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Reduction of olive fly, *Bactrocera oleae* (Rossi, 1790), damage by selecting native and exotic olive cultivars

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Abstract

Olive fly is the most dangerous pest in olive groves worldwide. Therefore the study of the most susceptible and resistant cultivars to olive fly can bring new information to diminish the olive flies harmful impacts. The main goal of the present study is to verify the olfactory response of olive fly to olive volatiles from five native Iranian cultivars (Fishomi, Mari, Rowghani, Shengeh, and Zard) and four exotic cultivars (Arbequina, Coratina, Koroneiki, and Manzanilla). Olfactometer bioassays were carried out in order to verify the attraction level of the volatiles of different cultivars to olive flies. A second experiment was performed with native cultivars in order to verify the preference between healthy olives and olives already infested by olive fly. The obtained results demonstrated that among native cultivars, Fishomi and Zard were those attracting higher number of olive flies, while cv. Rowghani showed to be the less preferred one. The exotic olive cultivars, Arbequina and Manzanilla attracted higher number of olive flies, while the volatiles of cv. Koroneiki showed a low attraction effect. According to the results of this study we suggest setting new strategies in cultivation of olives by spreading those cultivars less attractive to olive fly.

Keywords: *Olea europaea*; Olive fruit fly; Cultivar preference; Olfactory response; Volatile compounds.

Introduction

Olive production is heavily reduced each year worldwide due to the incidence of pests and diseases. The olive fruit fly, *Bactrocera oleae* (Rossi, 1790) (Diptera: Tephritidae) is the most dangerous pest, present in most olive groves, mainly in the Mediterranean Basin, leading to unprecedented losses (1). Olive products obtained from olives infested with olive fruit fly are commercially depreciated due to

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production losses by pulp consumption, fruit drop, and contamination of olives with microorganisms (2), leading to low commercial classification of olive products, reducing olive oil quality as well as altered composition and functional properties (3, 4).

The olive fruit fly is a monophagous pest (5). The female punctures the fruit with the ovipositor and deposits an egg beneath the skin. From the egg a larva hatches feeding exclusively inside the fruit creating galleries and destroying the interior. The larvae pass through three instars and then create an exit hole in the fruit escaping to pupate in the soil, or they pupate inside the fruit making the exit hole a route for escaping the adult fly.

The predominant method to control the olive fruit fly in the last years has been through the use of conventional pesticides. The continued use of such products has proven to be unsafe and to leave residues in olive products (6, 7). This leaded to concern in most olive growing countries and concerted effort to reduce the amount of pesticides being used to control pests in olive groves. Nowadays the search for eco-friendly and biological control strategies of olive fruit fly are increasing. The use of natural enemies, such as parasitoids (8), lure and kill and mass-trapping (9), use of semiochemicals (10), among other strategies have been implemented to control the olive fruit fly.

Olive fruit fly is known to display oviposition preference. Certain olive cultivars are heavily infested while others are significantly less attacked by this dipteran, being all inserted in the same olive orchard within the same agro-climatic conditions. Several aspects are believed to influence the oviposition preference, including chemical (11), physical (12), and molecular aspects (13). In terms of chemical factors, the volatile compounds emanating from the fruits play an important role in attracting olive fruit fly. The volatile profile emitted by each olive cultivar is specific, and those more susceptible to olive fly infestation are reported to release higher amounts of attractive volatiles to this tephritid (14). Numerous studies have been done on the biology, control, ecology and behavior of the olive fruit fly but there are few studies on its olfactory response to olive fruits of different cultivars. Most of olfactory studies are about the attraction of the olive fruit fly female to male extracts (15). So, the present study is the first addressing the olfactory response of olive fruit fly to different olive cultivars in order to assess olive fruit fly oviposition preference.

Since organic olive growing is increasing worldwide, the adoption of eco-friendly strategies against pests and diseases is a major line of research. The selection of the less susceptible olive cultivars in detriment of highly susceptible cultivars could be a good strategy to reduce the incidence of olive fruit fly, reducing the production losses. Therefore, the main objective of this study is to assess the olfactory response of olive fruit fly to the volatile emissions of some native (cvs. Fishomi, Mari, Rowghani, Shengeh, and Zard) and exotic cultivars (cvs. Arbequina, Coratina, Koroneiki, and Manzanilla) of the olive in Iran in order to distinguish susceptible (preferred by olive fruit fly females to oviposit) and less susceptible ones. The study of the susceptibility of olive cultivars to olive fly can give information about which cultivars should be increasingly cultivated and those that should be maintained as “natural-traps” in olive groves.

Materials and methods

Harvesting olives

Five representative native Iranian cultivars, i.e. Fishomi, Mari, Rowghani, Shengeh, and Zard as well as four exotic cultivars widely cultivated worldwide, i.e. cvs. Arbequina, Coratina, Koroneiki, and Manzanilla, were selected for study (Table 1).

About 5 kg of olive fruits were harvested per each cultivar (when available, except for cv. Arbequina due to low amount of olives) in Ghoushchi gardens (Tarom Sofla region, Qazvin province, Iran). Once in laboratory, olives were inspected visually to separate healthy from infested or injured olives. We used only healthy olives without signs of pests, diseases or any kind of damage.

Insect collection and rearing

Olive fly pupae and larvae were collected from infested olives collected in the field, stored olives and factories. The adults were reared in the insectarium of the University of Tehran. Once emerged, adults were transferred to cages (volume of 0.9 m^2) for age control purposes, under a photoperiod of 16:8 h (light:dark) at 27±1˚C and relative humidity above 60%. Both sexes were maintained together and adults were feed *ad libitum* with a honey solution (10% w/v), artificial diet (sucrose and yeast extract at a ratio 4:1), and water, being diet changed every two days.

Y-tube olfactory bioassays

The response of *B. oleae* to the volatiles emitted by the 9 olive cultivars in study was assessed by a Y-tube olfactometer. In this experiment all native cultivars were tested among each other in pairs. The same procedure was repeated for exotic cultivars. We refrained to compare the native and exotic cultivars with each other due to preserve the cultivation of native Iranian olive cultivars and select from the exotic cultivars those with less preference by olive fly.

For the olfactometer bioassays only females at least 13 days old were tested, in order to ensure that all flies were sexually mature and gravid. For each experiment we used 3 to 5 fruits depending on the size of fruits (3 olives for cv. Koroneiki, and 5 for cvs. Fishomi and Arbequina) in order to adjust the total volume of olives tested.

The olfactometer consisted of a Y-shaped Pyrex tube (2.5 cm diam.) with an entry arm (20 cm in length) and two side arms (18 cm in length) (Fig. 1). The air was first cleaned by activated charcoal and then blown through both arms at 5 m/s speed by a small electrical motor. The female insects were individually introduced at the basal end of the Y-tube by a small brush to initiate upwind movement. Glass cylinders (6.5×34 cm) containing the olives was connected to the end of each arm, without ocular contact between flies and fruits. For each cultivar comparison a total of 20 flies (unless otherwise stated) were tested separated in groups of 4 flies (a total of five replicates), and each fly was tested individually. After testing each group of flies, the Y-tube was cleaned with alcohol (70%) and left to be dried. Then a new group of flies was tested with a new set of olives. Negative controls were carried out for each cultivar against air with also 20 flies per cultivar (unless otherwise stated).

Each olive fly female was individually introduced into the tube and observed until it walked at least 9 cm up one of the arms or until 10 min had elapsed and the fly remained in that arm at least for 3 min after passing from the middle long arm towards the odor region. Females that did not choose a side arm within 10 min were recorded as ‘no choice’. To avoid any unforeseen asymmetry in environmental factors (*e.g.* light, temperature), odor sources were switched between the left and right side arms to minimize any spatial effect on choices. The experiments were conducted at 25±1ºC and with a relative humidity of 45±5%.

Other type of olfactometer bioassays were carried out with three native cultivars (cvs. Mari, Shengeh, and Zard) in order to verify if olive fly females are attracted to healthy or infested olives with *B. oleae* larvae. For this purpose, the same numbers of healthy and infested olives, with L3 instar larvae, were presented to olive fly females in the same conditions described before. For this experiment at least 15 flies were tested individually.

All experiments were carried out between 2 and 6 pm as this time is characterized by a higher activity of olive flies (16) with higher reaction to the odor sources.

Statistical analysis

To analyze the olfactometer results a replicated G-test was used against the null hypothesis that insect reach the end of the two arms with equal probability (1:1) (17).

Results

Olive fly attraction by volatiles in olfactometry bioassays

The results obtained from the olfactometer bioassays in native Iranian olive cultivars are presented in Fig. 2. Among the five cultivars, olfactometer bioassays showed that cvs. Fishomi and Zard were the ones attracted a higher number of olive flies. Each of the two olive cultivars attracted about 48.8% of all flies tested. Fishomi olives attracted less flies (8 flies) when tested against cv. Zard (9 flies) but without significant difference (P = 0.808). Compared to cv. Shengeh, cv. Fishomi attracted a considerably higher number of females, 13 against 5 attracted by cv.

Shengeh (Fig. 2), also without significant difference (P = 0.055). A similar result was observed when cvs. Zard and Shengeh were tested together, with 11 and 7 females attracted, respectively (P = 0.344). Again cv. Zard attracted higher number of flies compared to cv. Mari, 12 and 6 flies respectively (P = 0.153). In fact cv. Zard only attracted less flies when was compared with cv. Rowghani, (7 and 9 flies attracted respectively), but in this case a high number of flies did not opted for any cultivar, being registered as no-choice (4 flies; Fig. 2).

In a contrary trend, olive fly females were less attracted to the volatiles from cv. Rowghani (Fig. 2). In all bioassays, where this cultivar was present, only 37.5% of the flies (out of a total of 80 flies) were attracted by the volatiles of cv. Rowghani, the lowest percentage among the five cultivars (48.8% for cvs. Fishomi and Zard; 43.8% to cv. Shengeh 41.2% to cv. Mari, and 37.5% to cv. Rowghani). In fact a significant lower number of flies were attracted to cv. Rowghani compared to cv. Shengeh (P = 0.035); 5 and 14 attracted respectively (Fig. 2).

The results obtained from the olfactometer bioassays in exotic cultivars are presented in Fig. 3. The obtained results revealed that cv. Koroneiki is the one attracting lower number of flies at the conditions tested: 37.7% (20 flies out of 53; Fig. 3). Compared to cv. Coratina, cv. Koroneiki attracted considerably lower number of flies (6 and 10 flies) but without significant difference (P = 0.315). Olive volatiles of cv. Koroneiki attracted higher number of flies when compared to cv. Arbequina, with 6 against 5 flies (Fig. 3) and without significant difference again (P = 0.763).

Olive volatiles of cv. Arbequina attracted a higher number of flies compared to cvs. Manzanilla and Coratina (Fig. 3). This cultivar was the one, among exotic cultivars, that attracted a higher percentage of flies: 47.2% of all flies tested (25 out of 53 flies). Arbequina cultivar was followed by cv. Manzanilla. In our study, the volatile from cv. Manzanilla attracted about 43.3% of the total flies tested (26 out of 60 flies). Olives from cv. Manzanilla are normally produced as table olive, and in field conditions they are the first to be attacked by the flies due to their considerable size. In our study, visual cues are not considered; therefore volatiles from cv. Manzanilla can also be important for the attraction of olive fly.

Figure 1. Y-tube olfactometer for recording responses of olive fruit fly females to volatiles.

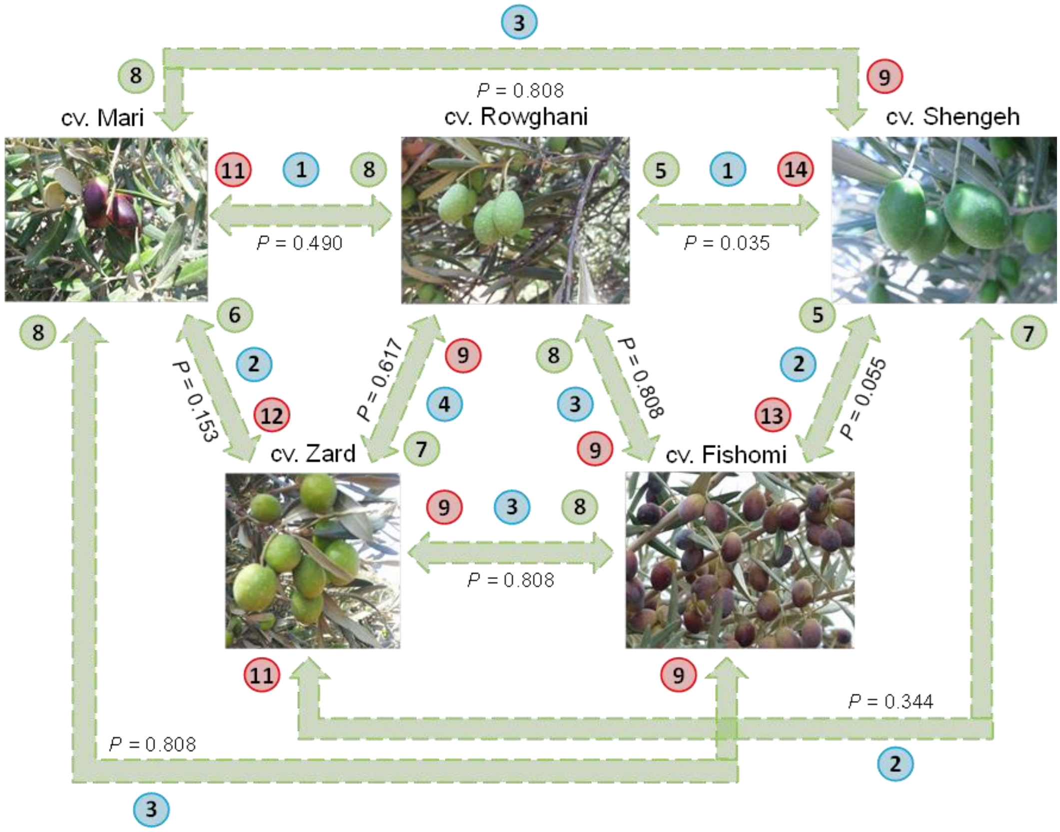

Figure 2. Olive fruit fly females attraction to different native Iranian cultivars based on the interaction between two cultivars at a time (values in red represent higher number of flies attracted in each interaction; values in green represent lower number of flies attracted in each interaction; values in blue are those for the number of flies without reaction).

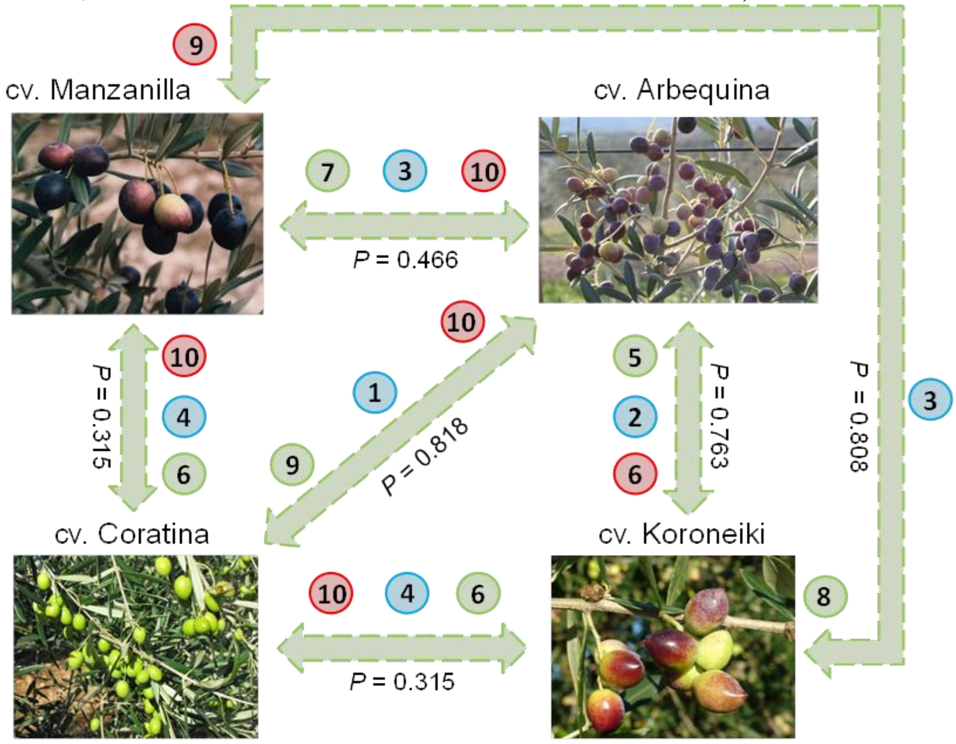

Figure 3. Olive fruit fly females attraction to different exotic olive cultivars cultivated in Iran based on the interaction between two cultivars at a time (values in red circles represent higher number of flies attracted in each interaction; values in green circles represent lower number of flies attracted in each interaction; values in blue circles are those for the number of flies without reaction).

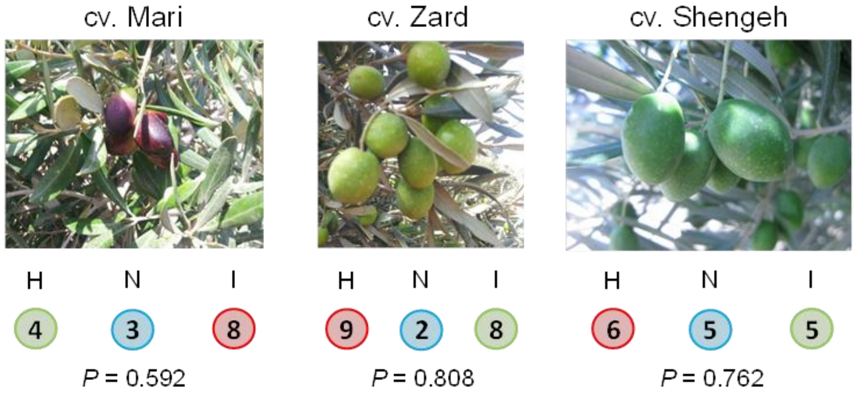

Figure 4. Olive fruit fly females' attraction to healthy (H) and infested olives (I) of cvs. Mari, Shengeh and Zard (values in red circles represent higher number of flies attracted in each interaction; values in green circles represent lower number of flies attracted in each interaction; values in blue circles (N) are those for the number of flies without reaction).

Discussion

Several mechanisms may influence the choice of a particular variety, such as plant colour, shape, size, and particularly the volatiles emitted by the fruiting tree (18) that may act as semiochemicals. The results obtained from both native and exotic cultivars are extremely related to the volatile cues exhaled by the olives.

Olive flies perceive volatile cues in the sensilla of their antenna (19), allowing them to interpret those signals and to efficiently choose habitat, food, mating partners, and host for oviposition (20).

Important factors in ovipositional preference include physical stimuli, such as fruit size, colour, and epicarp hardness (21) and chemical stimuli, mainly aliphatic waxes (22). This process of choice for a host results from the balance between visual, olfactory and tactile signs that act as attractants or deterrents for egg laying (23), and females may adjust their choice according to the available varieties and their phenological stages, in order to optimize reproductive success (24).

The chemical identification of oviposition deterrents/attractants may be a primary step for further studies to explore their mode of action, and to develop applications in pest control, such as the selective breeding of varieties or the use of infochemicals to disrupt oviposition behaviour, and the new monitoring methods based on the host-derived compounds.

Previous investigations reported specific volatile compositions for each olive cultivar, highly influenced by the maturation process (25). Several studies revealed the existence of specific volatiles related with the attraction of olive fly females, such as the sesquiterpene α-copaene (14). Malheiro et al. (25) also found a strong positive correlation between the amounts of α-copaene and the infestation levels found in olives of different cultivars. In the same research, the aromatic hydrocarbon, toluene, was also correlated with the infestation level. Toluene is also an attractant to olive fly females (26). Therefore, we believed that the characteristic composition of volatiles from each olive cultivar and their relative amounts, influenced by cultivar, are behind the obtained results.

The results obtained in the second experiment were performed to verify, if there was a preference of olive flies to healthy or infested olives (Fig. 4). Some hypothesis are raised in literature describing that when olive fly females lay an egg they also leave a signal to other females to warn them that olive is already infested (27). From the ecological point of view, this is a very important feature for the survival of the species, enabling two or more larvae to develop in the same olive, reducing the possibility of survival of the specie. The results obtained in the olfactometry bioassays revealed no specific attraction of olive flies to healthy or infected olives in cvs. Shengeh and Zard. In these two cultivars the difference between the number of flies attracted to healthy or infested olives was minor (Fig. 4). However, in cv. Mari, showing considerable attraction preference, 4 flies were attracted to healthy olives while the double were attracted to infested olives. Nevertheless for the three cultivars studied significant differences between the attraction of olive fly females to healthy or infested olives were not verified (P = 0.592, P = 0.762, and P = 0.808, for cvs. Mari, Shengeh, and Zard, respectively). Apparently the results obtained are contradictory to the hypothesis that olive fly leave a reconnaissance mark to other olive flies. Nevertheless we need to verify that the only cue present in our study was an olfactory cue. According to a former reference (28) volatiles are one of several cues that attract olive flies, showing volatiles to be important at medium-long range cues for olives. Other cues like physical ones are more important at medium-short range (29). Therefore, our results are merely indicative of attraction of olive fly to volatile cues and do not refer to oviposition. Other important aspect is that when larvae are consuming olive pulp, they release volatiles, mainly green leaf volatiles (GLV's), formed from the lipoxygenase pathway (LOX), like (*E*)-2-hexenal, and other volatiles like ethylene (30). In fact, (*E*)-2-hexenal is a repellent to olive fly females (26). By electroantennographic bioassays, Malheiro et al. (19) verified that (*E*)-2-hexenal elicited higher signals with females age, higher when they are gravid, showing that the repellent action may increase as females become older. This could explain the behavior of olive flies females towards cvs. Shengeh and Zard. However, for cv. Mari a higher number of flies were attracted to infested olives, in this variety, other volatiles rather that repellents could intervene in the olive flies' choice. Nevertheless, the hypothesis that olive flies do not lay eggs in fruits already infested, is not verified in some cases (2), in field conditions, when olive fly population are high and the olives production is low. Under these conditions, it is possible to observe olives with more than one larva inside in the field. Therefore, the hypothesis raised by several authors is yet controversial, and our results support that olive flies are attracted by both healthy and infested olives. In other tephritidae species, such as *Bactrocera tryoni* (Froggatt, 1897) and *Bactrocera jarvisi* (Tryon, 1927), this hypothesis was already validated (31).

Our results are merely indicative of the attraction of olive fly and do not refer to oviposition. Other factors, such as the color of olives and their hardness, might influence the oviposition of olive fly (12, 29). Therefore, in future works the infestation levels in the field will be monitored to verify real infestation levels to correlate with the data obtained in the current study. Also, the volatile composition of each cultivar will be analyzed also to correlate with the obtained results.

At the light of the obtained results we contribute to the knowledge of olive fly attraction by native and exotic cultivars. A sustainable olive growing starts in the design of olive grove and in the selection of productive olive cultivars with good characteristics for olive oil and table olives production, and also that could be less preferred by pests and diseases, in this case, resistant to olive fly. Therefore, our study indicates that an investment in the cultivation of cv. Rowghani and cv. Koroneiki could be a good option for new plantations. Nevertheless, the remaining olive cultivars should be cultivated as well, in a minor extent, in order to maintain the Iranian olive germplasm, but also to use them as "natural-traps". The inclusion of susceptible olive cultivars in the middle of new plantations will attract olive flies for these cultivars, maintaining them away from other cultivars with productive and economic importance.

Conclusion

Host plant finding and preference of fruit flies (Tephritidae: Diptera) on their hosts is determined by several factors. These factors may include the physical, chemical and nutritional properties of the plant (fruit) (32), climatic conditions (33), and phenology of the host plant (34). Among these factors, volatiles and olfactory stimuli play an important role in the behaviour, recognition, and attraction of tephritids towards hosts (35).

We concluded that preference of the olive fruit fly, *Bactrocera oleae* among native cultivars are cvs. Fishomi and Zard and among exotic cultivars cvs. Arbequina and Manzanilla. The olive fruits could be less preferred by some cultivars such as cv. Rowghani and cv. Koroneiki. Some hypothesis mentioned in literature about reduced attracting preference of already infested olives by olive fly, was not verified in our study, as the results with healthy and infested olives were similar.

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