracted to olive fruits more than one time, the number of landings were considered as response variable instead number of sterile males that landed on a fruit surface. All tests were conducted at 25 ± 2 °C and 65 ± 10% RH, with an L12 : D12 photoperiod and an artificial light intensity of 2300 ± 300 lux. Counting of number of sterile male landings was conducted by direct observations of fly behavior by 2 trained observers, one per treatment within same age (2 observers for each cage with two treatments of same age), while in the case of observations of three different varieties or three maturation stages, the number of sterile male landings was conducted by 3 trained observers, one per variety or one per fruit maturation stage (3 observers for each cage with 3 treatments of same age). The attraction of sterile *C. capitata* males to olive fruit odors included **three** separate experiments:

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Attraction of sterile males by olive fruit of different maturity stages:

The attraction of sexually immature (2-3 d) and sexually mature sterile *C. capitata* males (8-10 d) to olive fruit of the Oblica cultivar was evaluated for three maturity stages: BBCH codes (BBCH: 80, 85, and 89). After fruit selection based on fruit color (Sanz-Cortes et al., 2002), 10 fruits from each of 3 different maturity stages were placed on 3 individual Petri dishes. Fifteen minutes after sterile males were introduced into a cage, the Petri dishes containing the fruits were placed in the cage. For this purpose, two plastic cages (50 x 30 x 30 cm) were used as described above, one for each age of sterile males. 100 sterile males of the same age were placed in the test cages (each age in a separate cage). The number of number of landings of sterile males on a fruit surface and remained on the fruits for at least 30 seconds and displaying contact of the male mouthparts with the fruit surface or the juice from the cuts on mesocarp were consider response variable. Observation was done for 15-minute period. Each replicate was initiated at least half of hour after the termination of the previous one. There were 12 replicates for each age of sterile males. New set olive fruit and new group of sterile males were used for each replicate.

Attraction of immature sterile males to olive fruits with artificially made cuts on mesocarp:

In this experiment, fruits of three maturity stages (BBCH: 80, 85, and 89) with artificial cuts and without artificial cuts on the fruit surface were exposed to the immature sterile males (2-3 d). Fruits were artificially wounded by removing a ± 0.5 cm^2 slice approximately 1 mm deep at 5 different positions on the fruit mesocarp of each fruit with a sharp scalpel, similar to that described for citrus (Katsoyannos et al., 1997). These cuts resulted in exudation of liquid and volatile components from the mesocarp. 100 sterile males of the same age were placed in the test cages (each age in a separate cage). After the sterile males were placed in the cage, 10 artificially injured and 10 non-injured fruits were placed separately on 2 individual Petri dishes and transferred into the cage. Fruits were placed in the cages immediately after the artificial cuts were removed. The number of number of landings of sterile males on a fruit surface and remained on the fruits for at least 30 seconds and displaying contact of the male mouthparts with the fruit surface or the juice from the cuts on mesocarp were consider response variable. Observation was done for 15-minute period. Each replicate was initiated at least half of hour after the termination of the previous one. There were 12 replicates for each age of sterile males, using fresh olive fruit and sterile males each time.

Attraction of immature sterile males to three different olive cultivars:

The attraction of sexually immature sterile males (2-3 d) was evaluated for three different olive cultivars grown for different purposes: Ascolana Tenera (grown for table olive production, late variety), Oblica (grown for table olive and olive oil production, mid to late variety), and Leccino (grown worldwide for olive oil production, early variety). For this experiment, fruits at maturity stage BBCH 85 (increasing specific fruit coloration) were used. After selecting the most suitable fruits based on fruit color (Sanz-Cortes et al., 2002). Altogether 10 fruits from each of the 3 cultivars were distributed among 3 individual Petri dishes in the cage. 100 sterile males of the same age were placed in the test cages (each age in a separate cage). The number of number of landings of sterile males on a fruit surface and remained on the fruits for at least 30 seconds and displaying contact of the male mouthparts with the fruit surface or the juice from the cuts on mesocarp were consider response variable. Observation was done for 15-minute period. Each replicate was initiated at least half of hour after the termination of the previous one. New olive fruits and new sterile *C. capitata* males were used for each of the 16 replicates.

Statistical analysis

The findings of this study were subjected to analysis of variance using the SPSS Statistics version 19 from IBM Corp. (Armonk, NY, USA) (IBM Corp. 2016). The data were transformed using Friedman's Two-way

Analysis of Variance. The influence of the age of sterile male medflies on their attraction to olive fruits of three different maturity stages, the influence of artificially wounding olive fruit of three different maturity stages on the attraction of sterile males and attraction to three different olive cultivars were conducted using the Mann-Whitney U test. The overall attraction of sterile males to different maturity stage of olive fruit that were wounded or not wounded was conducted using the Mann-Whitney U test and Wilcoxon matched pairs test.

RESULTS

Attraction of sterile males by olive fruit of different maturity stages:

To test whether sterile *C. capitata* males respond to chemicals from the fruit surface of olives, sexually immature and mature males were given a choice between olive fruit from three maturation stages of Oblica cultivar. The behavior of sterile males, regardless of age, is presented through attractive response by landing on the fruit within 2-3 minutes after introducing them into the cages. Males were continuously observed lowering their heads and touching the surface of the fruit with their mouthparts. After landing on the fruit, majority of individuals remained there for a period of 5-7 minutes, touching the fruit surface with their mouthparts. Immature, sterile males were mostly sighted on semi-ripe olive fruit (BBCH 85) and very rarely on immature and fully matured fruits, exhibiting a statistically significant positive response to odors from the fruit surface (Table 1).

Direct observations revealed that significantly lower number of sterile *C. capitata* males responded to immature fruits (BBCH 80) and even, just few sterile males responded to fully mature olive fruits (BBCH 89). These results strongly suggest that sterile males were attracted to the semi olive fruit from a distance (BBCH 85) and thereafter arrested on it and consuming ingredients from the fruit surface with their mouthparts. Therefore, sexually immature sterile males were significantly more attracted to semi-mature olive fruit than to either immature ($P<0.05$) or fully mature ($P>0.05$) fruit. Sexually mature males were significantly attracted to both immature (BBCH 80) and semi-mature (BBCH 85) olive fruits with no significant difference between them ($P>0.05$), and they were significantly not attracted to fully mature fruits ($P<0.05$). Direct observation of the behavior of sexually mature sterile males confirmed the homosexual activity of males (mating attempts) on the fruit surface. Occasionally, some males left the fruit surface because they were disturbed by other males. Other characteristics of the behavior of mature sterile males in terms of timing of landing after the introduction of the fruits, consumption of the ingredients from the surface of the olive fruit were identical to the behavior of immature sterile males.

The results show that sterile males of both ages responded positively to semi matured fruits (BBCH 85) without significant difference. The response without statistical difference between ages, but in this case expressed as negative attractiveness, was observed for fully matured olive fruits (BBCH 89). In the response of

Table 1. Response of sterile *C. capitata* males of different ages to olive fruits of three maturity stages in laboratory cages during a 15-minute observation period

Maturity stage of fruits (BBCH)	BBCH code	Age of sterile males (days)	Mean number responding[1] ± SE
Fruit deep green color becomes light green, yellowish (immature)	80	2-3	9.3 ± 7.1 b
		8-9	26.8 ± 16.5 a
Increasing of specific fruit coloring (semi-mature)	85	2-3	22.3 ± 10.8 a
		8-9	21.3 ± 12.0 a
Harvest maturity, fruits get the typical variety color, (fully mature)	89	2-3	1.8 ± 1.5 c
		8-9	2.7 ± 2.5 c

[1] Means followed by the same letter are not significantly different (Mann-Whitney U test)

sterile males to immature fruits (BBCH 80), the results show a significant discrepancy in attractive response between immature and mature sterile males. Immature fruits were significantly more attractive to sexually mature than to sexually immature sterile males (*P*>0.05, Table 1).

Attraction of immature sterile males to olive fruits with artificially made cuts on mesocarp:

To compare whether sexually immature sterile *C. capitata* males respond differently in attractiveness to chemicals from olive fruit with artificial cuts (T) and fruit without artificial cuts (NT) on the fruit surface, sterile males were given a choice between these two treatments for three stages of olive fruit ripening. When olive fruit from three different maturation stages without artificial cuts on the fruit surface (NT) were compared with fruit with artificial cuts (T), differences in the responses of sterile males were not significant regardless of treatments. Nevertheless, the same characteristics of sterile male behavior were often observed in relation to landing and arresting, ingredients exploration and consumption with mouthparts on the fruit surface, or sap from artificial cuts. Table 2 shows that volatiles or compounds from fruit mesocarp had no additional effect on attraction of sexually immature sterile males or showed no increased response to chemicals from artificially wounded surfaces, whether the fruit was immature (*P*>0.05), semi-mature (*P*>0.05), or fully mature (*P*>0.05). Wounding of olive fruits by artificial cuts had no significant effect on attracting flies at different stages of fruit maturity stages.

In addition, the results in Table 2 confirm the results shown in Table 1 that immature (BBCH 80) and semi-mature (BBCH 85) fruits have the same tendency to attract sterile males.

Results in Table 3 show that treatment of the fruits with superficial cuts do not afftect response of sterile males regardless of gender, while sexually matured sterile males shows significantly stronger attraction to both treated or nontreated fruits. There is no significant difference between treated and nontreated fruits in the 2-3 days old sterile *C. capitata* males (*P*>=0,05, Mann-Whitney test) and 8-9 days old sterile *C. capitata* males (*P*>0,05, Mann-Whitney test).

Table 3. Response of sterile *C. capitata* males of different age to olive fruits treated and non-treated with superficial cuts

Age of sterile males	Mean number responding [1] ± SE	
	Treated ± SE	Nontreated ± SE
2-3 days old (sexually immature)	11.4 ± 12.5 b	10.4 ± 10.4 b
8-9 days old (sexually mature)	16.7 ± 15.9 a	17.2 ± 15.9 a

[1] Means followed by the same letter are not significantly different (Mann-Whitney U test

Attraction of immature sterile males to three different olive cultivars:

To evaluate male response to fruit of different olive cultivars, sterile *C. capitata* males were offered the semi mature (BBCH 85) fruits of Acsolana Tenera, Oblica, and Leccino olive cultivars.

Table 2. Response of immature sterile *C. capitata* males to olive fruits of three maturity stages treated or nontreated with superficial cuts in laboratory cages during a 15-minute observation period

Maturity stage of fruits (BBCH)	BBCH code	Treatment	Mean number responding [1] ± SE	Average number responding[2] ± SE
Fruit deep green color becomes light green, yellowish (immature)	80	T	17,0 ± 15,0 a	18,1 ± 15.1 a
		NT	19,2 ± 16,7 a	
Increasing of specific fruit coloring (semi-mature)	85	T	23,0 ± 13,3 a	21.8 ± 8.9 a
		NT	20,7 ± 9,0 a	
Harvest maturity, fruits get the typical variety color, (fully mature)	89	T	2,2 ± 2,2 b	2.3 ± 2.0 b
		NT	2,3 ± 1,9 b	

Legend: T – olive fruits treated with superficial cuts; NT – nontreated olive fruits
[1] Means followed by the same letter are not significantly different (Mann-Whitney U test)
[2] Means followed by the same letter are not significantly different (Wilcoxon matched pairs test)

Immature sterile males were more attracted to Ascolana Tenera ($P<0.05$) and Oblica ($P<0.05$) cultivars than to Leccino (Table 4). Direct observations revealed that sterile males landed on the fruits of Ascolana Tenera and Oblica almost immediately after fruit introduction, and most of them remained there for a period of more than 5 minutes, consuming the substances from the fruit surface. There was no difference in attraction between Ascolana Tenera and Oblica ($P>0.05$).

Table 4. Response of sterile *C. capitata* males of different ages to olive fruits of three maturity stages in laboratory cages during a 15-minute observation period

Cultivar name	Cultivar type/ maturation	Mean number responding[1] ± SE
Ascolana Tenera	Table olive / late cultivar	17.7 ± 13.4 a
Oblica	Table and olive oil / medium-late cultivar	20.2 ± 14.9 a
Leccino	Olive oil / early cultivar	4.17 ± 4.4 b

[1] Means followed by the same letter are not significantly different (Mann-Whitney U test)

DISCUSSION

The present study provides clear evidence that sterile *C. capitata* males respond strongly to surface and mesocarp of olive fruit, particularly immature and semi matured fruits. This result is consistent with the fact that the various fruit types, exudates and volatiles is found on the fruit surface or mesocarp (Papadopoulos et al., 2008). In this study, both sexually immature and sexually mature sterile males showed the weakest attraction to fully mature olive fruit, regardless of fruit surface or mesocarp, with no significant difference between their sexual maturity status. At the same time, both sexually immature and sexually mature sterile males were significantly more attracted to immature and semi-mature stages of fruits. A significant difference in the weakest attraction of sterile males to fully mature fruits, regardless of their age, could be explained by the changes of overall nutrition composition during the olive fruit ripening process or decreasing of the amount of a particular attractive compound for sterile *C. capitata* males (Shelly, 2001; Papadopoulos et al., 2008).

In the case of immature olive fruits in this study, the results show a significant discrepancy in attractive response between sexually immature (2-3 d) and sexually mature (8-9 d) sterile males. Immature olive fruits were significantly more attractive (almost three times as much) to sexually mature than to sexually immature sterile males. In contrast, sexual maturity of sterile males had no effect on their attraction to semi-mature or mature olive fruit.

The results of the present study could be related to olive fruit volatiles, and changes in amount of α-copaene during the fruit ripening process.

Up to the stage of olive fruit ripening, known as "hardening of the fruit pit", α-copaene is not detected, while the amount increases rapidly and reaches a maximum as the green fruit grows (BBCH 80-85) (Alfonso et al., 2014). The subsequent rapid decline of α-copaene amount is related to fruit maturity and this compound is no longer detected when the fruit begins to soften and colour become darker, corresponding to the fully mature fruit stage (BBCH 89) (Alfonso et al., 2014). Sterile *C. capitata* males were attracted more to late olive cultivars, which generally contain lower amounts of olive oil (in this study for table cultivars Ascolana Tenera and Oblica), than to Leccino, an early cultivar typical of oil production (Miljković, 1991). This is consistent with research showing that the amount of α-copaene may vary among cultivars and production sites (Alfonso et al., 2014) and, therefore, the attractiveness of sterile males to fruit of the three olive cultivars differ. The present results show a strong response of sterile males to unripe or semi-ripe fruits and are consistent with the explanation in other research of decreasing α-copaene content in olive oils obtained from fruits at different stages of ripeness (Wichi et al., 2010; Alfonso et al., 2014).

The use of semiochemical supplements to better manage the physical conditions and handling of sterile *C. capitata* males in fly emergence and release facilities would hold great potential to improve the cost-effectiveness of the SIT worldwide (Shelly and McInnis, 2001; Shelly et al., 2003; Briceno et al., 2007; Paranhos et al., 2010; Hendrichs and Pereira, 2013; Silva et al., 2013; Pereira et al., 2013, Bjeliš et al., 2013; Bjeliš et al., 2016). The quality of sterile males, and in particular their mating performance in the field, can be increased by exposing sterile males to certain scents prior to release. Oil from the roots of ginger, *Z. officinale* dramatically increases mating success of sterile medfly males when competing with wild males for attraction and mating with wild females (Shelly, 2001; Shelly et al., 2004; Papadopoulos et al., 2008; Paranhos et al., 2008). Moreover, exposure of sterile medfly males to ginger root oil, reduces medfly female re-mating (Morelli et al., 2013).

Experiments with citrus fruits documented a strong response of *C. capitata* males to fruit wounds, with males being significantly more attracted to fruit treated with wounds (Katsoyannos et al., 1997; Katsoyannos et al., 2002). Recent studies on the *C. capitata* have demonstrated an increase in male mating competitiveness following exposure to particular plant structures or products, including the fruit and fruit-derived oil of orange trees, the bark and fruits of guava trees, manuka oil and ginger root oil. Although it is not known which compound(s) was responsible for the enhanced mating success, all the performance-boosting substances tested thus far contain the sesquiterpene hydrocarbon α-copaene, and α-copaene tested alone was found to increase mating success in male *C. capitata* (Shelly, 2001; Shelly and Vilalobos, 2003; Shelly et al., 2003; Papadopoulos et al., 2008; Shelly et al., 2008). These results confirm that adult food availability in particular (as well as quality and quantity) is factors that significantly influence male reproductive success (Blay and Yuval, 1997; Field and Yuval, 1999; Katsoyannos et al., 2002). In contrast to the above examples, the present study found no significant difference in attraction to sterile males between artificially wounded and unwounded olive fruits, regardless of fruit maturity stage, suggesting that attractive compound is present on the fruit surface as well as in the fruit mesocarp.

CONCLUSIONS

This study provides clear evidence that sterile *C. capitata* males show strong attraction to compounds from the surface and mesocarp of olive fruits, especially from immature and semi matured fruits and the least to fully mature fruits. Both sexually immature and sexually mature sterile males showed the weakest attraction to fully mature olive fruit, regardless of fruit surface or mesocarp, with no significant difference between their sexual maturity status. At the same time, both sexually immature and sexually mature sterile males were significantly more attracted to immature and semi-mature stages of fruits. Attraction of sterile males to olive fruits follows the course of the maturation period of olive fruit. These results indicate the possibility of using olive fruits as a very cheap source of raw materials and further development for use in sterile insect programs. Therefore, incorporating olive fruit compounds into adult food may increase the effectiveness of the sterile insect technique and reduce the number of sterile *C. capitata* males released.

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