Qualitative Changes of Olive Oils Obtained from Fruits Damaged by *Bactrocera oleae* (Rossi)

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Abstract. Mature fruits of *Olea europaea* L. ‘Frantoio’ with different degrees of damage [from 0% to 100% of fruits with exit holes (EHs)] caused by the olive fruit fly (*Bactrocera oleae*), the key pest in Mediterranean olive orchards, were sampled to quantify the effects on free acidity, peroxide value (PV), and concentrations of secoiridoids and lignans of virgin olive oil (VOO). The total concentration of phenolic compounds and that of individual secoiridoids were negatively related to the degree of fruit damage, whereas the concentration of lignans, namely (+)-pinoresinol and (+)-1-acetoxypinoresinol, was unaffected. Free acidity was similar for the 0% and 10% EH treatments, increased sharply between 10% and 30% EH, and was similar again for the 60% and 100% EH treatments. Free acidity values were low and well within the limit for VOO classification even after 6 months of oil storage. Peroxide value responded to both *B. oleae* damage and storage conditions. Peroxide values increased between 10% and 30% EH treatments but changed little between the 30% and 100% EH treatments regardless of oil storage conditions. Secoiridoid concentrations closely reflected the degree of *B. oleae* damage when sources of variability such as cultivar and cultural practices were kept under control and conditions of processing and oil storage were optimal.

Olive trees are grown on ≈10 million ha in the world with 96% of production concentrated in the Mediterranean area (FAOSTAT, 2010), where the key pest of olive orchards is *Bactrocera oleae* (Rossi) (Raspi and Viggiani, 2008), commonly known as the olive fruit fly. The presence of *B. oleae*, a Tephritidae oligophagous on the fruits of a few *Olea* species, has long been documented in Mediterranean countries (Daane and Johnson, 2010; Tzanakakis, 2006). Recently, the olive fruit fly was discovered in southern California from where it spread almost throughout the entire state, posing a serious threat to the local olive industry (Daane and Johnson, 2010; Rice et al., 2003). Nowadays, *B. oleae* affects almost all the world olive production with few exceptions in isolated areas or where low temperatures limit its occurrence.

Olive fruit fly females lay their eggs in fruits of both cultivated and wild olives. Insect development occurs through three larval in-

stars: the hatched larva feeds and grows as a fruit borer in the mesocarp and, at the end of the third stage, either pupate in the olive or exit to pupate on the ground (Fletcher, 1987; Tzanakakis, 2003). In the field *B. oleae* larval development is largely temperature-dependent and the resulting number of annual generations depends on humidity as well as the availability and quality of olive fruits (Burrack and Zalom, 2008; Kounatidis et al., 2008).

Oviposition punctures by *B. oleae* cause a marked depreciation of fruits for table consumption (Tzanakakis, 2006), whereas the detrimental effects on oil production consist mainly in premature fruit drop, larval consumption of fruit pulp (estimated to range from 50 to 150 mg per larva, depending on cultivar) (Neuenschwander and Michelakis, 1978), and oil quality deterioration (Angerosa et al., 1992; Gomez-Caravaca et al., 2008; Tamendjari et al., 2009). The extent of the decrease in oil quality depends on the type of infestation, the percentage of damaged fruits, the fruit developmental stage, and the cultivar (Evangelisti et al., 1994). With regard to the type of infestation, a key role is played by the presence of EH produced by the full grown larvae, which destroy cellular integrity and expose the fruit inner tissues to oxygen (Angerosa et al., 1992; Gomez-Caravaca et al., 2008; Kyriakidis and Dourou, 2002). The resulting acceleration of hydrolytic and oxidative pro-

cesses determines an increase in free acidity and PV (Gomez-Caravaca et al., 2008). Current limits of free acidity and PV for VOO are 0.8% oleic acid and 20 mEq O₂/kg of oil, respectively (EU 1989/2003 modifying the ECC 2568/91; E.U. Off. J. Eur. Communities, 2003), whereas the concentration in phenolic compounds is not taken into account for oil classification, yet the modern concept of oil quality is mainly based on phenolic composition (secoiridoids and lignans in particular), which is closely related to the sensory and health properties of VOO (Servili et al., 2004). Although it is well documented that the phenolic content and oxidative stability decrease in oils obtained from fruits damaged by *B. oleae* attack (Angerosa et al., 1992; Evangelisti et al., 1994; Gomez-Caravaca et al., 2008; Pereira et al., 2004; Tamendjari et al., 2009), there are only few reports in which individual fractions of secoiridoids and lignans have been investigated (Gomez-Caravaca et al., 2008; Tamendjari et al., 2009). Moreover, in previous studies, the effects of *B. oleae* damage on qualitative characteristics of oils were difficult to quantify because of the many sources of sample variability (cultivar, orchard location, cultural practices, processing technology in Gomez-Caravaca et al., 2008) or the low number (0, 100, real infestation measured in the field) of treatments (Tamendjari et al., 2009). For instance, the high variability of samples led Gomez-Caravaca et al. (2008) to conclude that phenolic content was not a good indicator of *B. oleae* effects on oil quality.

The objective of the present study was to determine the effects of different levels of *B. oleae* damage, expressed as percentage of fruits with EH, on free acidity, PV, and concentrations of secoiridoids and lignans of VOO obtained from the widely cultivated cultivar Frantoio. Free acidity and PV were also measured during oil storage under different conditions of temperature and light to assess how storage conditions interacted with fruit damage caused by *B. oleae*.

Materials and Methods

Plant material. We used an irrigated olive (*Olea europaea* L. ‘Frantoio’) orchard (density of 513 trees/ha) at the experimental farm of University of Pisa at Venturina (lat. 43°01' N; long. 10°36' E) in 2008 and 2009 to sample fruits for the different experiments. Cultural conditions were as previously reported (Caruso et al., 2011). In brief, water was supplied during the summer by subsurface drip irrigation and only the trees that received full irrigation (1860 and 2134 m³·ha⁻¹, respectively) were used in both years. The orchard floor was permanently covered with grass that was mown three or four times a year. The trees were in full production and yielded ≈20 kg per tree in both years.

Pesticides were used only when strictly needed. To control diseases, copper oxychloride (Isagro, Milano, Italia) at a standard concentration of 5 g·L⁻¹ was sprayed once and twice a year in 2008 and 2009, respectively.

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The olive fruit fly pre-imaginal infestation was determined four times from the end of July through September by sampling 100 fruits from ≈ 50 trees. Fruits were taken to the laboratory and sectioned under a stereoscopic microscope (Wild® M5; Wild Ltd., Heerbrugg, Switzerland) to determine the type and extent of the *B. oleae* infestation. The percentage of fruits with active infestation (eggs, first and second instar larvae) and that with harmful infestation (third instar larvae, pupae, and EHs) was determined so that not only we could monitor larval population dynamics, but also the efficacy of pesticide applications. In fact, when the active infestation exceeded 15% of the fruits, Dimethoate (Isagro) at a standard concentration of $3 \text{ g} \cdot \text{L}^{-1}$ was sprayed to control the olive fruit fly. In 2008, active infestation was 21% and 24% on 22 July and 11 Sept., respectively, and therefore two larvicidal spray applications were done. In 2009 a single spray was applied on 22 July (active infestation 26%).

Olive fruit samples. Three experiments, mainly differing on harvest dates and storage conditions, were performed over the 2 years of study. Fruits were harvested at two dates (23 Oct. and 17 Nov.) in 2008 (Expts. 1 and 2, respectively) and on 25 Oct. 2009 (Expt. 3). In the first experiment, fruits (16 kg) were harvested from one tree, in the second one fruits were harvested from three trees (20 kg from each tree), in the third one only 8 kg of fruits was sampled from one tree. In both years care was taken that a large number of the harvested fruits had EH by *B. oleae*. To reduce the age variability of EH, before harvest, the tree branches were shaken to facilitate the abscission of fruits with old infestations and these fruits were discarded. Olive fruits presumably with the presence of EH of recent formation only were sampled directly from the tree. Healthy and damaged fruits were immediately taken to the laboratory, where they were divided into three categories after examining under a stereoscopic microscope: 1) fruits with EH; 2) fruits with ovipositional scars only; and 3) healthy fruits (neither holes nor scars). Once the extent of damage was assessed, fruits were divided into groups based on their skin color.

In Expt. 1, fruits were assorted to obtain samples differing in the percentage (0%, 10%, 20%, and 30%) of fruits with EH. An additional treatment consisted of fruits without ovipositional scars (WOS). In Expt. 2, we compared six levels of infestation (0%, 10%, 20%, 30%, 60%, and 100% EH) with WOS samples. Treatments of Expt. 3 differed in the level of infestation (0%, 10%, 20%, and 30% EH). Each treatment consisted of three replicates of 500 g fresh weight (FW) each. The maturation index of 50 fruits randomly taken from each replicate was measured according to standard methodology, whereby the skin and flesh colors were scored using a 0 to 7 scale (Caruso et al., 2011). The same fruits were also used to determine average fruit FW.

Oil extraction and analysis. To reduce potentially negative effects on oil quality resulting from factors other than olive fruit fly, the

experiments were carried out under optimal conditions for fruit storage and processing. In all experiments, the oil was obtained from the different fruit samples within 24 h from harvest using an Abencor system, consisting of a hammermill (MM-100), a thermo-mixer (TB-100), and a centrifugal machine (CF-100) (MC2, Ingenieria y Systemas, Sevilla, Spain). The fruit samples were washed with tap water, crushed with a hammer crusher, and the paste mixed at 28°C for 30 min. The malaxed paste was centrifuged at $1784 g_n$ for 3 min and the oil separated by decantation in a glass cylinder within 8 min. To avoid any contamination from the water phase below, only the top layer of the oil was collected ($\approx 50 \text{ mL}$) and stored at 4°C in the dark until analysis.

Free acidity and PV of oils were determined using an Oxitester unit (Olive Oxytester; CDR, Ginestra Fiorentina, Italy). This equipment allowed to determine both parameters rapidly (within 8 min) on small samples. An aliquot of oil sample (2.5 and $5.0 \mu\text{L}$ for free acidity and PV, respectively) was added to a pre-filled cuvette where fatty acids react with a chromogen developing a color with an optical density at 630 nm that is proportional to the fatty acid concentration. As for PV, radical peroxides oxidize Fe^{2+} and the resulting Fe^{3+} forms a red complex, the absorbance of which, measured at 505 nm, is directly proportional to the concentration of peroxides in the oil. Free acidity and PV determined by the Oxitester method had been preliminarily checked against the official method of analysis (EU 1989/2003 modifying the ECC 2568/91) using oil samples over a wide range of values. Regression equations between the two methods were highly significant (Fig. 1).

Phenolic compounds were determined only on oils from Expt. 2. The individual phenolic fraction of oils of Expt. 2 was determined by liquid-liquid extraction (Montedoro et al., 1992) and analyzed by high-performance liquid chromatography (HPLC). Solvents and reference compounds for liquid-liquid extraction and HPLC analyses, the solvents methyl alcohol, n-hexane, acetonitrile, acetone, and glacial acetic acid, were obtained from Carlo Erba (Milan, Italy). Water of both analytical and HPLC grade was obtained by using purification units. The dialdehydic form of eleonic acid linked to 3,4-DHPEA or p-HPEA (3,4-DHPEA-EDA and p-HPEA-EDA, respectively), the isomer of oleuropein aglycon (3,4-DHPEA-EA), (+)-1-acetoxypinoresinol, and (+)-pinoresinol were extracted from VOO and separated by semipreparative HPLC according to the procedure reported previously (Montedoro et al., 1993). The nuclear magnetic resonance (NMR) data of 3,4-DHPEA-EDA, p-HPEA-EDA, and 3,4-DHPEA-EA were consistent with those reported by Montedoro et al. (1993), whereas the NMR data relative to (+)-1-acetoxypinoresinol and (+)-pinoresinol were in accordance with those obtained by Owen et al. (2000). The 3,4-(dihydroxyphenyl)ethanol (3,4-DHPEA) was obtained from Cabru S.p.A. (Milan, Italy), and p-HPEA was purchased from Fluka (Milan, Italy).

Fig. 1. The relationship between free acidity (A) or peroxide value (B) measured according to the Official method (EU 1989/2003 modifying the ECC 2568/91) and the Oxitester. Regression equations: (A) $y = 1.06x + 0.001$, $R^2 = 0.996$; (B) $y = 1.09x - 0.21$, $R^2 = 0.995$.

The extraction of VOO phenols was performed as previously described by Montedoro et al. (1992). The HPLC analyses of phenolic extracts were conducted with an Agilent Technologies Model 1100 system, consisting of a vacuum degasser, a quaternary pump, an autosampler, a thermostatic column compartment, a diode-array detector, and a fluorimetric detector, as reported in Servili et al. (2011). The C18 columns used were Spherisorb ODS-1 $250 \text{ mm} \times 4.6\text{-mm}$ columns with a particle size of $5 \mu\text{m}$ (Phase Separation Ltd., Deeside, U.K.); the injected sample volume was $20 \mu\text{L}$.

Storage conditions. Free acidity and PV of oils from Expt. 1 were measured immediately after oil extraction. Different storage conditions were imposed only in Expts. 2 and 3. During storage, 1 mL of oil from each replicate was put in Eppendorf cuvettes and kept under different light and temperature conditions for ≈ 6 months. In particular, the storage conditions in Expt. 2 were: 1) 4°C in the dark; 2) growth chamber (average temperature of $23 \pm 0.1^\circ\text{C}$) under continuous neon light ($11 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ photosynthetic photon flux density); 3) growth chamber (average temperature of $23 \pm 0.1^\circ\text{C}$) in the dark; 4) average temperature of $15 \pm 1.3^\circ\text{C}$ with light turned on for short periods (5 min every 2 weeks) mimicking cellar conditions; and 5)

temperature like in treatment (4) but in the dark. The photon flux density was measured with an Integrating Quantum Sensor (LI-188 B; LI-COR, Lincoln, NE). Dark conditions were obtained by wrapping each Eppendorf cuvette with aluminium foil. In the 44-d study, only 0%, 20% and 60% EH levels stored at $23 \pm 0.1^\circ\text{C}$ (either in the dark or under continuous light) were compared. In Expt. 3, oil samples were stored under (2), (3), (4), and (5) conditions.

Statistical analysis. Fruits were randomly harvested from trees similar in size and productivity. Treatment means were separated by least significant differences at $P \leq 0.05$ after analysis of variance using a completely randomized design. Linear regression equations were calculated using Costat (CoHort Software, Monterey, CA).

Results

In all experiments, samples were prepared with fruits of similar FW and maturation index across all levels of *B. oleae* damage (Fig. 2A–B) to minimize effects resulting from size or ripening stage. Average fruit size was greater in Expt. 3 than in the two other experiments. Fruits from Expt. 3 were at a more advanced stage than those from Expts. 1 and 2, which had less than 50% of their skin pigmented. Free acidity and PV were affected by the percentage of fruits with *B. oleae* EH (Fig. 2C–D). Values of free acidity increased from 0.1% of WOS fruits to above 0.3% of samples when at least 60% of fruits were damaged. There were no differences in both free acidity and PV between 60% and 100% EH treatments.

Peroxide value increased from 2.3 mEq O_2/kg of oil of WOS fruits to 5.2 mEq O_2/kg of oil samples with 30% EH (Expts. 1 and 2); PV was higher in Expt. 3 and ranged from 5.1 to 6.7 mEq O_2/kg for the 0% and 30% levels of EH, respectively (Fig. 2D). In all samples, free acidity and PV were well within the limits of VOO classification (EU 1989/2003 modifying the ECC 2568/91).

There was a highly significant negative correlation between the total concentration of phenolic compounds (or the individual fractions of secoiridoids) and the degree of fruit damage (Fig. 3). In particular, although there were little differences in phenolic concentrations between WOS and 0% EH fruits, total phenols concentration decreased markedly as the number of fruits with EH increased (Fig. 3A). In general, the decrease was sharp between 0% and 60% EH treatments, but phenolic concentrations were similar over the 60% to 100% EH range. The phenolic concentrations of oils obtained from fruits with 30% and 100% EH were 59% and 16% those of fruits with 0% EH, respectively. Similar patterns were apparent for individual secoiridoid compounds with the exception of p-HPEA-EDA, which showed a decrease between 0% and 20% EH, and similar values beyond 20% level (Fig. 3). The concentrations of lignans, namely (+)-pinosresinol and (+)-1-acetoxypinosresinol, were unaffected by the degree of *B. oleae* fruit damage (Fig. 3F–G).

Free acidity of oils obtained from samples with 0% and 20% EH was unaffected by duration of storage or light exposure over the 44-d study period (Fig. 4A). On the other hand, free acidity of oils from 60% damaged fruits

had values that were between 1.8 and 2.2 times higher than those of the 0% and 20% damage levels, respectively. At the beginning of storage, average PV of oils was 4.9 mEq O_2/kg of oil and similar for all treatments but, after 1 week of storage in the light, PV had overcome the 20 mEq O_2/kg limit for VOO classification regardless of the degree of initial *B. oleae* damage (Fig. 4B). After 12 d, free acidity of the 60% EH treatment was 0.39% (Fig. 4A). When oils were stored in the dark at 23°C , the increase in PV was directly related to the degree of fruit damage but remained within the limit of VOO classification throughout the experimental period (Fig. 4B).

Changes in oil-free acidity driven by *B. oleae* damage followed a sigmoidal pattern after 3 and 6 months of storage (Fig. 5A–B). The acidity was similar for the 0% and 10% EH treatments, but beyond 10% EH increased sharply. Free acidity remained stable beyond the 60% EH level and, hence, no differences were measured between 60% and 100% EH. Free acidity values were low and well within the limit for VOO with a very small increase within each level of *B. oleae* damage between 3 and 6 months of storage (Fig. 5A–B).

Peroxide value responded to both *B. oleae* damage and storage conditions. However, the effect of light exposure and temperature during storage was prevalent over that of the olive fruit fly (Fig. 5C–D). Although PV changed little between the 30% and 100% EH treatments at both sampling dates regardless of storage conditions, there was a marked increase in PV values between 10% and 30% EH treatments when storage temperature was 4 or 15°C and between 0% and 20% EH treatments at 23°C . Similar results were obtained in Expt. 3 over the 0% to 30% range of damage, although PV was higher across all treatments and exceeded 15 mEq O_2/kg for samples stored at 15 or 23°C (data not shown). It should be noted that PV data for the storage treatment under continuous light exposure at 23°C are not shown in Figure 5 because PV of all samples exceeded the 40 mEq O_2/kg limit of detection of the equipment.

Discussion

Free acidity, peroxide values, and secoiridoid concentrations responded readily to increasing levels of fruit damage determined by *B. oleae* infestation. Both free acidity and PV increased as the degree of damage increased, as already reported for other cultivars (Angerosa et al., 1992; Gomez-Caravaca et al., 2008). Tamendjari et al. (2009) reported similar values of free acidity, which ranged from 0.2% to 0.4% oleic acid in oils from fruits from 0% to 44% to 63% EH, but higher PV probably as a result of the late date of harvest. In our study, free acidity was little affected by oil storage conditions, whereas differences in the light regime or temperature during storage markedly influenced PV. Three months of storage at 23°C in the dark or at 15°C with occasional exposure to feeble light were sufficient to double the initial PV of samples with at least 20% EH. Peroxide values of samples exposed to continuous light exceeded the 20 mEq O_2/kg

Fig. 2. Fruit fresh weight (A), maturation index (B), free acidity (C), and peroxide value (D) of oils obtained from fruits (cv. Frantoio) without ovipositional scars (WOS) or with different percentages of exit holes by *Bactrocera oleae* at harvest. Harvest dates were 23 Oct. 2008, 17 Nov. 2008, and 25 Oct. 2009 for Expts. 1, 2, and 3, respectively. Symbols are means $\pm$ SDs of three replicate samples.

Fig. 3. Concentrations (mg·kg⁻¹ oil) of the total phenols, the isomer of oleuropein aglycon (3,4-DHPEA-EA), the dialdehydic form of elenolic acid linked to 3,4-DHPEA or p-HPEA (3,4-DHPEA-EDA and p-HPEA-EDA, respectively), the hydroxytyrosol (3,4-DHPEA), the tyrosol (p-HPEA-EDA), the (+)-pinosresinol, and the (+)-1-acetoxypinosresinol in virgin olive oils obtained in Expt. 2 from fruit without ovipositional scars (WOS) or with different percentages of exit holes by *Bactrocera oleae*.

limit for VOO classification within 1 week regardless of *B. oleae* infestation. Light is a strong oxidative agent as a result of the occurrence of chlorophylls that activate photo-oxidation in the oil. The photo-oxidation process is not controlled by primary antioxidants such as secoiridoids and lignans and, for this reason, light can produce a strong oxidative damage in VOO (Hamilton, 1994). The lack of an effect of storage conditions on free acidity can be explained by the relatively low values of free acidity measured initially in the samples. The relationship between free acidity evolution during storage and its initial values is well known (Hamilton, 1994) and 6 months evidently were not sufficient to induce the autocatalytic phase of hydrolytic cleavage of fatty acids from the glycerol backbone.

Secoiridoid concentrations in the oil were very sensitive to the presence of fruits with EH, but not to that of oviposition scars. In fact, concentrations of most fractions were similar in WOS and 0% EH samples. Concentrations of 3,4 DHPEA-EDA and p-HPEA-EDA

showed a marked decrease in oils obtained from 10% of fruits with EH, whereas for 3,4-DHPEA-EA and 3,4-DHPEA, a clear decrease was measured only when at least 20% of fruits had EH. The presence of EH induces the beginning of oxidative reactions that result in degradation of phenolic compounds. The different response of secoiridoids and lignans to the percentage of fruits with EH can be explained by the activation of endogenous oxido-reductases such as peroxidases and polyphenoloxidases that are characterized by a strong oxidative affinity for the secoiridoids (Servili et al., 2008). These enzymes, in fact, use preferably ortho- and paradiphenols, which are characteristic of the secoiridoid structure as substrates (Robinson and Eskin, 1991). The activity of oxygen-induced enzymes like polyphenoloxidases, present mainly in the mesocarp, also explains the lack of an effect between WOS and 0% EH treatments because it is the presence of the EH that massively exposes the inner tissues of the mesocarp to the action of oxygen (Servili

Fig. 4. Free acidity and peroxide value of olive oils obtained from fruits (cv. Frantoio) with different degrees of infestation (0%, 20%, 60% of exit holes by *Bactrocera oleae*) in Expt. 2 and stored at 23 °C under either continuous light or in the dark in a growth chamber. Symbols are means – SDS of three replicates.

et al., 2007b, 2008). The presence of EHs might have determined a greater contamination by fruit macrobiota, which have been shown to decrease the concentration of oleuropein derivatives in the oil (Vichi et al., 2011). Concentrations of phenols above 200 mg·kg⁻¹ oil were measured in oils obtained from fruits with up to 60% EH. The 200 mg·kg⁻¹ value is currently considered the threshold above which phenolic compounds exert their nutraceutical effects as antioxidants that help to maintain healthy low-density cholesterol level (European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies, 2011).

From the practical point of view, free acidity reflected the degree of *B. oleae* infestation when damage was severe. In addition, secoiridoid concentrations appeared to be good markers of olive fruit fly damage when sources of variability (cultivar, cultural practices, and presumable age of EH) were kept under control and conditions of processing and oil storage were optimal. The negative correlation between oil phenolic concentration and the percentage of fly attack had already been shown by Gomez-Caravaca et al. (2008), but their conclusion was opposite to ours probably because of the variability of sampled material in that study. On the contrary, lignans were

Fig. 5. Free acidity (A–B) and peroxide value (C–D) of olive oil obtained from fruits (cv. Frantoio) with different degrees of infestation of *Bactrocera oleae* in Expt. 2 measured after 3 (A, C) and 6 (B, D) months of storage under different light and temperature conditions. Symbols are means of three replicates. Vertical bars represent least significant differences ($P \leq 0.05$) calculated after analysis of variance within each infestation level.

unaffected by *B. oleae* damage and cannot be proposed as indicators of olive fruit fly infestation. Lignans have been shown to be cultivar-dependent and are little affected by changes in environmental conditions or cultural practices (Servili et al., 2004, 2007a).

Reducing chemical input in the orchard is indispensable in modern olive growing. Previous studies identified thresholds of active infestation (i.e., eggs, first and second instar larvae) by the olive fruit fly beyond which pesticides should be applied to prevent major decreases in yield and oil quality. For this purpose, larval population levels causing economic losses have been proposed for some cultivars (Kapatos and Fletcher, 1983; Neuenschwander and Michelakis, 1978). Proposed thresholds of active infestation also take into account the fact that once oil degradation processes are induced, they progress rapidly during storage, especially if storage conditions are not optimal (Neuenschwander and Michelakis, 1978; Pereira et al., 2004; Torres-Villa et al., 2003). However, since these thresholds were determined, there has been a substantial evolution of the concept of oil quality. Nowadays oil quality includes analytical parameters as well as sensory evaluation by a panel test that are crucial for assessing the genuineness, sensory impact, and health properties (Servili et al., 2004). Defects of fustiness have been reported in oils produced from late-harvested fruits with EH (Tamendjari et al., 2009). Angerosa et al. (1992) showed the direct relationship between some volatile compounds associated with defective characteristics and the percentage of fruits with EH as well as the attenuation of volatiles responsible for the fruity notes of VOO.

In conclusion, we quantified the relationship between level of fruit damage by olive fruit fly and phenolic concentrations in the oil and showed that secoiridoids are sensitive indicators of fly attack. Moreover, most secoiridoids appeared to respond more readily than free acidity at low levels of olive fly infestation. Levels of fruit damage not exceeding 10% EH appear tolerable for the oil quality parameters we considered provided that fruits are processed within 24 h from harvest and conditions of processing and oil storage are optimal. In this respect, we confirmed that light is deleterious during storage and that temperature should not exceed 15 °C (Hamilton, 1994). Our results are preliminary evidence that thresholds of active infestation currently used in integrated pest management of olive orchard are probably too restrictive.

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