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Tree Planting Density and Canopy Position Affect 'Cerasuola' and 'Koroneiki' Olive Oil Quality

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Abstract: To maximize orchard production and tree crop efficiency, optimization of both maximum orchard light interception and radiation distribution within the tree canopy are important strategies. To study the influence of planting density and fruit position within the canopy on oil quality from 'Cerasuola' and 'Koroneiki' olive (*Olea europaea* L.), fruits were harvested from the upper and lower canopy layers of trees in hedgerow planting systems at two densities: High at 1000 trees ha^{-1} (HD) and Medium at 500 trees ha^{-1} (MD). Tree crop efficiency and fruit weight, water and fat content were measured together with olive oil standard quality parameters, phenolic and volatile composition. Fruits in the upper layers of the canopy always showed a higher maturity index, 6% more fat content, and 4% less water content than lower layers. Upper layers of HD trees showed the highest phenol content, whereas lower layers of MD trees showed the lowest phenol content (36% less than the upper layers of HD). HD trees showed the largest differences in fruit maturation, water and fat content between upper and lower canopy positions, increasing quality and oil yield variability at harvest. 'Koroneiki' showed more stable oils with a 28% higher MUFA/PUFA ratio and 12% higher phenol content than 'Cerasuola' oils. This study provides further evidence of the fact that cultivar, planting density, and canopy architecture may be strong determinants of olive oil yield and composition in hedgerow planting systems.

Keywords: *Olea europaea*; fat content; fatty acid profile; phenolic content; volatile compounds; hedgerow planting systems

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1. Introduction

Due to a global increase of olive oil consumption and labor costs many olive (*Olea europaea* L.) growers are moving towards the use of hedgerow super-intensive planting systems. Earlier bearing, increased yield, and a reduction of alternate bearing, as well as cost and facilitation of complete mechanization, have spurred adoption of new and super-intensive planting systems. Sicily represents an important center of olive oil production in the Mediterranean basin, where 'Cerasuola' is one of the most common olive cultivars [1]. The variety adapts well to poor soils, is drought-resistant, and provides excellent results under optimal nutritional conditions [2,3]. The oil content in the drupes of 'Cerasuola' is relatively high (20–25%), and, according to sensory evaluations, generally falls in the category of medium intensity fruitness along with the taste sensations of bitter, pungent,

and sweet [3]. Recently, the international cultivar 'Koroneiki' has been introduced in Sicily. 'Koroneiki' is a cultivar of high vigor with an upright growth habit that partially satisfies requirements for super-intensive planting systems. Its fruits are rather small, and the olive oil produced by 'Koroneiki' has excellent quality and fragrance, classified as a very fruity oil with green-apple notes, medium level aroma of leaves and grass, bitter and pungent. It is also astringent with a touch of almond, fig, and bark [4].

Due to its nutritional and health-promoting effects, the consumption of olive oil has been increasing worldwide, even in countries where it is not produced [5]. The nutritional and health promoting effects of olive oil have been associated with the optimal balance between saturated, mono, and polyunsaturated fatty acids as well as to minor components such as chlorophyll, polyphenols, and tocopherols [6]. Marketing of high quality olive oils is based on the chemical and sensory attributes, which are strongly affected by genotype, environment, fruit maturity at harvest, and their interaction among other factors [7–9]. Previous studies evidenced that high quality olive oil requires harvesting olive fruit at the optimum time [10–12]. The rate of oil synthesis and the length of the oil accumulation period can be responsible for the final oil content in the olive fruit [13,14].

High production costs, especially for harvest, have played a key role in the redesigning of olive orchards during the last 30 years [15]. For an optimal yield and maximum light interception, optimum planting density should be determined [16]. In addition to tree spacing, cultivar, climate, harvest method, tree training system, fertilization, irrigation management, and soil conditions should be properly considered. It was reported that along with the reduction of row spacing (ranging from 7 to 3 m), the management of orchard light interception should be taken into consideration [16–18]. Moreover, increasing tree planting density alters interception of solar radiation and distribution of radiation within the tree canopies during the orchard development [17,18]. This allows for managing the efficiency of solar radiation used for different processes including photosynthesis, flower bud formation, growth, and fruit quality. Jackson [17] indicated that to maximize orchard production and efficiency, both interception of maximum amount of radiation and optimization of the radiation distribution within the canopy are important factors. In olive trees, fruits located on the periphery of the canopy which exploited more solar radiation were bigger and with higher oil contents compared to fruits from internal parts of the canopy [19]. In 'Arbequina' hedgerows, fruits from the upper part of the canopy showed more advanced maturity and larger size. Furthermore, oil content increased by nearly 50% from lower to upper layers (Gómez-Del-Campo et al., 2009b). Hence, it can be concluded that intercepted radiation determines some of these differences, such as fruit size and oil content. In addition, differences in oil quality can result from rapid growth and early maturation in the upper layers of the tree canopy. Indeed, previous studies indicated that irradiance received in different hedgerow positions and orientations influenced fruit development and oil quality in olive. Fruits receiving more radiation showed the highest fruit weight, mesocarp oil content, maturity index, and total polyphenols in virgin olive oil [20]. Recently a study of 'Arbequina' also found that oil extracted from the upper layers presented higher concentration of oleuropein and ligstroside aglycone [12]. These compounds, besides having high antioxidant capacity, also contribute to the bitter and pungent flavor of the oil [21]. On the other hand, several studies reported that biomass production was directly related to canopy light interception in other fruit trees such as apple and peach [22–25]. In 'Arbequina' olive trees trained to 2.5–2.9-m hedgerows, the fatty acid composition of the extracted oils was significantly affected by the amount of intercepted light [26].

The aim of this study was to evaluate the influence of fruit position in the canopy and planting density on the production, fruit characteristics, and oil quality parameters such as phenol contents and volatile compounds from 'Koroneiki' and 'Cerasuola' olives. 'Koroneiki' was included as a reference cultivar since it is already implemented in super-intensive planting systems while 'Cerasuola' is a major Sicilian cultivar usually grown in traditional planting systems and appreciated for its quality oils.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Fruits were obtained from olive trees grown in an experimental field located in the southwest region of Sicily (37°31′ N, 13°03′ E, about 120 m a.s.l.). The orchard was planted in 2012 using 1-year-old self-rooted olive trees of the cultivars Cerasuola and Koroneiki. Trees of the two cultivars were planted in a single north–south row at two planting densities: 2 × 5 m (1000 trees/ha, HD) and 4 × 5 m (500 trees/ha, MD). The trees were pruned lightly during the first 5 years after planting and trained to free palmette, a two-dimensional tree shape, to facilitate mechanical harvesting. Two self-compensating in-line drippers per plant, delivering 16 L/h, were used for weekly irrigation, from July through mid-September. The total seasonal application rate was 640 and 320 m^3/ha/year, for HD and MD plantings, respectively. Fruit yield (kg/tree), yield efficiency (kg cm^{-2}), and yield (t ha^{-1}) were calculated for each tree as a biological replicate. All other measurements performed in the fruit and oil were determined separately in the upper and lower layers of the canopy.

2.2. Olive Harvest

On 15 November (for 'Cerasuola') and 17 November (for 'Koroneiki') of 2017, 28 trees for each cultivar were selected based on similarities for fruit load, number of branches and light distribution in canopy. To test the effect of the fruit position in the canopy fruits were harvested separately from the upper half of the canopy (1.5–3.0 m) or the lower half of the canopy (0–1.5 m). Fruits were hand harvested and placed in 1-ton bins for processing. Fruits were weighed and processed with a commercial two-phase mill (Toscana Enologica Mori-TEM) with a working capacity of 400 kg of olives/run. The oil extracted from each combination of factors (cultivar, planting density and canopy position) was subsequently weighed, and subsamples taken for chemical analyses.

2.3. Fruit Measurements

Fruits from upper and lower canopy layers at each planting density were assessed for fresh weight of 100 fruits (g) and maturity index based on skin and pulp pigmentation [27]. Fruit moisture content (% of fresh weight) and fat content (% of fresh weight) were determined by near infrared (NIR) measurements using an Olivia NIR analyzer (FOSS, Hillerød, Denmark). Percentage of black peel cover color was determined by digital image analysis as described in Farina et al. [28] using 32 photo replicates per cultivar (two per tree from 16 trees), each containing 45 fruits. Specifically, we used an algorithm that converts images from RGB to CIE (Commission Internationale de l'Eclairage) L*a*b* format, extracts the fruit from the image (removing the image background), and quantifies color characteristics as the weighed distance of each pixel in the image from a reference sample (darkest area interactively chosen from a well colored fruit). A green–red threshold algorithm was added to the previous procedure to obtain a separation of the total fruit area (in number of pixels) into two subregions: black color (closer to red) and ground color (closer to green). The pixel ratio between the red-colored area and total fruit area was used to quantify the percentage of black peel color (or degree of veraison).

2.4. Oil Quality Traits

Free acidity (% of oleic acid) and peroxide value (mEq O_2 kg^{-1}) were measured according to the European Union standard methods (UE, 1989/2003 modifying the ECC 2568/91). According to the limits established by the International Olive Oil Council [29] for free acidity, peroxide value, and organoleptic characteristics, all oils studied were classified as extra virgin olive oils (EVOOs) (Table 1). Chlorophyll and carotenoid contents were measured colorimetrically using a Beckman DU 640 UV spectrophotometer at 476 and 670 nm, as described by Mineo et al. [30]. The chlorophyll and carotenoid contents were expressed as mg of chlorophyll a and β-carotene per kg of oil, respectively.

Table 1. Main olive oil quality traits. Average and standard deviation (SD) of all olive oil samples (n = 24). Limits for classification of extra-virgin olive oil by the International Olive Council.

Main Quality Traits	Limits Described in	Mean ± SD
	IOOC/T.15/NC No 3/Rev. 11	
Free acidity (%m/m expressed in oleic acid)	≤0.8	0.4 ± 0.18
Peroxide value (in milleq. O_2 per kg/oil)	≤20	7.0 ± 3.8
K_{232}	≤2.50	1.35 ± 0.18
K_{270}	≤0.22	0.10 ± 0.02
ΔK	≤0.01	0.001 ± 0.0013

2.5. Phenolic Compounds

Identification and quantification of phenolic compounds was performed for each olive oil sample. Phenols were extracted according to IOC procedure (COI/T.20/Doc. No 29/Rev.1 2017) and identified according to Grilo et al. [3]. Specifically, in a centrifuge tube, 2 g of olive oil were mixed with 5 mL of a solution of methanol and water (80:20 v/v). The tube was vortexed for 1 min, held in an ultrasonic bath for 15 min, and centrifuged at 5000 rpm for 25 min. The supernatant was filtered with a 0.45 µm PTFE syringe filter and kept at 4 °C. Triplicate samples of olive oil were used for each cultivar, planting density, and canopy position. Phenolic compounds were identified by ultra-high performance liquid chromatography, heated electrospray coupled with high resolution mass spectrometry (UHPLC-HESI-MS) analysis using a quadrupole Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). UHPLC analysis was performed using a Dionex Ultimate 3000 System (Dionex Softron GmbH, Germering, Germany) equipped with an autosampler controlled by Chromeleon 7.2 Software (Thermo Fisher Scientific, Bremen, Germany). A UHPLC column (Phenomenex Luna C18(2) 50 × 1 mm, 2.5 µ) was used for separation of the selected compounds at 35 °C. The mobile phases used were 0.1% formic acid in water (v/v) (A) and methanol (B). The elution gradient program was: 0–5 min 10% B; 5–50 min linear increase to 99% B, 50–56 min 10% B coming back to the initial conditions until full stabilization. The column temperature was set at 30 °C and the injection volume at 1 µL. The flow rate was 50 µL min^{-1}. The total UHPLC-HESI-MS method runtime was 60 min. Detection was based on calculated exact mass and on retention time of target compounds, and data were evaluated by Quan/Qual browser Xcalibur 3.0 (Thermo Fisher Scientific, San Jose, CA, USA). Reference phenolic compounds including hydroxytyrosol, tyrosol, oleocanthal, were purchased from Sigma-Aldrich (Steinheim, Germany). Linearity of the MS response was verified with solutions containing all standards at six different concentration levels over the range from 0.250 to 5 ppm. Each point of the calibration curve corresponded to the average of five independent injections.

2.6. Fatty Acid Profile

Fatty acids of oil samples were determined as methyl esters by gas chromatography using the method described by the International Olive Oil Council (IOOC/T20 doc. 33). Quantification was carried out using a Focus GC equipped with a MEGA 10 column (50 m × 0.32 mm × 0.25 µm, Agilent Technologies, Santa Clara, CA, USA) and helium as carrier gas. Data were expressed as percentage of total area of the picks from each chromatogram.

2.7. Sensory Evaluation

Sensory evaluation of the oils was performed according to the panel test method (IOOC/T20 Doc. 15/Rev. 10) by trained panelists from the Assessorato Regionale dell'Agricoltura, dello Sviluppo Rurale e della Pesca Mediterranea (Sciacca, Italy). This method is only applicable to olive oils and is based on the intensity of attributes perceived by a group of tasters trained and monitored as a panel. The main positive attributes are fruity, bitter, and pungent. Four

samples were evaluated in each session. Samples were evaluated in individual sensory booths, using standard blue glasses. Judges could re-taste the oils as often as necessary and had to rinse their mouth with water and eat crackers in between sample tastings. Each session lasted approximately 60 min. Oils were tasted two times on different days and results are expressed in median of the sensory attribute.

2.8. Volatile Profile

Volatile compounds were analyzed using a gas chromatograph (GC) coupled with a mass spectrometer (MS) according to Sacchi et al. [31]. Each sample (3.0 ± 0.1 g), spiked with 4-methyl-2-pentanol as internal standard (2 mg/kg), was weighed into a 10 mL glass vial, and sealed with a PTFE/silicon septum. After 10 min at 40 °C, a solid-phase microextraction (SPME) fiber (DVB/CAR/PDMS, Sigma-Aldrich, St. Louis, MO, USA) was exposed to the sample headspace for 30 min for volatile extraction. The volatile analysis was performed on a GC/MS Shimadzu model QP5050A (Kyoto, Japan). A Supelcowax 10 (60 m × 0.32 mm × 0.5 µm, Sigma-Aldrich, St. Louis, MO, USA) was used for separation of compounds. After sampling, the fiber was thermally desorbed in the GC injector for 10 min at 240 °C. Helium was used as carrier gas at a flow rate of 1 mL/min. GC oven temperature started at 40 °C and ramped at 3.5 °C/min after 4 min to a final temperature of 240 °C. Ionization energy of 70 eV was adopted and the ions were analyzed in the m/z range from 40 to 400. Quantification was determined in relation to the internal standard. Peak areas were calculated by using the Labsolution acquisition system (GC-MS Solution version 1.20; Shimadzu, Kyoto, Japan). A blank test was performed prior to each analysis and all analyses were performed in triplicate. C5 (1-penten-3-one and 1-penten-3-ol) and C6 (Hexanal, *(E)*-2-hexenal, 3-hexen-1-ol acetate, 1-hexanol, 3-hexen-1-ol and 2-hexen-1-ol) volatile compounds were identified.

2.9. Statistical Analysis

Analysis of variance (ANOVA) at $p \leq 0.05$ was performed to identify the effects of cultivar and planting density on yield parameters (two-factor ANOVA), and the effects of cultivar, planting density, and canopy position on all quality parameters (three-factor ANOVA) using Systat software (Chicago, IL, USA). When appropriate, ANOVA was followed by Tukey's multiple comparison test ($\alpha \leq 0.05$) to separate means.

3. Results and Discussion

3.1. Production and Fruit Quality

The influence of cultivar, planting density, and canopy position on production and fruit characteristics is presented in Table 1. Fruit yield ranged between 4.9 and 9.2 kg/tree. MD trees of both cultivars showed higher fruit yield than HD trees. The same trend was observed for yield efficiency with MD giving the highest values (0.2 kg cm^{-2}). The highest yield per hectare was obtained with 'Cerasuola' at HD. These results are in agreement with a long-term study on 'Arbequina', where fruit and oil production per tree responded markedly to row spacing after six years from planting with higher values at a greater planting distance [32]. In 'Cerasuola', fruit yield expressed in t ha^{-1} indicated that lower fruit yield per tree of HD was compensated by the larger number of trees per hectare. In contrast, 'Koroneiki' did not show significant differences between HD and MD, suggesting that yield potential of a single 'Koroneiki' tree was reached at lower densities. Results on the percentage of fruit yield harvested in the upper or lower layers of the canopy demonstrated that the majority of the production occurred in the upper layers. 'Cerasuola' presented the biggest differences in fruit distribution across the canopy. Percentage of fruit production in the lower layers of the canopy was higher than previously described by Castillo-Ruiz et al. [33] for 'Arbequina' in the south of Spain (17%), indicating that cultivars (growth habit, canopy density, and vigor) and cultural practices (mainly pruning) have a great influence on fruit distribution within the canopy.

'Cerasuola' presented higher fruit weight than 'Koroneiki' (Table 2). Fruit weight was not affected by planting density or canopy position, suggesting that any possible difference in fruit growth must have been canceled by the fruit's ability to attract assimilates from distant areas of the canopy during development [34].

Table 2. Production and fruit quality of 'Cerasuola' and 'Koroneiki' olive trees planted at 1000 (HD) and 500 (MD) trees/ha. Production described as fruit yield (kg/tree), yield efficiency (kg cm^{-2}), yield (t ha^{-1}), percentage of total fruit harvest distributed on the upper and lower layers of the canopy, and oil yield (kg of oil 100 kg^{-1} of fruit). Fruit conditions at harvest described by maturity index, percentage of black peel cover (veraison), fresh weight (g), moisture and fat content (g 100 g^{-1} of fresh weight).

Cultivar	**'Cerasuola'**				**'Koroneiki'**			
Planting Density	**HD**		**MD**		**HD**		**MD**	
Canopy Layer	**Upper**	**Lower**	**Upper**	**Lower**	**Upper**	**Lower**	**Upper**	**Lower**
Fruit yield	6.82 ab [z]		8.03 a		4.40 b		9.23 a	
Yield efficiency	0.23 ab		0.26 a		0.16 b		0.28 a	
Yield	6.82 a		4.02 b		4.40 b		4.62 b	
% fruit production	60.7 a	39.3 c	61.6 a	38.4 c	58.4 ab	41.6 bc	52.5 abc	47.5 abc
Fruit weight	1.35 a	1.35 a	1.35 a	1.38 a	0.87 b	0.82 b	0.77 b	0.79 b
Maturity index	2.56 c	2.15 d	3.06 ab	2.79 bc	3.18 a	2.69 bc	2.68 bc	2.54 cd
% black peel	87.3 ab	62.7 c	95.8 a	79.6 ab	87.7 ab	72.6 bc	65.9 bc	71.6 bc
Oil yield [y]	15.7	15.4	18.5	14.9	21.2	15.6	17.3	13.1
Moisture content	49.5 c	53.7 a	51.4 bc	52.5 ab	49.5 c	50.5 bc	49.9 c	51.7 abc
Fat content	27.4 a	24.8 d	27.1 a	25.5 cd	27.6 a	25.8 bcd	26.9 ab	26.4 abc

[z] Values followed by the same letters in each row are not significantly different among cultivars and planting densities for the yield parameters, and among cultivars, planting densities and canopy position for fruit quality parameters by Tukey's test at $\alpha < 0.05$ (n = 20). [y] Only a single oil extraction per treatment combination, no statistical analysis.

During maturation, most of the chlorophyll is degraded and replaced by anthocyanins, producing a change in color from green to black in the olive fruit, commonly visually evaluated by the maturity index (MI). MI ranged between 2.2 and 3.3 and differences between upper and lower layers of the canopy were larger in HD than in MD (Table 2). This is likely due to a less homogeneous distribution of the light intercepted by the canopy when the trees are close to one another [35].

Percentage of black peel was assessed by image analysis. The same method applied in this study was previously used to study peel color and maturity level of oranges, apples, peaches, and nectarines at harvest [28,36,37]. In olive, percentages of black peel did not follow the same trend as MI values. In 'Cerasuola', MI of fruits from upper canopy layers of HD trees was significantly lower than fruits from MD trees, while their percentage of black peel was not significantly different. Differences between these methods can be explained by the color changes in the peel of the fruit from both cultivars and the methodology applied. In 'Cerasuola' fruits, the darkening of the peel starts on one side of the fruit and then spreads to the whole fruit, creating higher variability in the percentage of black peel. While in 'Koroneiki', the black peel starts from the tip of the fruit and uniformly expands around the fruit. To determine MI, fruits were separated into groups based on the amount of dark skin color, and in fruits where the color was more uniform, like 'Koroneiki', the MI relates better to the percentage of black peel. On the other hand, in 'Cerasuola', the MI methodology allows for determination of the intermediate color break between green and dark, detecting a greater difference between samples than the percentage of black peel. These results show how subjective the MI can be and how limiting the use of a nonsubjective method is at higher maturity stages. Furthermore, to decide the harvest date, many producers use MI in the field, but then the industry uses optical sensors to carry out a preselection of the fruits before oil extraction. Therefore, the results also showed how important the calibration of these sensors is to decrease the possible discrepancies between MI done in the field and the quality of the oil extracted.

Olives from the upper layers of the canopy produced more oil on average, regardless of cultivar and planting density (Table 2). However, fruit fat content did not follow the same pattern as oil yield, suggesting that other factors (i.e., fruit characteristics) can influence the oil yield even when the same extraction conditions are used [14,38]. Planting density and canopy position did not affect fruit moisture content of 'Koroneiki'. In contrast, 'Cerasuola' fruit showed higher moisture content in the lower than the upper canopy layers, but only at HD.

'Cerasuola' fruit showed higher fat content at the upper than the lower canopy layers independent of planting density, whereas 'Koroneiki' fruit showed higher values in the upper layers only at HD. These observations agree with findings from previous studies showing that fruits from lower canopy positions accounted for approximately one-fourth of tree fruit production (26%) and oil yield (25.2%) [33], while fruits from the upper canopy layers showed the highest weight, ripening index, and oil content [12,26,32]. A possible explanation for a higher oil accumulation is that fruits from upper layers were more exposed to incident light which has been shown to increase the number of oil bodies inside the fruit mesocarp [39].

3.2. Oil Quality and Minor Compound Composition

Chlorophylls are mostly located in the skin of the olive fruit, where the highest photosynthetic activity is observed. Due to their liposolubility, chlorophylls migrate to the oil phase during the extraction process [40]. The final concentration in the oil is affected by the initial concentration in the fruit, but also by extraction variables [14,41]. Cultivar and planting density showed no effect on chlorophyll and carotenoid contents in the oil (Table 3). However, these pigments were significantly higher in oils from the lower than the upper layers.

Table 3. Oil chlorophyll, carotenoid content, ratio of unsaturated and saturated fatty acids (UFA/SFA), ratio of monounsaturated and polyunsaturated fatty acids (MUFA/PUFA), and main phenols in 'Cerasuola' and 'Koroneiki' olive trees planted at 1000 (HD) and 500 (MD) trees/ha. Chlorophyll, carotenoids, 3,4-DHPEA-EA, 3,4-DHPEA-EDA, *p*-HPEA-EDA, *p*-HPEA-EA, hydroxytyrosol, tyrosol, sum of total phenols, sum of C5 volatiles, and sum of C6 volatiles were determined in olives from the upper and lower layers of the canopy and expressed as mg kg^{-1}.

Cultivar	**'Cerasuola'**				**'Koroneiki'**			
Planting Density	**HD**		**MD**		**HD**		**MD**	
Canopy Layer	**Upper**	**Lower**	**Upper**	**Lower**	**Upper**	**Lower**	**Upper**	**Lower**
Chlorophyll	13.1 b *	17.3 a	9.52 b	20.0 a	10.2 b	14.1 a	13.0 b	18.0 a
Carotenoids	10.2 b	12.5 a	8.03 b	14.2 a	7.75 b	10.7 a	9.89 b	13.2 a
UFA/SFA	5.67 b	6.26 a	4.70 d	6.15 a	4.97 cd	5.20 c	5.21 c	5.68 b
MUFA/PUFA	5.40 d	5.45 d	5.18 d	5.63 d	8.05 ab	7.11 bc	8.35 a	6.73 c
3,4 DHPEA-EA	368 b	187 d	248 c	169 d	434 a	383 b	462 a	185 d
3,4 DHPEA-EDA	75.1 a	70.3 b	66.9 b	59.0 c	21.6 e	20.8 e	25.9 d	12.3 f
p-HPEA-EDA	23.8	23.8	23.5	23.6	23.8	23.7	23.7	23.7
p-HPEA-EA	116 d	137 bc	127 cd	112 d	165 a	117 d	148 b	87.4 e
Hydroxytyrosol	9.68 e	9.80 e	15.0 d	7.46 f	6.69 g	21.7 b	16.1 c	29.2 a
Tyrosol	30.5 b	25.2 bc	22.3 c	25.8 c	27.0 bc	39.9 a	38.2 a	30.5 b
Σ phenols	734 c	564 e	626 d	507 f	776 b	705 c	816 a	467 g
Σ C5	0.62 a	0.22 b	0.59 a	0.74 a	0.34 b	0.25 b	0.32 b	0.10 b
Σ C6	21.2 b	13.5 bc	43.1 a	12.8 bc	10.4 bc	7.86bc	10.7 bc	3.13 c

* Values followed by the same letters in each row are not significantly different by Tukey's test at $\alpha < 0.05$ ($n = 3$).

Identified fatty acids were myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1n9), margaric (C17:0), stearic (C18:0), oleic (C18:1n9), linoleic (C18:2n6), linolenic (C18:3n3), arachidic (C20:0), gadoleic (C20:1n9), behenic (C22:0), and lignoceric (C24:0) acids. In order to facilitate the interpretation of fatty acid compositional changes in response to the studied factors, fatty acids were grouped according to their saturation level into saturated fatty acids (SFA; myristic, palmitic, margaric, stearic, arachidic, behenic, and lignoceric acids)

and unsaturated fatty acids (UFA; palmitoleic, oleic, linoleic, linolenic, and gadoleic); and further into monounsaturated fatty acids (MUFA; palmitoleic, oleic, and gadoleic) and polyunsaturated fatty acids (PUFA; linoleic and linolenic). MUFAs are the predominant fatty acids in olive oil, with oleic acid being the most abundant, ranging from 55% to 83% of total fatty acids [42]. The monounsaturated profile of fatty acids is one of the factors that may explain the healthy benefits of olive oil in the Mediterranean Diet [43,44]. In all of the measured samples, oleic acid was always the predominant fatty acid (70.03–72.47%). Cultivar showed a main effect on fatty acid ratios, with 'Cerasuola' showing a significantly higher UFA/SFA but a lower MUFA/PUFA than 'Koroneiki'. Canopy position also affected fatty acid ratios, with oils from lower layers showing significantly higher UFA/SFA. In 'Koroneiki', higher MUFA/PUFA in the oils from upper layers than in lower layers was due to a decrease of linoleic acid rather than changes of oleic acid. In contrast, an increase of linoleic acid in the oils from upper canopy layers was previously reported in 'Arbequina' and 'Frantoio' and justified by more mature olives [12,45]. There is no easy explanation for this discrepancy between our results and the previous literature, but a combination of greater light interception and lower crop load of the upper canopy layers in 'Koroneiki' may be in part related to the observed MUFA/PUFA trends.

Oleuropein and ligstroside are the most relevant phenolic compounds identified in olive fruit [46]. These substances are hydrolyzed after crushing by the enzyme β-glucosidase, leading to the formation of oleacein and oleuropein aglycon (respectively, 3,4-DHPEA-EDA and 3,4-DHPEA-EA) and oleocanthal and ligstroside aglycon (respectively *p*-HPEA-EDA and *p*-HPEA-EA), which exhibit a higher lipophilicity and constitute the most abundant phenolic compounds in virgin olive oil [47]. In addition to β-glucosidase, polyphenol oxidase and peroxidase degrade phenolic compounds during the extraction, also shaping the phenolic profile of the oil [48]. Cultivar significantly affected the phenolic composition of the oils (Table 3). 'Cerasuola' presented significantly lower phenol contents compared to 'Koroneiki', except for 3,4-DHPEA-EDA, which was higher in the former cultivar. There was no difference in *p*-HPEA-EDA level between cultivars. The phenol content was significantly higher in oils from HD than MD trees, except for hydroxytyrosol that showed higher values in oils from MD trees. Tyrosol was not affected by planting density. Canopy position significantly affected the phenol content in the oil. Oils from fruits in upper layers showed the highest total phenol content due to higher 3,4-DHPEA-EA, 3,4-DHPEA-EDA, and *p*-HPEA-EA, but lower hydroxytyrosol. Tyrosol and *p*-HPEA-EDA were not affected by canopy position. These results are in accordance with previous studies on 'Arbequina' and 'Frantoio', where canopy position was a determining factor for phenol concentration, increasing with height [12,45].

Volatile compounds are responsible of the fruity and green aroma of fresh olive oil [49]. These compounds are synthesized during processing from free polyunsaturated fatty acids, through an enzymatic pathway involving two main enzymes, lipoxygenase and hydroperoxide lyase. Lipoxygenase catalyzes the oxygenation of polyunsaturated fatty acids (linoleic and linolenic) to produce their corresponding 13-hydroperoxides. Hydroperoxide lyase catalyzes the cleavage of linoleyl and linolenyl 13-hydroperoxides, yielding C5 and C6 aldehydes, the main compounds identified in olive oil [50]. C6 volatiles presented the highest concentration in all of the samples, with *(E)*-2-hexenal being the major volatile compound. *(E)*-2-Hexenal concentration ranged between 13.73 and 0.89 mg kg^{-1} for 'Cerasuola' and between 3.58 and 0.47 mg kg^{-1} for 'Koroneiki', in oils from HD and MD trees, respectively. In this study, C5 and C6 volatile compounds were affected by cultivar and canopy position. Both families of volatile compounds were highest in oils from 'Cerasuola' and upper layers of the canopy (Table 3). Fruit from upper layers of the canopy of 'Arbequina' were previously reported to have higher volatile compounds concentration than fruit from lower layers after November and a MI higher than 1 [12].

3.3. Oil Sensory Attributes

From a sensory point of view, all the samples can be classified as extra virgin olive oil with medium intensity of the positive attributes. The artichoke (*Cynara cardunculus*), almond (*Prunus dulcis*), and tomato attributes, very common in Sicilian olive oils, were also found in the extracted oils. 'Cerasuola' oils were characterized by higher artichoke, almond, tomato, and oregano (*Origanum vulgare*) attributes than 'Koroneiki' oils (Table 4). On the other hand, 'Koroneiki' oils were distinguished by low fruity and banana (*Musa* spp.) attributes. Bitter and pungent sensations were previously positively related to the amount of secoiridoid compounds [51]. In particular, *p*-HPEA-EDA is known to be responsible for pungency in the oil [51]. In this study, values of *p*-HPEA-EDA were not affected by cultivar, planting density, or canopy position although a slight increase of pungency was found in oils from upper canopy layers and HD. C6 volatiles were previously associated with bitterness and pungency in olive oil [52]. In our study, from the upper layer of the canopy, the highest C6 volatile concentration was followed by an increase in pungency in the oils. Moreover, most C5 volatile compounds in addition to the C6 volatiles contribute to almond (mainly *(E)*-2-hexenal), tomato (mainly *(E)*-2-hexen-1-ol), and banana (mainly *(Z)*-3-hexenyl acetate) odor notes [53]. The contribution of volatile compounds to the overall aroma of virgin olive oil depends not only on their concentration but also on their sensory threshold values [54,55]. Consequently, a high concentration of volatile compounds is not necessarily related to a major contribution to the oil aroma [54,56].

Table 4. Quantitative descriptive profile for sensory evaluation of 'Cerasuola' and 'Koroneiki' oils obtained from two planting densities and two fruit canopy positions. Numbers represent the median of the attributes (n = 8) according to the international olive oil council (COI/T.20/DOC.15/Rev.10) standards.

Cultivar	**'Cerasuola'**				**'Koroneiki'**			
Planting Density	**HD**		**MD**		**HD**		**MD**	
Canopy Layer	**Upper**	**Lower**	**Upper**	**Lower**	**Upper**	**Lower**	**Upper**	**Lower**
Fruity	5	6	5	5.5	3	3	3	2.8
Bitter	5	6	5.5	5	5	5	5	4
Pungent	6	5.5	6	5	5	4	4	3
Artichoke	3	3.5	3	3.5	2	nd [z]	2	nd
Almond	2	2	2.5	nd	1	nd	1	nd
Grass	2.5	3	nd	3	nd	nd	nd	nd
Green tomato	2.5	3	nd	3.5	nd	1	nd	nd
Banana	nd	nd	nd	nd	2	nd	1	1
Oregano	nd	1	nd	2	nd	nd	nd	nd
Chicory	nd	1	1	1	nd	nd	nd	nd

[z] nd = not detected.

4. Conclusions

Given the relevance of harvest costs to the overall cost of olive oil production, tree density in newly planted olive orchards has been increasing steadily as part of the intensification of olive tree cultivation. Data obtained in this study showed that higher planting density increased yield per hectare of the Sicilian cultivar 'Cerasuola', which was proven to adapt to higher density orchards, without losing the quality and peculiar sensory attributes. Upper layers of the canopy were characterized by higher crop loads and more mature fruits with higher fat content. All the major variables had an influence on fatty acid, phenol, and volatile composition. Canopy position was the primary factor that influenced most of the measured parameters. The differences in production, fruit maturity, and fat content between upper and lower canopy layers increased at higher densities. On the other hand, treatment effects on oil quality did not follow the same trend in both cultivars, showing that genetic factors interacted with environmental conditions to influence oil quality. Taken together, the impact of the interactions between cultivar, planting density, and canopy position on oil quality and sensory properties established in this study provide new insights

into the relationships between yield and tree structure in modern high and medium density hedgerow olive planting systems.

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