PAPER

PRELIMINARY DATA ON VOLATILE COMPOSITION OF OLIVE FRUITS OF CV. "SIMONA" AND POSSIBLE RELATIONSHIP TO RESISTANCE TO FLY OVIPOSITION

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ABSTRACT

By characterizing the volatile compounds of the olive cultivar "Simona" from southern Italy (Apulia region), we identified a way to analyze characteristics possibly linked to this olive's well-known resistance to fly oviposition. The pool of volatile compounds in the unripe and ripe fruits was identified, and even if the relative amounts of these compounds tended to vary with ripening, the fly repellent action appeared to be related to the sesquiterpenes such as α-copaene, cycloisosativene, α-muurolene, β-cubebene and hydrocarbons such as (*E*)-2-dodecene, undecane, tridecane, and 3-methyl undecane.
In agreement with the concept that the collective pool of volatile substances can enhance olfactory pleasure/repulsion more than would the effects of a single compound, the pool of volatile compounds identified in this paper may be among the possible characteristic mixtures with repellent action against *Bactrocera oleae*.
The selection of volatile compounds made by the cuticle and responsible of the headspace quality surrounding the whole olive fruit is also demonstrated to be markedly different from the headspace volatile compounds produced by the extracted oil.

Keywords: cv. Simona, olive fruits, olive oil, HS-SPME-GC/MS, *Bactrocera oleae*

1. INTRODUCTION

Olive growing in the Apulia region (southern Italy) has led to a specific varietal population selected through centuries. About 50 varieties have been developed, most of low agronomic interest. The major crops are limited to no more than about 15 widely grown cultivars, whereas some were abandoned for low productivity, despite their specific sensory and nutritional properties. Today, the promotion of local products is a common marketing trend, and the defined sensory characteristics represent the greatest attraction to consumers.

In recent years, some authors have noted that temperature can influence the acidic composition of olive oils in each cultivar and that various cultivars respond differently to different environmental conditions (FIORINO *et al*., 2003a; FIORINO *et al*., 2003b; PANNELLI, 2006).

Recently, a very old cultivar, "Simona," long grown in southern Italy, in the Apulia region, and particularly in the southeast of the province of Bari, in the territories of the towns of Castellana Grotte, Conversano, Monopoli, and Polignano, has been reconsidered for quality characteristics. GODINI *et al*. (2002) described the essential phenological and morphological traits of this "Simona" cultivar, which produces yellow oil and is slightly fruited, with fruits that are small, ellipsoidal, pruinose, and black violet at maturity. The origin is unknown, but presumably it is native to the Apulia region.

The main defect of this olive is the low oil output (GODINI *et al*., 2002): farmers identify the mean oil extraction value at 12%, *i.e*., about 20% less than the other cultivars. However, until 1960, the cv. "Simona" represented about 33% of the olive cultivation in the region, probably because the resulting oil preserves its sensory characteristics for more than one year (TATEO and BONONI, 2016).

The cv. "Simona" has not been well described. LOMBARDO *et al*. (2008), studying the triacylglycerol composition of 188 Italian cultivars from 2001 to 2005, observed an extreme variability of percentage of each fatty acid, making each cultivar peculiar in its triacylglycerol composition. For example, in the same year 2003, the percentage of oleic acid varied from a minimum of 49.8% (cv. "Orbetana") to 78.2% (cv. "Simona"), while the percentage of linoleic acid ranged from 3.9% (cv. "Simona") to 23.9% (cv. "Racioppella"). The paper confirmed the great sensitivity of olives to climatic changes.

The "Simona" cultivar shows good resistance to the fly *Bactrocera oleae*. Studies on the influence of oviposition have been carried out for some species of pest flies (FOSTER and HARRIS, 1997), but for "Simona" cultivars, no studies have addressed how the olive fruit affects susceptibility to *B. oleae*. The "Simona" cultivar is particularly resistant to *B. oleae* and its oil is of lower acidity than other oils produced in the same growing area from different cultivars (for example, "Oliastra", "Pasola"). Therefore, these characteristics justify the studies of the "Simona" olive fruit to discover the parameters that influence its resistance to *B. oleae* attack.

For plants, volatile organic compounds are largely recognized as having roles in reproduction, tritrophic interactions, below ground defense, and abiotic stress (DUDAREVA *et al*., 2006; PARÈ and TUMLINSON, 1999; TAMIRU *et al*., 2011; UNSICKER *et al*., 2009; WU and BALDWIN, 2010). Moreover, *B. oleae* appears to demonstrate cultivar preference in attacking specific olive cultivars (BURRACK and ZALOM, 2008; GONÇALVES *et al*., 2012; IANNOTTA *et al*., 2007; NAVROZIDIS *et al*., 2007). Other authors believe that oviposition is conditioned from host selection based on chemical, physical, and molecular features (CORRADO *et al*., 2012; KOMBARGI *et al*., 1998; IMPERATO *et al*., 2012; MALHEIRO *et al*., 2016; NEUENSCHWANDER *et al*., 1985; RIZZO *et al*., 2012; SPADAFORA *et al*., 2008). The amount of sesquiterpenes has been correlated to growth of olives; for example, in the case of cv. "Serrana," SPME-GC-MS analyses have

indicated an increase in α-copaene while the green fruit grows followed by a decrease related to fruit ripening (DE ALFONSO *et al*., 2014). On the other hand, a cultivar effect for sesquiterpenes and terpenes has been observed; for example, in cv. "Cobrançosa", the relative proportion of these compounds increased during olive maturation (MALHEIRO *et al*., 2015). The same authors cited the cv. "Verdeal Transmontana" as the most susceptible and the cv. "Cobrançosa" as the least susceptible to olive fly oviposition and affirmed that the susceptibility differences could be ascribed to olive volatile content and composition. Thus, the volatile composition of olives may depend on the olive cultivar and be influenced by olive maturation. Other authors (BURRACK and ZALOM, 2008; GONÇALVES *et al*., 2012) also support the view that a combination of several factors may affect the ovipositional preference of *B. oleae*.
Considering that cv. "Simona" shows a particularly resistance to *B. oleae,* the aim of this work was the determination of volatile compounds released from the whole unripe and ripe olives harvested in an area of Castellana Grotte (Bari, Italy). Moreover, the determination of volatile compounds in the oil obtained has been carried out in order to distinguish the selection of volatiles effected by the cuticle of whole fruits.
We determined that the volatile compounds surrounding the whole olive fruits were represented by sesquiterpenes and hydrocarbons, while the volatile compound composition of the olive oil was characterized by aldehydes, alcohols, esters.
The volatile compounds selected by the cuticle may represent a possible natural adaptation useful to reduce the susceptibility to olive fly attack.
This preliminary study was focused on the headspace characterization of whole olive fruit of cv. Simona which shows a particularly high resistant to fly attach.

2. MATERIALS AND METHODS

2.1 Description of the geographical area

We evaluated the volatile compounds found in whole unripe and ripe olives harvested in an area of Castellana Grotte (latitude and longitude 40.86907314710559 and 17.1785811334848, respectively). In this area (2.4 ha), owned by the "Agriculture" foundation, 170 "Simona" olives are grown with other cultivars (e.g., "Oliastra", "Coratina", and "Pasola") in lower quantity. The olive production period covered in this study was October 2015 to January 2016.

2.2. Evaluation of fly infestation level and maturity index

Following MALHEIRO *et al.* (2016), to assess the fly infestation level of cv. "Simona" in comparison with two other cv. "Pasola" and "Oliastra" growing in the same area, 20 random hand-picked fruits were collected in early November 2015 from five olive trees per cultivar, and the resulting 100 fruits per cultivar were inspected for signs of infestation in a stereo microscope SMZ-168 TH – 1:6.7 Zoom Ratio (Motic-Italy) equipped with a PrimoCam 5 HD Zeiss digital camera (TiEsseLab-Italy).
For calculation of the maturation index, we followed the method described by HERMOSO *et al*. (2001), separating 100 olive fruits (20 fruits per tree) in levels from 0 to 7, and examining the epidermis and pulp color. If the epidermis was green, the fruit was classified as "0". Fruits showing epidermis of yellowish-green were classified as "1". Fruits showing red spots on less than half fruit were classified as "2"; epidermis red or purple on more than half the fruit led to classification "3", and fruits with black epidermis and white pulp were classified as "4". The level "5" was due to fruits with black epidermis

and purple on less than half the pulp and the level "6" was due to fruits with black epidermis and purple on more than half the pulp but not reaching the stone. The level "7" is due to fruits with black epidermis and whole pulp purple, reaching the stone. Using the letters a, b, etc., to identify the number of fruits and the seven levels cited before, the maturation index is calculated as follows: MI = (a x 0 + b x 1 + c x 2 + d x 3 + e x 4 + f x 5 + g x 6 + h x 7)/100.

2.3. Oil extraction process

Within 12 h of fruit harvest, oil was extracted using an "Oliomatic 150" system, equipped with a hammer crusher, a vertical malaxator, and a two phase decanter (Enoagricola Rossi - Perugia), operated at 25 °C and with 3% of water. The oil was not filtered after the extraction.

2.4. Olive oil samples

Oil was produced by olives of cv. "Simona" at a maturation index between 3.5 and 5.5 and stored in a 5 litre dark glass bottle at 12 - 15 °C for 4 days before the HS-SPME analysis.

2.5 HS-SPME analysis of whole olive fruits and oil and GC-MS conditions

For olive fruits, three aliquots of 80 ripe or unripe whole olive fruits were placed in a Pyrex 250 mL round media bottle sealed with a screwcap equipped with a septum.
For olive oil samples of cv. "Simona", three 100 g aliquots were weighed and placed into the same type of media bottle.
For whole fruit and oil, the sealed bottle was placed in a thermostatic bath at 40 °C, with a magnetic stirrer for the oil aliquots. After 1 h, an SPME fiber covered with 2 cm of DVB/CAR/PDMS, 50/30 mm (divinylbenzene/carboxen/polydimethylsiloxane) (Supelco, Milan, Italy) was inserted through the septum and left in the headspace for 5 h. With respect to the repeatability of the results, the headspace equilibrium, from to the cuticle of whole olive fruit, was reached in the time adopted.
The volatiles absorbed by the fiber were thermally desorbed in the injection port of a GC/MS (gas chromatography/mass spectrometer) for 10 min at 220 °C in a gas chromatograph with a split/splitless injection port. The injector was operated in split mode (1:5). Before sampling, the fiber was reconditioned for 15 min in the GC injection port at 240 °C, and blank runs were carried out before every analysis. The efficiency of the fiber was periodically verified by monitoring the signal of the internal standard.
Gas chromatography/mass spectrometry (GC/MS) analyses were performed in a Shimadzu 2010 gas chromatograph coupled to a Shimadzu QP-2010 MSD quadrupole mass spectrometer (Shimadzu, Milan, Italy). The gas chromatograph was equipped with a Supelcowax -10 30 m × 0.25 mm column, 0.25 mm film thickness (Supelco – Italy). The operating conditions for the GC/MS were: helium flow 1.0 ml min^{-1}, and oven temperature 40 °C for 5 min, increased to 240 °C at a rate of 3 °C min^{-1}. The temperature of the ion source was 200 °C, the electron energy was 70 eV, and the interface temperature was 240 °C. Mass spectra were acquired over the mass range 40–300 a.m.u.

2.6. Volatile compound identification and quantification

Volatile compounds in the headspace of the whole olive fruits and the olive oil were identified by matching their mass spectra with the reference mass spectra of a private library (Tateo-Bononi Oils) and the NIST 147 library.

To verify that the fly repellent action produced by headspace of whole olive fruits was maintained by the same pool of volatile compounds identified in the unripe and ripe stage, we compared data expressed as normalized area counts from whole unripe and ripe olive fruits. These data were compared to the volatile composition of the oil extracted from cv. "Simona", expressed as normalized area counts. All data were normalized to the most represented volatile compound (a-copaene in unripe whole olive fruits).

2.7. Statistical analysis

All analyses were performed in triplicate and the results are reported as mean values. The significance of the differences between data of whole olive fruits and olive oil was confirmed using Student's *t* test. A p-value < 0.05 was set as a statistical threshold for significance.

3. RESULTS

In the year 2015, the fly infestation measured with the method described in *2.2*, and concerning our samples collected in the limited area described in 2.1, was not particularly high: the fruits of cv. "Simona" showed no oviposition sites or exit holes, while the two other cv. showed 25% ("Oliastra") and 18% ("Pasola") *B. oleae* infestation. These data represent the mean of three replicate measurements. So, we can affirm that the values reported in this paper correspond to a meaningfully different behavior of cv. "Simona" with respect of other cultivars considered for comparison from the same area.
Considering the headspace for whole olive fruit samples at two maturation index values 0.0 and 5.2, as shown in Fig. 1 and in Table 1, we deduced that the fly repellent action was maintained by the same pool of volatile compounds, even though their amounts were naturally modified by ripening. These compounds are sesquiterpenes and hydrocarbons, the two most highly represented classes of volatiles in the headspace of olive whole fruits. Data reported also showed the substantially different composition between the headspace produced by whole olive fruits (ripe or unripe) and by olive oil. Indeed, the olive oil headspace contained a very important fraction of more volatile oxygen-containing compounds such as alcohols ((*Z*)-3-hexenol, (*E*)-2-hexenol, octanol), aldehydes (hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, nonanal), and some other typical compounds such as 5-ethyl-2(5*H*)-furanone. In contrast, the volatile compounds released through the cuticle of the whole unripe olive fruits were almost exclusively represented by sesquiterpenes (a-copaene, cycloisosativene, α-muurolene, and β-cubebene, in decreasing order of representation) and hydrocarbons ((*E*)-2-dodecene, undecane, tridecane, and 3-methyl undecane, in decreasing order). This result suggests that the effective fly repellent action is related to the pool of these volatile compounds.
The data obtained demonstrate that GC/MS identification of the volatiles starting from whole fruits is fundamental for evaluating volatile compounds responsible for the fly repelling activity.
However, the volatile composition reported in Fig. 1 and derived from whole fruit of cv. "Simona" appeared to be specific and not comparable to volatile composition of the olive oil, and the characterized pool of sesquiterpenes and hydrocarbons could be identified as repellent to *B. oleae*.

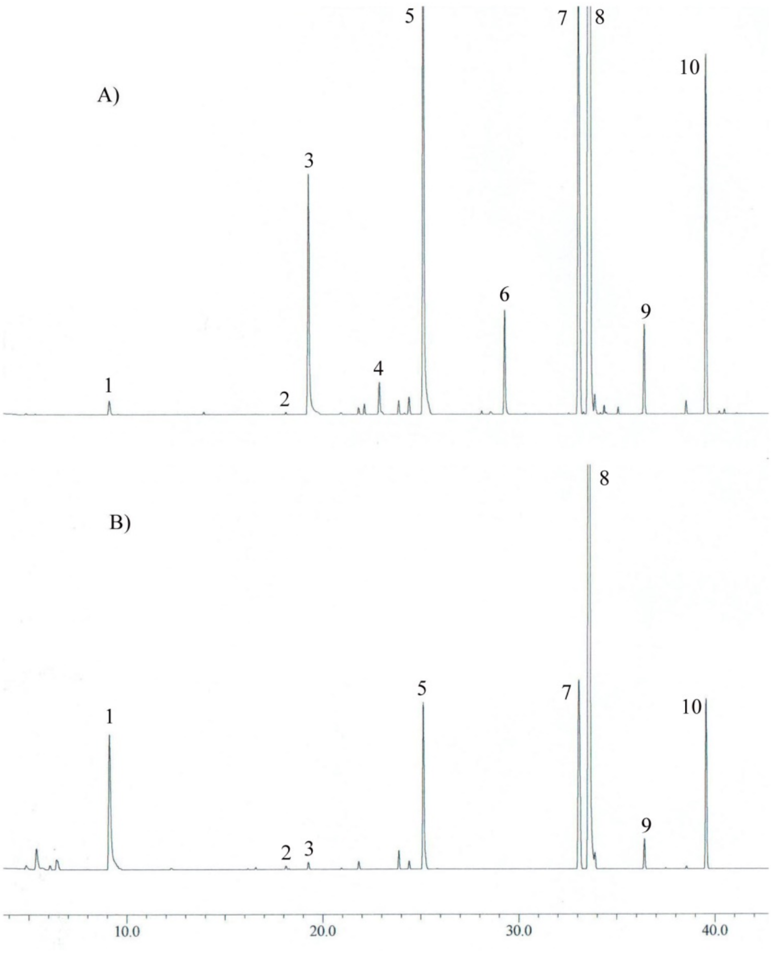

Figure 1. HS-SPME-GC/MS trace of unripe A) and ripe B) whole olive fruits cv. "Simona." Peak identification: 1) ethyl-2-methyl butyrate; 2) (*Z*)-3-hexenyl acetate; 3) undecane; 4) 3-methyl undecane; 5) (*E*)-2-dodecene; 6) tridecane; 7) cycloisosativene; 8) α-copaene; 9) β-cubebene; 10) α-muurolene.

Table 1. Volatile compound identification and comparison of normalized for whole unripe (M.I. = 0.0) and ripe (M.I. = 5.2) olive fruits and olive oil (M.I. 3.5 - 5.5). Values are normalized to the most represented volatile compound (α-copaene in unripe whole olive fruits). Data reported demonstrate the substantially different composition between the headspace produced by whole olive fruits (ripe or unripe) and by olive oil.

cv. "Simona"	unripe fruits	CV%	ripe fruits	CV%	oil	CV%
hexanal					0.2	0.01
(*Z*)-3-hexenal					2.8	0.03
(*E*)-2-hexenal					33.6	0.48
(*Z*)-3-hexenol					7.0	0.34
(*E*)-2-hexenol					2.9	0.03
(*Z*)-3-hexenyl acetate			0.1	0.04	10.3	0.42
D^3carene					1.8	0.02
5-ethyl-2(5*H*)-furanone					2.3	0.03
ethyl-2-methyl butyrate	0.4	0.02	5.3	0.62		
octanol					0.2	0.01
nonanal					5.0	0.28
3-methyl undecane *(H)*	0.7	0.05				
undecane *(H)*	6.7	0.92	0.2	0.07		
dodecane *(H)*					4.6	0.25
(*E*)-2-dodecene *(H)*	15.9	0.74	4.3	0.73	26.3	0.51
tridecane *(H)*	2.5	0.03				
phenyl ethyl alcohol					1.2	0.02
2,2-dimethyl-3-heptanone					6.7	0.13
cycloisosativene *(S)*	15.0	0.81	6.7	0.74	5.3	0.20
α-copaene *(S)*	100.0	1.20	47.0	0.82	45.8	0.40
α-muurolene *(S)*	8.8	0.58	4.1	0.05	17.0	0.32
α-farnesene *(S)*					46.4	0.42
β-cubebene *(S)*	2.1	0.07	0.7	0.08		
Total	152.1		68.4		122.4	
Σ sesquiterpenes *(S)*	125.9		58.5		114.5	
Σ hydrocarbons *(H)*	25.8		4.5		30.9	

(S)= sesquiterpenes
(H) = hydrocarbons

4. CONCLUSIONS

The resistance activity shown by cv. "Simona" to fly oviposition was evidenced in a well-defined growing area of 2.4 ha as described in 2.1 where other cultivars were present but did not show the same repellent activity against *B. oleae*.
Considering that as of today the factors associated with repelling a fly attack have not been identified with certainty, we think that a concrete approach to investigate the fly repellent action could be the identification of the pool of active specific volatile compounds starting from whole fruit of other cultivars showing resistance to fly attach, following the method suggested in this paper. The analysis of whole fruit versus oil of cv. "Simona" yielded two very different patterns of volatiles: the data demonstrate that the

whole fruit does not convey into the headspace the same qualitative and quantitative abundance of volatile compounds produced in the pulp, and by selective permeation, only selected volatile substances that are truly active in repelling the flies are conveyed into the headspace.
This identification of a mixture of substances that can reduce the negative consequences of *B. oleae* attack is in accord with a fundamental principle of flavoring science and technology: no single volatile compound can always produce the best olfactory response, and often, various volatile substances collectively produce a more effective olfactory stimulus. Thus, it is reasonable to suggest that a synergistic effect of a mix of various flavorings might underlie the repellence of *B. oleae*, and in the case of cv. "Simona," the active substances able to prevent the fly attack seem to be a pool of sesquiterpenes and hydrocarbons and no other volatile oxygen compounds.
This proposed line of research is compatible with the accepted conclusion of many authors that fly repellence is related to the specificity of the cultivar.
This analytical approach, based on the evidence of selective permeation of volatile compounds through the cuticle of the whole olive fruit, could be used to evaluate the composition of headspace surrounding the whole olive fruits for other cultivars traditionally recognized as not being susceptible to infection or as being more resistant to fly attack.

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