JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Soil Water Availability in Rainfed Cultivation Affects More than Cultivar Some Nutraceutical Components and the Sensory Profile of Virgin Olive Oil

Pierluigi Bucelli,*[,†] Edoardo A. C. Costantini,[†] Roberto Barbetti,[†] and Elena Franchini[§]

[†]Agricultural Research Council, Research Centre for Agrobiology and Pedology, Piazza M. D'Azeglio 30, 50121 Firenze, Italy

[§]National Research Council, Trees and Timber Institute, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy

ABSTRACT: This research considered the varieties 'Frantoio' and 'Moraiolo' growing in rainfed olive trees (*Olea europaea*) and took place in Tuscany, central Italy. Soil moisture was monitored during the very meteorologically contrasting years 2002 and 2003 in two nearby olive groves. The plots had the same morphological and climatic conditions, but different soil types. Monocultivar oil samples were analyzed to determine fatty acids, minor polar compounds, and tocopherols content and were submitted to organoleptic analysis by a panel of trained tasters. The results highlighted that soil water regimen affects some nutraceutical components and the sensory evaluation of olive oil. Cultivar also affected yield components, polyphenols, and tocopherols content, but less than soil water availability. The plants on the soil inducing a relatively more intense and longer water deficit during summer (a Skeleti Calcaric Regosol) had an early ripening and gave the best results in terms of phenolic compounds and, consequently, antioxidant properties of the olive oil. The sensorial properties of the oil obtained from both cultivars on the Regosol were superior in both years of the trial.

KEYWORDS: hydropedology, polyphenols, organoleptic characteristics, antioxidant properties, Frantoio, Moraiolo

INTRODUCTION

Virgin olive oil (VOO), produced by physical pressure and without any chemical processing, is particularly rich in natural antioxidants, preventing the insurgence of cardiovascular and cancerogenous pathologies. The nutritional quality and the healthy benefits of virgin olive oil are above all due to the specific fatty acid composition[1,2] and the presence of antioxidant substances in the nonsaponifiable fraction, such as tocopherols and phenolic compounds.[3−7] Tocopherols are present in olive oil with the forms α, β, γ, and δ, but 90% of them are found in the α form, the most biologically active, namely, vitamin E. The most important phenolic components of virgin oil belong to the classes of the lignans, phenolic acids, flavones,[8,9] and secoiridoids, which are found only within the family of the Oleaceae.[10] The phenolic composition of olive oil is conditioned by multiple factors, among which there are the level of maturation of the drupe,[9] the technology employed in the process of oil production,[11] and the environmental conditions. Irrigation, in particular, seems to affect polyphenols and *o*-diphenol contents and, consequently, the bitterness index and oxidative stability, which increase when the amount of irrigation water decreases.[12,13] Some studies have been carried out about the effect of irrigation on the concentration of phenolic compounds in olive oil. In a semiarid area of Tunisia, the different irrigation regimens applied to the cv. Arbequina affected both the total amount of phenols and their HPLC profiles.[14] In northern Tunisia, Dabbou et al.[15] studied the impact of different irrigation water amounts on the quality and quantity of virgin olive oils from the cultivar 'Koroneiki'; however, they did not observe consistent effects of irrigation on phenol contents. The impact of short-term water stress on plant physiological processes, crop yield, and oil quality was investigated in Marlborough, New Zealand.[16] Drier treatments showed a reduced yield, as berry and pit weight were lowered by almost 50% at harvest, had a minor oil percentage, and were poorer in phenolics. In Spain (Castilla-La Mancha) Gómez-Rico et al.[17] studied the influence of different irrigation strategies on the composition and quality of Cornicabra virgin olive oil. The total phenol content decreased significantly as the amount of supplied water increased. More recently, also the physiological stress caused by the use of different saline water irrigation levels on virgin olive oil was the object of study. In Crete (cultivars 'Koroneiki' and 'Mastoidis') and in Tunisia (cv. 'Chemlali') data showed an increase in total phenol concentration in VOO under saline water irrigation.[18,19]

Variety also seems to play an important role in olive oil quality. Aguilera et al.[20] worked on the characterization of VOOs from the Italian cultivars 'Frantoio' and 'Leccino', grown in two different lands of Andalusia. They found significant differences between the oils from both cultivars when grown in the different environments, in terms of tocopherols and oleic and linoleic acid contents, stability, and sensorial characteristics. As for the phenolic compounds, in particular, the environment affected each cultivar in a different way.

Although there is a wealth of studies on the influence of irrigation and cultivar on the phenolic fraction of VOO, nothing is known about the influence of soil water availability on the nutraceutical components and organoleptic properties of the virgin olive oil produced in rainfed conditions. Actually, olive tree water nutrition is very different whether regulated or not by irrigation,[21−23] and we hypothesized that this could also affect significantly oil components.

The aim of the present work was to verify the influence of soil water availability on some nutraceutical components and on the sensory profile of the VOO obtained from the varieties 'Frantoio'

Received: March 31, 2011
Revised: June 20, 2011
Accepted: June 30, 2011
Published: June 30, 2011

Table 1. Classification and Main Characteristics of the Two Benchmark Soil Profiles[a]

soil profile	P5			P6			
soil classification (World Reference Base)	Skeleti Calcaric Regosol			Haplic Calcisol			
soil classification (Soil Survey Staff, 1998)	Typic Ustorthents clayey-skeletal, mixed, calcareous, mesic, superactive			Udic Calciustepts coarse-loamy, mixed, mesic, superactive			
rooting depth until the limiting layer (m)	0.80			0.80			
available water until the root limiting layer (mm)	74			111			
texture class	clay			loam			
slope (%)	25			20			
aspect	north/northeast			north/northeast			
class of internal drainage	well drained			moderately well drained			
class of runoff	medium			medium			
soil horizon and limits (cm)	Ap1 0−15	Ap2 15−35/80	Ckr 35/80−120	Ap1 0−10	Ap2 10−40	Bk 40−80	BCk 80−140
clay (dag kg^{-1})	41	40	40	19	18	12	18
sand (dag kg^{-1})	21	21	21	46	47	51	33
rock fragments (% v v^{-1})	10	15	50	2	2	0	0
consistency	RE	RE	RE	FR	FR	RE	RE
structure	SB	SB	MA	G	SB	SB	MA
FC (g g^{-1})	0.263	0.220	0.220	0.202	0.172	0.176	0.209
WP (g g^{-1})	0.157	0.161	0.161	0.073	0.066	0.083	0.079
AWC (mm m^{-1})	191	148	148	167	137	120	168
bulk density (g cm^{-3})	1.27	1.44	nd	1.25	1.33	1.45	nd
pH (H_2O)	8.0	8.1	8.1	8.0	8.1	8.3	8.5
total $CaCO_3$ (dag kg^{-1})	10.5	10.2	nd	12.0	25.0	28.0	27.7
active $CaCO_3$ (dag kg^{-1})	2.8	2.3	nd	3.7	4.2	6.2	5.3
organic matter (dag kg^{-1})	1.82	1.23	nd	1.96	1.29	0.23	0.17
el cond (dS m^{-1} (1:2.5))	0.168	0.147	0.120	0.207	0.184	0.146	0.121
CEC (mequiv 100 g^{-1})	nd	23.1	nd	nd	11.3	nd	nd
Ca + Mg (mequiv 100 g^{-1})	nd	22.62	nd	nd	11.05	nd	nd
Na (mequiv 100 g^{-1})	nd	0.14	nd	nd	0.14	nd	nd
K (mequiv 100 g^{-1})	nd	0.34	nd	nd	0.14	nd	nd
Fe (mg L^{-1})	nd	7.4	nd	nd	7.6	nd	nd
Mn (mg L^{-1})	nd	2.6	nd	nd	2.8	nd	nd
Cu (mg L^{-1})	nd	5.4	nd	nd	6.4	nd	nd
Zn (mg L^{-1})	nd	1.0	nd	nd	0.5	nd	nd
C/N	nd	7.0	nd	nd	8.2	nd	nd

[a] Consistency: FR, friable; RE, resistant. Structure: SB, subangular blocky; G, granular; MA, massive. FC, water content at field capacity. WP, water content at wilting point. AWC, available water capacity. El. cond, electrical conductivity. CEC, cationic exchange capacity. nd, not determined.

and 'Moraiolo' cultivated in rainfed conditions. The results of the trial might support the differentiation of lands within a territory in terms of potential soil suitability for high-quality olive oil production.

MATERIALS AND METHODS

The trial was carried out in central Italy at Cinciano, in the territory of the municipality of Poggibonsi (province of Siena, Chianti Classico DOP Olive oil zone) during the years 2002 and 2003. The benchmark experimental site was chosen after a soil survey of the whole province of Siena and is representative of the most widespread and suited soils for olive tree cultivation in the area.[24] Two rainfed olive groves were selected, which were homogeneous in age (about 20 years), plant density (6 × 6 m), permanent grass cover, and agricultural husbandry. The two olive groves were adjacent and had the same morphological and climatic conditions, but they differed in soil types (identified with the acronyms P5 and P6) (Table 1). The cultivars 'Frantoio' and 'Moraiolo' were chosen for the trial, because they are the most widespread in Tuscany (48 and 22%, respectively, of all olive tree varieties)[25] as well as in the studied area. The two olive groves had both varieties in the same field, as it is a rule in the studied territory. In both soils, 10 homogeneous plants were selected.

Soil Analysis. After the soil mapping of the farm, two benchmark profiles were described and analyzed to characterize the soil types present in the studied olive groves. The two soil profiles (P5 and P6) were located close to the plant chosen for the trial. The profiles were described according to the Italian methods, which permit soil classification according to the main international standards.[26] Laboratory analyses were carried out on each soil horizon up to the rooting depth. Available water capacity (AWC) was estimated as the difference between soil water content at field capacity (FC) and wilting point (WP), as determined in the laboratory using the Richards pressure plate extractor.[27] FC, WP, and AWC (mm m^{-1}) were measured on the fine earth, whereas available water until the root limiting layer was reduced by taking into account the skeleton content. Layers without measured bulk density were beyond the rooting depth. Bulk density was obtained with the core method, averaging two or three replicated samples. Routine analyses were carried out following the Italian official methods.[28] In particular, soil texture was tested in the laboratory by the sieve and pipet method. $CaCO_3$ content was measured gas volumetrically, by the addition of HCl in a Dietrich−Frühling calcimeter. Active $CaCO_3$ was analyzed with a solution of ammonium acetate; this is the more active fraction of $CaCO_3$, which easily dissolves and precipitates. Soil organic carbon content was determined by using the Walkley−Black procedure; pH and electrical conductivity were measured in a 1:2.5 (w w^{-1}) water suspension; cation exchange capacity (CEC) was measured by use of 1 M sodium acetate solution at pH 7.0; exchangeable bases were extracted with 1 M NH_4^+ acetate solution at pH 7.0 and measured by flame photometry (Na, K, and Ca) and atomic absorption spectrometry (Mg); Fe, Mn, Zn, and Cu were measured in the solution with diethylenetriaminepentaacetic acid (DTPA) at pH 7.3, according to the method of Linsday and Norvell.[29] The soil profiles were classified in agreement with the Soil Survey Staff[30] as Typic Ustorthents clayey-skeletal, mixed, calcareous, mesic, superactive (P5) and

Udic Calciustepts coarse-loamy, mixed, mesic, superactive (P6) and according to the World Reference Base[31] as Skeleti Calcaric Regosol and Haplic Calcisol, respectively (Table 1).

The water regimen of the two soils was monitored for 2 years, close to the studied plants. The water content was measured using the gravimetric method. Three samples were taken with a hand auger every 2 weeks at depths of 0.1−0.3 and 0.4−0.7 m. In fact, the experimental plots were unrestricted, and the use of permanent equipment, such as neutron probes or transducer tensiometers, was not possible.

Transpirable soil water (TSW) from the surface to 0.70 m was calculated. At the two studied depths, the TSW was the difference between the measured gravimetric soil water content and the absolute minimum value measured at the depth during the two years of testing. This system was introduced by some authors to take into account the fact that some species are able to take up and transpire water at tensions beyond the standard wilting point.[32] The TSW of each plot at the date of measurement was computed as the weighted average between the surface and 0.7 m. The measurements were also averaged to obtain a mean monthly value. Therefore, available soil water was estimated through two ways: (i) as the difference between the measured soil moisture and AWC, that is, the value of water content at standard wilting point obtained in the laboratory (potential water deficit) and (ii) as the difference between the measured moisture and the absolute minimum soil water content at the sampling depth (TSW).

Redox potential assessment at 0.15 m (hand-held Barnant pH/mV/ORP meter, two measurements) was replicated every 2 weeks during the rainy season. Electrode calibration followed the instruction of Barnant Co. (Barrington, IL) using solutions buffered to pH 7 and 4 with Quinhydrone. Redox potentials were normalized at pH 7 according to Patrick and collaborators.[33]

Olive Ripening Evaluation. The olive ripening was evaluated by observing the pigment of the drupe, monitoring the fresh weight (g) and oil content (% of fresh matter) of drupes. For this purpose, in each year and soil, two samples of 100 olives each, taken from plants of 'Frantoio' and 'Moraiolo' close to the soil monitoring sites, were randomly harvested, and ripeness olive index, fresh weight, and olive oil content were determined. Ripeness index was evaluated according to the method of Uceda and Frias,[34] splitting the olive samples into eight classes of color (from deep green to black).

Olive Oil Extraction and Chemical and Organoleptic Analyses. Olive fat content was evaluated by the Soxhlet extractor. Two samples of approximately 10 kg of olives each, taken from the plants of 'Frantoio' and 'Moraiolo' close to the monitoring sites, were harvested each year toward the second half of October. The olives were immediately brought to a small-scale two-phase oil mill, reproducing the industrial process.[11,35] The olives were washed and rapidly crushed to a fine paste by a hammer crusher to proceed to the extraction of the oil. During the extraction, the olive paste was kneaded, without addition of water, for a standardized time of 35 min at 24−25 °C. Then the paste was pumped into a horizontal centrifuge, where it was separated into oil and wet pomace. The oil was immediately filtered and bottled.

All oil samples were analyzed for free acidity, peroxide index, UV extinction coefficients, and fatty acids according to European Official Methods of Analysis.[36] In particular, the acid composition was determined through gas chromatographic analysis at high resolution (HRGC), and the values were expressed as relative percentage of the whole chromatogram. Phenolic compounds were determined by means of liquid chromatography (HPLC 1050 Hewlett-Packard equipped with DAD), after extraction with methanol/water, and expressed as mg kg^{-1} of oil, according to the method of Rovellini and Cortesi.[37] About 2 g of oil was weighed in a screw cone tube, and 1 mL of hexane for HPLC and 2 mL of internal standard were added. The internal standard was syringic acid (0.010 mg mL^{-1}) in methanol/water 60:40, v/v. The solution was vigorously shaken for 2 min and centrifuged at 4000 rpm for 10 min. The extraction was repeated twice. The extracts, dried by a Rotavapor, were dissolved with methanol before analysis. For the quantitative analysis, syringic acid was used as internal standard and phenols were expressed as tyrosol. The identification of some phenolic components was carried out by comparing the peak retention times with those obtained by injection of pure standards (hydroxytyrosol and tyrosol, purchased at Extrasynthese, Genay Cedex, France) and analyzing the obtained spectra and by LC-MS analysis. The identification of the other phenolics was made on the basis of previous studies in the literature.[38−40] The total tocopherols were also determined by means of liquid chromatography (HPLC-DAD 1050 Hewlett-Packard), following the IUPAC standard method.[41] The quantitative determination was performed by injection of external standards of α-, γ-, and δ-tocopherols. Oil samples (100 mg) were dissolved in 10 mL of hexane and directly analyzed. Results were expressed as mg kg^{-1} of total tocopherols.

The oils were finally submitted to a quantitative descriptive analysis (QDA) by a panel of eight trained tasters to perform virgin olive oil sensory analysis, in accordance with the current EU regulation.[36] Oil samples (15 g) were presented to the tasters in amber-colored glasses at room temperature. For a better description of the VOOs, the following descriptors were proposed to the assessors: fruity, pine nut, almond, apple, ripe olive, spicy, green grass, green olive, artichoke, and green wood. The intensity of each property was graded using a line scale for each descriptor and thus converted to numerical score by measuring the position of the placed mark along a 10 cm line.

Statistical Analysis. The data were submitted to ANOVA and principal component analysis (PCA) by means of the software Statistica (StatSoft Inc., Tulsa, OK). Three replicates were made for each parameter analyzed in the oil sample. The studied characteristics showed very different ranges of values, sometimes having not normal distributions; therefore, they were all normalized to populations with 0.0 mean and 1.0 standard deviation. Tukey's test was used to determine significant differences in the means of ripeness index, weight and oil content of drupes, oil fatty acid, minor polar compounds, and total tocopherols in each year, cultivar, and soil. Factorial ANOVA was performed to check the hypothesis that the same variables were affected by year, cultivar, soil, and their interactions. The coefficient of variation (CV%) was used to evaluate the variability of soil water content. A PCA model was built to verify the relationship between TSW, potential water deficit, phenolic components, and sensorial data of VOO. The analytical data were put in a matrix with the rows corresponding to the samples (n objects) and the columns corresponding to the analytical parameters (k variables). The results of PCA modeling are presented in graphical form.

RESULTS

Soil Characterization. The two soils lay on slopes with similar steepness and had similar rooting depth, as a consequence of the deep plowing carried out before tree planting. Actually, the two soils were very similar, apart from their physical and hydrological properties. In fact, P5 was more clayey than P6, but it also had many more rock fragments; therefore, the AWC of P5 was slightly higher than that of P6, when referred to fine earth, but much lower after taking into account the skeleton (Table 1). Skeleton gives P5 also a better internal drainage. On the other hand, the difference between chemical characteristics was negligible, that is, the pH was moderately alkaline in both soils, total lime was relatively less in P5, but always higher than 10%, whereas active lime was similar and not excessive in either soil. The CEC, although more elevated in P5, exceeded in both cases the threshold of 10 mequiv 100 g^{-1}, believed to be the minimal reference for olive tree cultivation.[42] Exchangeable elements were dominated by calcium, whereas potassium was rather low (<2% of the CEC), especially in P6. Total nitrogen was also rather low, as were micronutrients. The carbon to nitrogen ratio indicated a rapid decomposition of the organic matter in both

soils. The low electrical conductivity showed the absence of salinity in both soils.

Meteorological Conditions and Soil Water Regimen during the Trial. Long-term (1961−1990) meteorological conditions of the study area are typical of the Mediterranean climate. Mean annual precipitation in the study area is 753.3 mm; November is the rainiest month, July the driest. Long-term mean annual air temperature is 13.9 °C, with July and August the hottest months and January and February the coldest. Like in all of the Mediterranean basin, meteorological conditions of every year are very variable. During the trial, the meteorological conditions differed from the long-term averages and, especially, between the two studied years. The year 2002 was relatively humid and cool, whereas 2003 had very hot and dry spring and summer (Figure 1). Although it is well-known that the olive tree is adapted to the long dry summer period and even able to survive with annual rains of only 200 mm,[43] the meteorology of 2003 was rather exceptional and could represent rather extreme conditions for the olive tree cultivation in the area. In fact, if water supply is very limited, roots absorb less nourishment, causing a decrease in plant vegetation growth.[44] The different meteorological conditions of the two studied years span about 80% of the long-term climatic variability of the area.

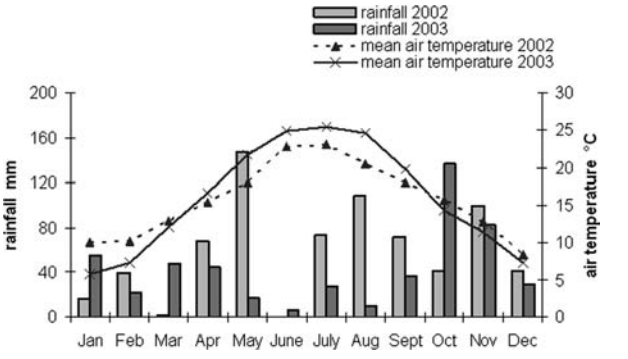

Figure 1. Precipitation and air temperature during the study period.

The soil water regimen of P5 was characterized by a redox potential of >300 mV and a water content higher than FC throughout both years (Figure 2a). Good drainage of the soil prevented waterlogging during the winter season. On the other hand, the soil water content was less than standard WP in summer. The mean monthly difference between measured moisture and soil water content at standard WP (potential water deficit) was rather high: 5.0 mm from June to September 2002 and 10.4 mm from May to October 2003 in the first 0.3 m; 8.6 mm from June to September 2002 and 18.2 mm from May to October 2003 at 0.4−0.7 m.

The CV% of the soil moisture values of the replicated samples varied from 0.94 to 20.39 (Figure 2b). As a whole, in-depth replications were more variable, without any marked seasonal influence.

P6 showed an average moisture that was higher than water content at FC during autumn and winter, reaching mean redox values which <300 mV during January. The moderately low redox potential in the surface horizon may indicate the occurrence of nitrogen losses from soil caused by denitrification, but excluded stronger reductive processes. Average potential water deficit was limited to July in 2002 and from July to October in 2003. There was no deficit in the first horizon, only in depth; in

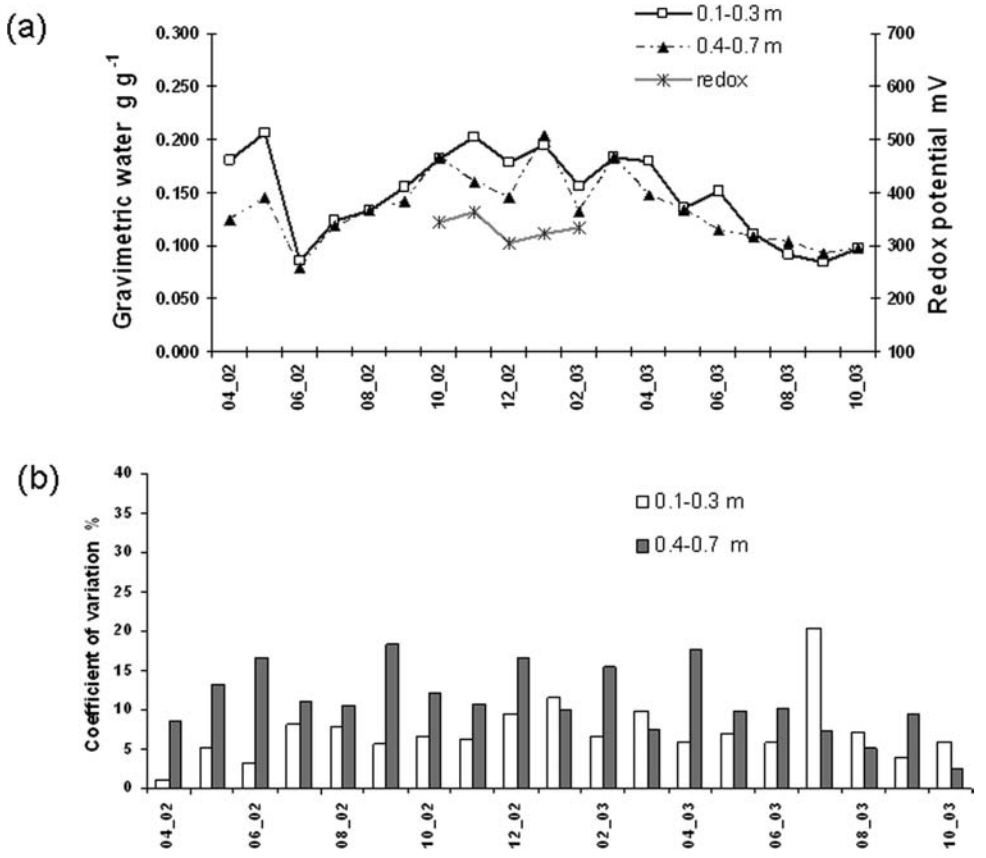

Figure 2. Soil P5 (a) gravimetric water content (g g^{-1}) at 0.1−0.3 and 0.4−0.7 m soil depths and surface redox potential (mV); (b) CV% of soil water content at depths of 0.1−0.3 and 0.4−0.7 m.

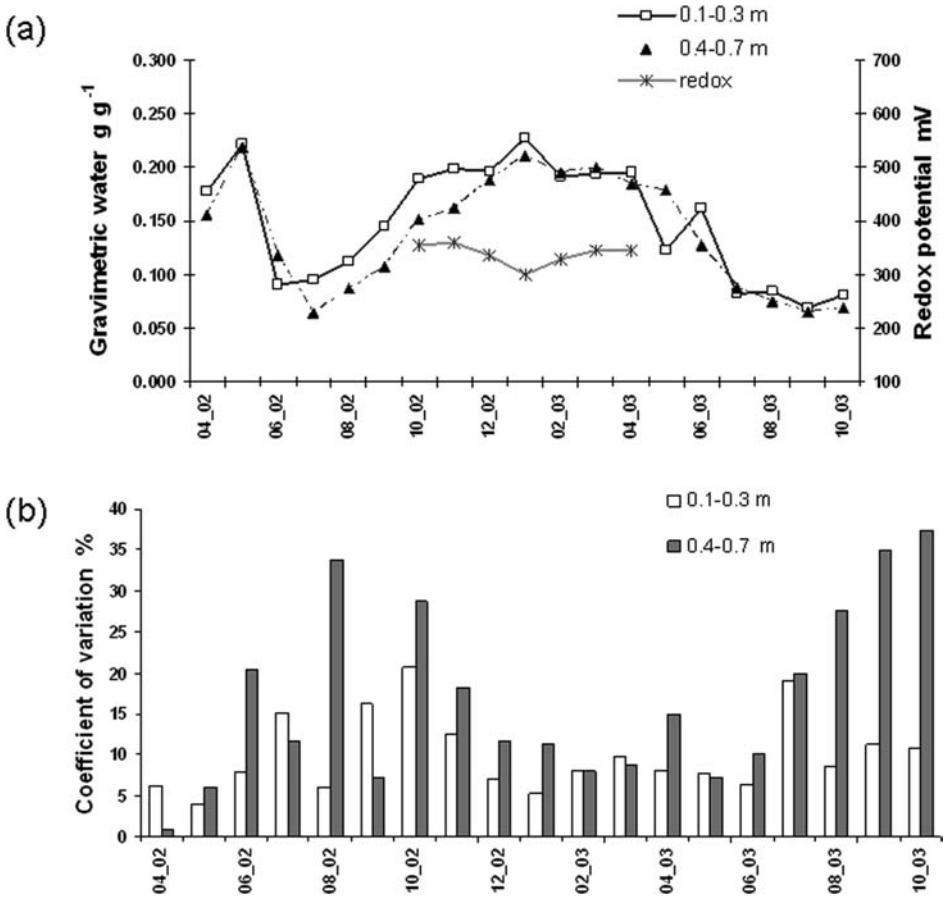

Figure 3. Soil P6: (a) gravimetric water content (g g^{-1}) at 0.1−0.3 and 0.4−0.7 m soil depths and surface redox potential (mV); (b) CV% of soil water content at depths of 0.1−0.3 and 0.4−0.7 m.

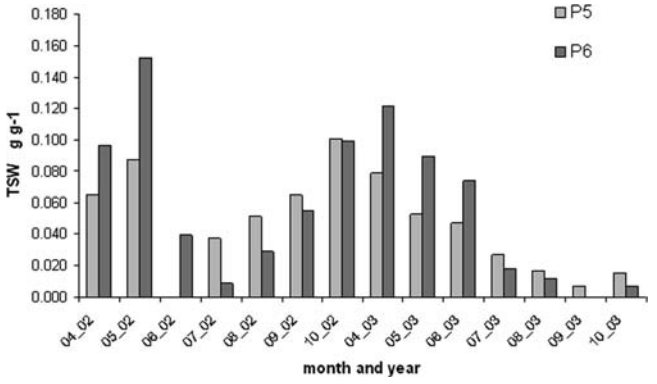

Figure 4. Mean monthly TSW (g g^{-1}) from the surface to 0.70 m from April to October in the two studied years.

particular, the mean monthly potential water deficit at 0.4−0.7 m was only 1.1 g g^{-1} in 2002 but 2.3 g g^{-1} in 2003 (Figure 3a). The CV% of soil water content was greater in soil P6 than in P5. The replicated measurements of soil water content varied seasonally, resulting in higher CV% during the summer and the beginning of autumn (Figure 3b).

Hence, we can assume that the plants in P6 did not suffer from water stress. More in detail, taking into account the TSW from April to October in both years, the mean monthly value from the soil surface to 0.70 m was lower in P5 than in P6 (Figure 4). In particular, in soil P6, it was 4.82 g g^{-1} in 2002 and 3.21 g g^{-1} in 2003, whereas in soil P5, it was 4.07 g g^{-1} in 2002 and 2.45 g g^{-1} in 2003.

Table 2. Ripeness Index, Mean Weight, and Oil Content of 100 Drupes in 2002 and 2003, Frantoio and Moraiolo Cultivars, P5 and P6 Soils[a]

		ripeness index	mean weight (g)	oil content (%)
year	2002	3.3 ± 0.17 ns	193.8 ± 14.1 a	15.4 ± 0.71 b
	2003	3.6 ± 0.21 ns	149.5 ± 13.5 b	18.8 ± 1.11 a
cultivar	Frantoio	3.2 ± 0.22 b	212.6 ± 11.4 a	16.0 ± 0.93 ns
	Moraiolo	3.8 ± 0.25 a	130.6 ± 7.4 b	18.0 ± 0.98 ns
soil	P5	3.9 ± 0.21 a	162.4 ± 15.3 ns	17.9 ± 1.08 ns
	P6	3.1 ± 0.22 b	180.8 ± 15.5 ns	16.1 ± 1.07 ns

[a] In each column and for each factor (year, cultivar, soil), values with different letters are significantly different at $P < 0.05$ (Tukey's test; means of three replicates ± SE); ns, not significant.

Yield Components. The yield components were influenced by cultivar, soil, and year (Table 2). 'Moraiolo' was characterized by significantly smaller olives than 'Frantoio' (on average 130.6 vs 212.6 g, respectively), but was always earlier (ripeness index, on average, 3.8 vs 3.2, respectively; $P < 0.05$). The oil content was on average higher in 'Moraiolo' than in Frantoio' olives, but without statistical

dx.doi.org/10.1021/jf201278r |J. Agric. Food Chem. 2011, 59, 8304–8313

Table 3. Mean Percentage Values (± SE) of Fatty Acids in Monocultivar Frantoio and Moraiolo Oils, 2002 and 2003, P5 and P6 Soils[a]

	cultivar		year		soil	
	Frantoio	Moraiolo	2002	2003	P5	P6
palmitic acid (C16:0)	12.39 ± 0.44 ns	12.74 ± 0.24 ns	12.11 ± 0.22 ns	13.02 ± 0.39 ns	12.49 ± 0.39 ns	12.64 ± 0.33 ns
palmitoleic acid (C16:1)	0.88 ± 0.06 ns	0.83 ± 0.05 ns	0.80 ± 0.02 ns	0.92 ± 0.07 ns	0.81 ± 0.06 ns	0.90 ± 0.04 ns
heptadecanoic acid (C17:0)	0.036 ± 0.002 ns	0.036 ± 0.003 ns	0.041 ± 0.001 a	0.031 ± 0.001 b	0.036 ± 0.003 ns	0.036 ± 0.002 ns
heptadecenoic acid (C17:1)	0.081 ± 0.007 ns	0.078 ± 0.007 ns	0.093 ± 0.005 a	0.066 ± 0.005 b	0.073 ± 0.006 ns	0.086 ± 0.007 ns
stearic acid (C18:0)	1.94 ± 0.10 a	1.79 ± 0.08 b	1.71 ± 0.10 b	2.02 ± 0.03 a	1.75 ± 0.04 b	1.98 ± 0.12 a
oleic acid (C18:1)	75.53 ± 1.03 ns	75.89 ± 0.79 ns	77.71 ± 0.31 a	73.71 ± 0.68 b	75.92 ± 1.04 ns	75.50 ± 0.78 ns
linoleic acid (C18:2)	7.80 ± 0.41 ns	7.15 ± 0.56 ns	6.41 ± 0.27 b	8.54 ± 0.33 a	7.20 ± 0.56 ns	7.75 ± 0.41 ns
linolenic acid (C18:3)	0.60 ± 0.02 ns	0.63 ± 0.09 ns	0.60 ± 0.02 ns	0.63 ± 0.09 ns	0.66 ± 0.03 ns	0.57 ± 0.08 ns
arachidic acid (C20:0)	0.33 ± 0.01 a	0.27 ± 0.03 b	0.30 ± 0.01 ns	0.30 ± 0.04 ns	0.33 ± 0.02 ns	0.28 ± 0.03 ns
eicosenoic acid (C20:1)	0.29 ± 0.01 ns	0.27 ± 0.01 ns	0.29 ± 0.01 ns	0.27 ± 0.01 ns	0.28 ± 0.01 ns	0.29 ± 0.01 ns

[a] In each row and for each factor (cultivar, year, soil), values with different letters are significantly different at $P < 0.05$ (Tukey's test; means of three replicates ± SE); ns, not significant. The values for cultivar and soil are means of two years (2002 and 2003).

Table 4. Mean Phenolic and Total Tocopherols Composition (Millgrams per Kilogram) of Monocultivar Frantoio and Moraiolo Oils, 2002 and 2003, P5 and P6 Soils, and Interaction Cultivar versus Year[a]

	cultivar		year		soil		interaction, cultivar vs year
	Frantoio	Moraiolo	2002	2003	P5	P6	*P* value
3,4-DHPEA	7.6 ± 1.6 b	23.1 ± 5.9 a	7.7 ± 1.3 b	22.9 ± 6.0 a	15.0 ± 3.3 ns	15.7 ± 6.6 ns	0.001
p-HPEA	6.8 ± 1.4 b	10.8 ± 2.9 a	3.7 ± 0.5 b	13.9 ± 2.1 a	8.5 ± 1.9 ns	9.1 ± 2.9 ns	0.005
EA	64.8 ± 12.8 ns	62.4 ± 10.2 ns	84.1 ± 8.5 a	43.2 ± 8.8 b	77.5 ± 10.7 a	49.8 ± 9.9 b	0.021
EA derivates	91.1 ± 7.7 a	76.5 ± 5.6 b	67.5 ± 7.1 a	42.4 ± 2.9 b	63.6 ± 6.4 a	46.4 ± 6.5 b	0.011
3,4-DHPEA-EA	48.6 ± 8.9 b	76.5 ± 12.9 a	49.2 ± 3.7 b	75.9 ± 15.4 a	79.0 ± 13.5 a	46.1 ± 6.6 b	0.097
3,4-DHPEA-EDA	59.7 ± 11.9 ns	61.6 ± 13.6 ns	83.4 ± 8.4 a	37.8 ± 10.4 b	80.7 ± 9.4 a	40.6 ± 11.1 b	0.161
secoiridoid derivates	161.9 ± 27.2 a	134.8 ± 26.6 b	187.1 ± 12.2 a	109.6 ± 19.2 b	183.3 ± 19.9 a	113.4 ± 27.5 a	0.876
lignans	90.1 ± 28.9 a	31.7 ± 11.4 b	13.2 ± 1.5 b	108.5 ± 23.6 a	47.8 ± 19.2 b	73.8 ± 28.2 a	0.001
total polyphenols	500.6 ± 38.4 ns	449.3 ± 40.2 ns	492.1 ± 32.9 ns	457.9 ± 42.4 ns	555.4 ± 19.5 a	394.5 ± 32.3 b	0.978
total tocopherols	189.0 ± 2.7 b	278.0 ± 2.9 a	467.0 ± 9.6	nd	245.0 ± 7.9 ns	222.0 ± 6.7 ns	nd

[a] In each row and for each factor (cultivar, year, soil), values with different letters are significantly different at $P < 0.05$ (Tukey's test; means of three replicates ± SE); ns, not significant; nd, not determined. The values for cultivar and soil are means of two years (2002 and 2003). Tocopherols was determined only in 2002.

evidence. The effect of the year was reflected only in the oil content, which was higher in 2003 than in 2002 (18.8 vs 15.4% respectively, $P < 0.05$).

The soil significantly affected the ripeness index. The olive ripening of the two cultivar was lower in P6 rather than in the P5 in both years, but above all in the drier 2003. In fact, the ripeness index was on average 3.1 in P6 and 3.9 in P5. On the other hand, the differences between the two soils in terms of olive mean weight and oil content were not statistically significant.

Oil Composition. All experimental oil samples were extra virgin olive oil. The acidity was <0.5% of oleic acid, peroxide index < 8 mequiv O_2 kg^{-1}, and UV extinction coefficients K_{232} < 1.70 and K_{270} < 0.10 (absorbance 1%, 1 cm optical path, at 232 and 270 nm). The values of the acidic composition of oils did not indicate important variability in the comparison between cultivars, with the exception of the stearic and arachidic acid, which were significantly higher in the oil produced from the cultivar Frantoio (Table 3). As expected, the oleic acid content was significantly higher in the moister year 2002 than in the drier 2003. On the contrary, the linoleic and stearic acids were significantly higher in the year 2003.

Total tocopherols content did not seem to depend on the soil characteristics. The variety instead affected the concentration of the tocopherols in the oil. In fact, the cv. Moraiolo produced oils that were significantly richer in total tocopherols (Table 4).

However, soil properties affected polyphenol content. Total polyphenols in general, and secoiridoids in particular, were significantly influenced by the soil effect. The oil produced in P5 showed a total polyphenol concentration that was about 30% more elevated than in P6, due to more elevated values of oleuropein aglycon (3,4-DHPEA-EA), deacetoxy oleuropein aglycon (3,4-DHPEA-EDA), and secoiridoid derivate products elenoic acid (EA) and its derivates (Table 4).

As expected, the year had a significant effect on nearly all of the phenolic components. The concentration of secoiridoid derivates, 3,4-DHPEA-EDA, EA, and EA derivates was significantly greater in the moist year 2002 than in the dry 2003. In addition, smaller quantities of hydroxytyrosol (3,4-DHPEA), tyrosol (p-HPEA),

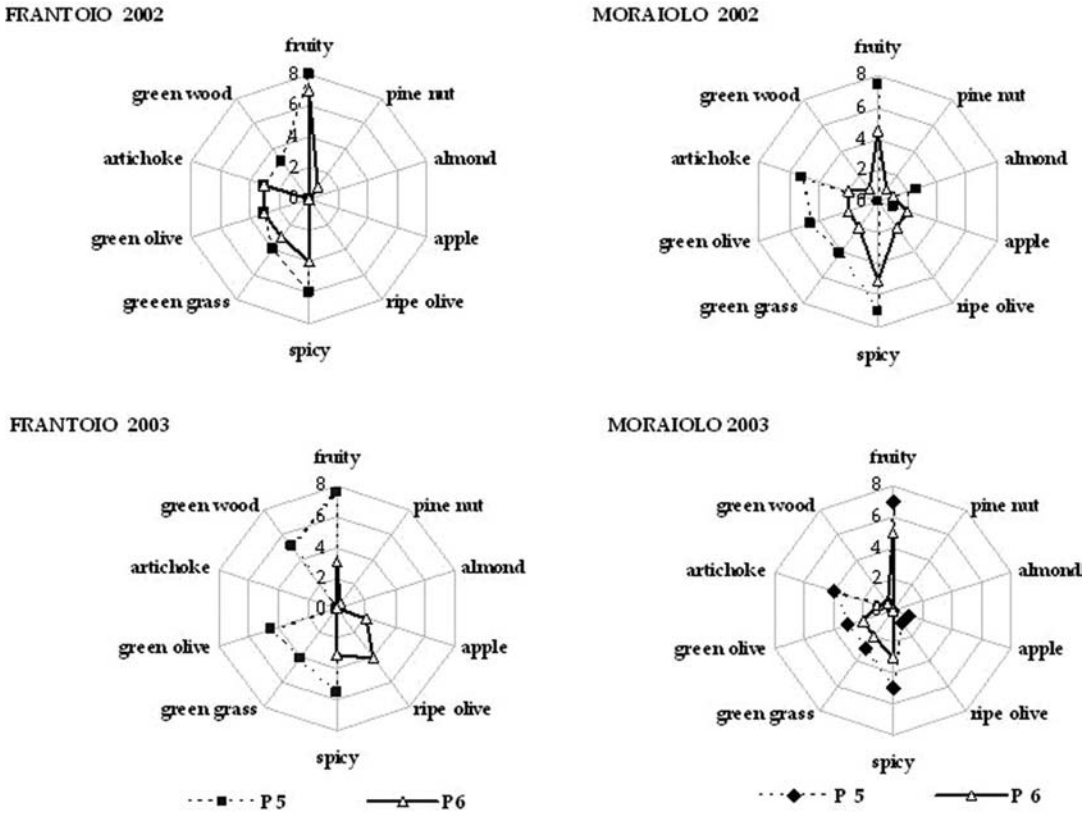

Figure 5. Average sensorial profile of monocultivar Frantoio and Moraiolo oil in P5 and P6 soils in 2002 and 2003.

3,4-DHPEA-EA, and lignans were recorded in 2002. The opposite trend of the phenolic substances determined the not significant differences between the total polyphenol content in the two years. The Frantoio and Moraiolo cultivars significantly influenced mainly 3,4-DHPEA and lignans. Significant interactions resulted for cultivar versus year on 3,4-DHPEA, p-HPEA, EA and its derivates, and lignans (Table 4). These interactions indicate that the cultivar effect was enhanced by the year: the hot and dry 2003 incremented the prevalence of 'Moraiolo' in terms of 3,4-DHPEA, p-HPEA, and lignans; the cool and humid 2002 exalted the predominance of 'Frantoio' for EA and its derivates.

Other significant interactions, not reported in the table, resulted for cultivar versus soil on p-HPEA, for year versus soil on 3,4-DHPEA-EA and lignans; and for cultivar versus soil versus year on 3,4-DHPEA. Therefore, the soil effect was enhanced respectively by the cultivar ('Moraiolo' and P6 for p-HPEA), by the year (2003 and P5 for 3,4-DHPEA-EA; 2003 and P6 for lignans), and by both cultivar and year ('Moraiolo', 2003, and P6 for 3,4-DHPEA).

Sensorial Evaluation. In the year 2002, the oils of the 'Frantoio' olive trees obtained positive judgments when produced on both soils. Nevertheless, the oil was qualitatively superior on soil P5 because of the more intense fruity and spicy smell-gustative notes, together with more marked herbaceous flavors (green grass and green olive notes). The same was true for the Moraiolo cultivar. On P5, 'Moraiolo' oil was judged decidedly superior to that on P6: more fruity and spicy, with more intense herbaceous and artichoke flavors (Figure 5).

Also in the year 2003 the oils of the cultivar Frantoio proved to be different according to the soil. The oil obtained in P5 was judged very young, that is, slightly unripe and spicy, with detached herbaceous and green wood aromas, whereas the oil produced in P6 had less intense aromatic notes and a mature fruit taste, namely, apple and ripe olive. Like Frantoio, the cultivar Moraiolo showed prominent differences in the two soils; in particular, the oil from P5 was more intensely fruity and spicy than that from P6 and had marked herbaceous and artichoke flavors.

TSW, Potential Soil Water Deficit, and Oil Quality. The multivariate statistical analysis of the results highlighted a significant relationship between TSW and potential soil water deficit from the surface to 0.70 m and some important parameters of oil quality (Figure 6). It is remarkable that the first two principal components of the model explained about 73% of the total variance, that is, 51.16% factor 1 and 21.68% factor 2. Potential water deficit was positively related, and TSW negatively, to the concentrations of 3,4-DHPEA-EA, 3,4-DHPEA, and p-HPEA, important phenolic substances for their antioxidant activity and sensory qualities.[3,8,45] Also, herbaceous (green grass, green olive, green wood) and fruity flavors opposed TSW, whereas they went along with the potential water deficit. In contrast, TSW was significantly related to mature fruit taste, namely, apple and ripe olive. Therefore, it was the soil with relatively lower values of TSW and higher potential water deficit that was associated to some nutraceutical substances and the most appreciated sensorial characteristics of VOO. On the other hand, total polyphenols, EA, EA derivates, secoiridoid derivates, 3,4-DHPEA-EDA, lignans, and the spicy and artichoke smell-gustative notes were significantly influenced neither by TSW nor by potential soil water deficit.

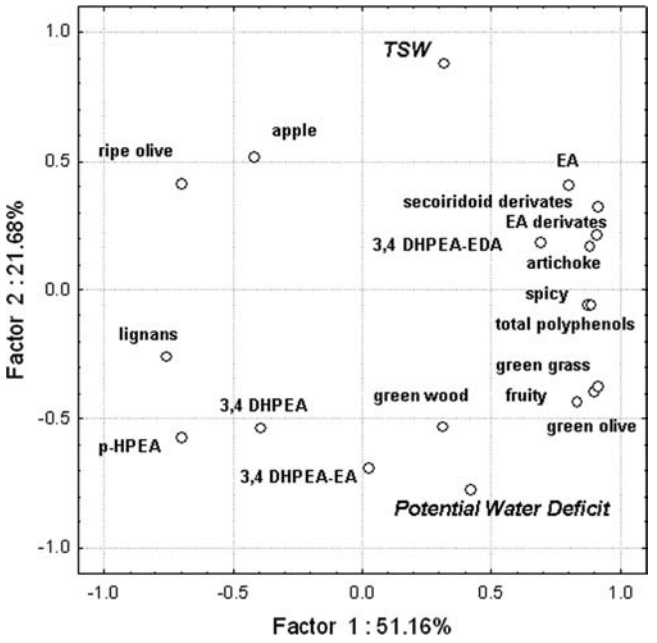

Figure 6. Plot of the first two principal components of PCA between TSW and potential soil water deficit from the surface and 0.70 m, along with some important parameters of oil quality.

DISCUSSION

The trial gave some significant insights into the soil properties that can affect olive ripening, the concentration of some nutraceutical substances, and the sensory profile of virgin olive oil produced in rainfed conditions. Boundary conditions of the experiment can be considered representative of the study area and typical of the Mediterranean olive-growing zone; in fact, rainfall amounts, more than temperatures, differentiated the study years.

The selected soils changed only for their physical and hydrological characteristics; therefore, we had postulated that the observed different phenologies of the olive trees cultivated on them was caused by different water availabilities. This was actually verified by means of a monitoring activity, which lasted two climatically contrasted years. Soil P5 showed a prominent potential summer water deficit in both years (on average, a cumulative deficit of 15.4 mm, in the first 0.3 m of soil and 42.2 mm in the first 0.7 m) and a reduced mean monthly TSW (3.4 g g^{-1} from the surface to 0.3 m and 7.7 g g^{-1} in the first 0.7 m, during the same period).

To complete the interpretation of the results of the monitoring, it is important to emphasize the role played by water tension on the availability of transpirable water. In fact, the two soils not only held different total amounts of TSW during the summer (Figure 4), but the water was held at different tensions, depending on their contrasting particle sizes and structures. In fact, as reported in Table 1, WP of P5 was on average 0.160 g g^{-1}, whereas it was 0.070 g g^{-1} in P6; therefore, a similar amount of gravimetric soil water was kept at higher tension in P5 than in P6.

As a consequence of the different soil water regimens, the olive trees in P5 were induced to ripen earlier and with better results in terms of polyphenolic substances (about 30% more) than in P6.

The acidic composition of oils did not indicate important variations in the comparison between soils, with the exception of stearic acid, which was significantly higher in the oil produced in P6. Moreover, the soil did not affect the total tocopherols content.

On the other hand, the soil water regimen influenced the polyphenolic oil composition. In P5, where available water was lower than in P6, 3,4-DHPEA-EA, 3,4-DHPEA-EDA, secoiridoid derivate products, and EA and its derivates were more abundant. Consequently, the antioxidant properties of the olive oil also were more pronounced in P5. However, limited soil water availability significantly lowered lignan production. The lower soil water availability of P5 showed a more pronounced effect on 3,4-DHPEA-EA and lignans in the hot and dry year 2003.

As a rule, 3,4-DHPEA-EA, 3,4-DHPEA, and p-HPEA were negatively related to TSW and positively related to higher potential soil water deficit. The effect on oleuropein aglycon is very important for oil sensorial quality, as well as for antioxidant properties. In fact, 3,4-DHPEA-EA is very bitter and astringent,[8] giving very appreciated sensorial hints of fresh and young to VOOs. Thus, despite the very different climatic conditions during the studied years, the sensorial properties of the oil obtained from both cultivars on the soil with a more pronounced summer water deficit were qualitatively superior, regardless of cultivar and year of trial, and characterized by a good fruity, balanced spicy taste and marked herbaceous notes.

Ultimately, we can conclude that the soil properties determining water availability, together with the climate of the year, were the most important factors affecting some nutraceutical components and the sensorial quality of the virgin olive oil that we obtained in rainfed cultivation. The variety influenced ripeness index and olive mean weight, as well as polyphenols and tocopherols content, but less than soil water availability.

AUTHOR INFORMATION

Corresponding Author

*E-mail: pierluigi.bucelli@entecra.it. Phone: +390552491227. Fax: +39055241485.

ACKNOWLEDGMENT

We acknowledge Antonio Cimato, Graziano Sani, and Cristina Attilio of IVALSA - CNR, Firenze, for the help given in the olive harvest and analysis and the farm Cinciano-Le Fonti (Siena) for its kind hospitality.

ABBREVIATIONS USED

HRGC, high-resolution gas chromatography; HPLC-DAD, high-performance liquid chromatography—diode array detection; FC, field capacity; WP, wilting point; AWC, available water capacity; TSW, transpirable soil water; CEC, cationic exchange capacity; CV%, coefficient of variation; ANOVA, analysis of variance; PCA, principal component analysis; RPM, revolutions per minute; VOO, virgin olive oil; IUPAC, International Union of Pure and Applied Chemistry; QDA, quantitative descriptive analysis.

REFERENCES

(1) Kris-Etherton, P. M.; Pearson, T. A.; Wan, Y.; Hargrove, R. L.; Moriarty, K.; Fishell, V.; Etherton, T. D. High—monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. *Am. J. Clin. Nutr.* **1999**, *70*, 1009–1015.

(2) Teres, S.; Barcelo-Coblijn, G.; Benet, M.; Alvarez, R.; Bressani, R.; Halver, J. E.; Escriba, P. V. Oleic acid content is responsible for the reduction in blood pressure induced by olive oil. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 13811–13816.

(3) Carrasco-Pancorbo, A.; Cerretani, L.; Bendini, A.; Segura-Carretero, A.; Gallina-Toschi, T.; Lerker, G.; Fernandez-Gutierrez, A. Evaluation of individual antioxidant activity of single phenolic compounds on virgin olive oil. *Prog. Nutr.* **2006**, *8*, 28–39.

(4) Caruso, D.; Galli, G.; Visioli, F. Biological activities and metabolic fate of olive oil phenols. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 677–684.

(5) Fabiani, R.; De Bartolomeo, A.; Rosignoli, P.; Servili, M.; Montedoro, G. F.; Morozzi, G. Cancer chemoprevention by hydroxytyrosol isolated from virgin olive oil through G1 cell cycle arrest and apoptosis. *Eur. J. Cancer Prev.* **2002**, *11*, 351–358.

(6) Owen, R. W.; Giacosa, A.; Hull, W. E.; Haubner, R.; Spiegelhalder, B.; Bartsch, H. The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. *Eur. J. Cancer* **2000**, *36*, 1235–1247.

(7) Visioli, F.; Galli, C.; Galli, G.; Caruso, D. Biological activities and metabolic fate of olive oil phenols. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 677–684.

(8) Andrewes, P.; Busch, J. L. H. C.; De Joode, T.; Groenewegen, A.; Alexandre, H. Sensory properties of virgin olive oil polyphenols: identification of deacetoxy-ligustroside aglycon, a key contributor to pungency. *J. Agric. Food Chem.* **2003**, *51*, 1415–1420.

(9) Servili, M.; Selvaggini, R.; Esposto, S.; Taticchi, A.; Montedoro, G. F.; Morozzi, G. Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. *J. Chromatogr.* **2004**, *1054*, 113–127.

(10) Montedoro, G. F.; Servili, M.; Badioli, M.; Selvaggini, E.; Miniati, E.; Macchioni, A. Simple and hydrolysable compounds in virgin olive oil. 3. Spectroscopic characterization of the secoiridoids derivates. *J. Agric. Food Chem.* **1993**, *41*, 2228–2234.

(11) Servili, M.; Selvaggini, R.; Taticchi, A.; Esposto, S.; Montedoro, G. F. Volatile compounds and phenolic composition of virgin olive oil: optimization of temperature and time of exposure of olive pastes to air contact during the mechanical extraction process. *J. Agric. Food Chem.* **2003**, *51*, 7980–7988.

(12) Servili, M.; Esposto, S.; Lodolini, E.; Selvaggini, R.; Taticchi, A.; Urbani, S.; Montedoro, G. F.; Serravalle, M.; Gucci, R. Irrigation effects on quality, phenolic composition and selected volatiles of virgin olive oils cv. Leccino. *J. Agric. Food Chem.* **2007**, *55*, 6609–6618.

(13) Tovar, M. J.; Romero, M. P.; Alegre, S.; Girona, J.; Motilva, M. J. Composition and organoleptic characteristics of oil from Arbequina olive (*Olea europaea* L.) trees under deficit irrigation. *J. Sci. Food Agric.* **2002**, *82*, 1755–1763.

(14) Dabbou, S.; Chehab, H.; Faten, B.; Dabbou, S.; Esposto, S.; Selvaggini, R.; Taticchi, A.; Servili, M.; Montedoro, G. F.; Hammami, M. Effect of three irrigation regimes on Arbequina olive oil produced under Tunisian growing conditions. *Agric. Water Manage.* **2010**, *97*, 763–768.

(15) Dabbou, S.; Dabbou, S.; Chehab, H.; Brahmi, F.; Taticchi, A.; Servili, M.; Hammami, M. Chemical composition of virgin olive oils from Koroneiki cultivar grown in Tunisia with regard to fruit ripening and irrigation regimes. *Int. J. Food Sci. Technol.* **2011**, *46*, 577–585.

(16) Greven, M.; Neal, S.; Green, S.; Dichio, B.; Clothier, B. The effects of drought on the water use, fruit development and oil yield from young olive trees. *Agric. Water Manage.* **2009**, *96*, 1525–1531.

(17) Gómez-Rico, A.; Salvador, M. D.; Moriana, A.; Pérez, D.; Olmedilla, N.; Ribas, F.; Fregapane, G. Influence of different irrigation strategies in a traditional Cornicabra cv. olive orchard on virgin olive oil composition and quality. *Food Chem.* **2007**, *100*, 568–578.

(18) Stefanoudaki, E.; Williams, M.; Chartzoulakis, K.; Harwood, J. Olive oil qualitative parameters after orchard irrigation with saline water. *J. Agric. Food Chem.* **2009**, *57*, 1421–1425.

(19) Ahmed, C. B.; Rouina, B. B.; Sensoy, S.; Boukhriss, M. Saline water irrigation effects on fruit development, quality, and phenolic composition of virgin olive oils, cv. Chemlali. *J. Agric. Food Chem.* **2009**, *57*, 2803–2811.

(20) Aguilera, M. P.; Beltrán, G.; Ortega, D.; Fernández, A.; Jiménez, A.; Uceda, M. Characterisation of virgin olive oil of Italian olive cultivars: 'Frantoio' and 'Leccino', grown in Andalusia. *Food Chem.* **2005**, *89*, 387–391.

(21) Costagli, G.; Gucci, R.; Rapoport, H. F. Growth and development of fruits of olive Frantoio under irrigated and rainfed conditions. *J. Hortic. Sci. Biotechnol.* **2003**, *78*, 119–124.

(22) Dag, A.; Ben-Gal, A.; Yermiyahu, U.; Basheer, L.; Nir, Y.; Kerem, Z. The effect of irrigation level and harvest mechanization on virgin olive oil quality in a traditional rain-fed Souri olive orchard converted to irrigation. *J. Sci. Food Agric.* **2008**, *88*, 1524–1528.

(23) Iniesta, F.; Testi, L.; Orgaz, F.; Villalobos, F. J. The effects of regulated and continuous deficit irrigation on the water use, growth and yield of olive trees. *Eur. J. Agron.* **2009**, *30*, 258–265.

(24) Costantini, E. A. C.; Barbetti, R.; Bucelli, P.; Cimato, A.; Franchini, E.; L'Abate, G.; Pellegrini, S.; Storchi, P.; Vignozzi, N. *Zonazione Viticola ed Olivicola della Provincia di Siena*; Grafiche Boccacci: Colle Val d'Elsa, Italy, 2006; pp 224.

(25) Cimato, A.; Cantini, C.; Sani, G.; Romani, A.; Autino, A.; Cresti, M. *L'Olivo in Toscana: il Germoplasma Autoctono*; ARSIA-Regione Toscana, CNR: Florence, Italy, 2004; pp 55.

(26) Costantini, E. A. C. *Linee Guida dei Metodi di Rilevamento e Informatizzazione dei Dati Pedologici*; CRA Research Centre for Agrobiology and Pedology: Florence, Italy, 2007; pp 280.

(27) Kassel, D. K.; Nielsen, D. R. Field capacity and available water capacity. In *Methods of Soil Analysis*, Part 1, 2nd ed.; Klute, A., Ed.; American Society of Agronomy: Madison, WI, 1986; pp 901−926.

(28) MIPAF (Ministero delle Politiche Agricole e Forestali). *Metodi di Analisi Chimica del Suolo*. Angeli, F., Ed.: Milan, Italy, 2000; fasciculated.

(29) Linsday, W. L.; Norvell, W. A. Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Sci. Soc. Am. J.* **1978**, *42*, 421–428.

(30) Soil Survey Staff. *Keys to Soil Taxonomy*; SMSS, Technical Monograph 19; Pocahontas Press: Blacksburg, VA, 1998.

(31) IUSS Working Group WRB. World Soil Resource Reports 103. In *World Reference Base for Soil Resource*, 2nd ed.; FAO: Rome, Italy, 2006; pp 132.

(32) Lebon, E.; Dumas, V.; Pieri, P; Schultz, H. R. Modelling the seasonal dynamics of the soil water balance of vineyards. *Funct. Plant Biol.* **2003**, *30*, 699–710.

(33) Patrick, W. H.; Gambrell, R. P.; Faulkner, S. P. Redox measurement of soils. In *Methods of Soil Analysis*, Part 3. Chemical Methods, Book Series 5; Soil Science Society of America: Madison, WI, 1996; pp1255−1276.

(34) Uceda, M.; Frias, L. Harvest dates: evolution of the fruit oil content, oil composition and oil quality. In *Proceedings Segundo Seminario Oléicola Internacional*; COI: Cordoba, Spain, 1975; pp 125−130.

(35) Boskou, D. *Olive Oil: Chemistry and Technology*, 2nd ed.; American Oil Chemists Society Press: Champaign, IL, 2006; pp 268.

(36) European Union Commission. Regulation 1989/03. Modify at Regulation EEC/2568/91. Characteristics of olive and olive-pomace oils and on their analytical methods. *Off. J. Eur. Communities* **2003**, *L295*, 57−77.

(37) Rovellini, P.; Cortesi, N. Liquid chromatography-mass spectrometry in the study of oleuropein and ligustroside aglycons in virgin olive oil: aldehydic, dialdehydic forms and their oxidized products. *Riv. Ital. Sostanze Grasse* **2002**, *1*−*2*, 1–14.

(38) Brenes, M.; Garcia, A.; Garcia, P.; Garrido, A. Rapid and complete extraction of phenols from olive oil and determination by means of a coulometric electrode array system. *J. Agric. Food Chem.* **2000**, *48*, 5178–5183.

(39) Gómez-Alonso, S.; Mancebo-Campos, V.; Desamparados Salvador, M.; Fregatane, G. Evolution of major and minor components and oxidation indices of virgin olive oil during 21 months storage at room temperature. *Food Chem.* **2007**, *100*, 36–42.

(40) Gómez-Rico, A.; Fregapane, G.; Desamparados Salvador, M. Effect of cultivar and ripening on minor components in Spanish olive fruits and their corresponding virgin olive oils. *Food Res. Int.* **2008**, *41*, 433–440.

(41) IUPAC. Determination of tocopherols and tocotrienols in vegetable oils and fats by HPLC. In *Standard Methods for the Analysis of Oils Fats and Derivatives*, 7th ed.; IUPAC Applied Chemistry Commission on Oils Fats and Derivatives; Paquot, C., Hautfeenne, A., Eds.; Blackwell Scientific: Oxford, U.K., 1992; Method 2432.

(42) Euroconsult. *Agricultural Compendium for Rural Development in the Tropics and Subtropics*; Elsevier Science Publishers: Amsterdam, The Netherlands, 1989; pp 740.

(43) Franchini, E.; Cimato, A.; Costantini, E. A. C. Olive tree (*Olea europea L.*). In *Manual of Methods for Soil and Land Evaluation*; Costantini, E. A. C., Ed.; Science Publishers: Plymouth, U.K., 2009; pp 401−449.

(44) Dichio, B.; Sofo, A.; Xiloyannis, C. Drought resistence mechanism in olive tree. In *Proceedings 5th International Symposium on Olive Growing*, Izmir, Turkey, 2004; International Society for Horticultural Science: Leuven, Belgium, 2008; pp 761.

(45) Bendini, A.; Cerretani, L.; Carrasco-Pancorbo, A.; Gómez-Caravaca, A. M.; Segua-Carretero, A.; Fernández-Gutiérrez, A.; Lerker, G. Phenolic molecules in virgin olive oil: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of last decade. *Molecules* **2007**, *12*, 1697–1719.