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Irrigation Effects on Quality, Phenolic Composition, and Selected Volatiles of Virgin Olive Oils Cv. Leccino

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Field-grown olive trees (*Olea europaea* L. cv. Leccino) were used over two growing seasons to determine the effect of deficit irrigation regimes on virgin olive oil (VOO) quality. Drip irrigation was managed to maintain a predawn leaf water potential (PLWP): (a) higher than −1.1 MPa (full irrigation: FI); (b) between −1.0 and −3.3 MPa (deficit irrigation: DI); (c) higher than −4.2 MPa (severe deficit irrigation: SI). The fruit yield and oil yield of DI trees were over 90% of those of FI treatments in both years, respectively, whereas yields of SI trees ranged from 61 to 76%. The irrigation regime had minor effects on the free acidity, peroxide value, and fatty acid composition of VOO. The concentrations of phenols and *o*-diphenols in VOO were negatively correlated with PLWP. The concentrations of the dialdehydic form of decarboxymethyl elenolic acid linked to (3,4-dihydroxyphenyl)-ethanol (3,4-DHPEA-EDA), the isomer of the oleuropein aglycon (3,4-DHPEA-EA), and the dialdehydic form of decarboxymethyl elenolic acid linked to (*p*-hydroxyphenyl)ethanol (*p*-HPEA-EDA) were lower in FI than in SI treatments. The concentrations of lignans (+)-1-acetoxipinoresinol and (+)-1-pinoresinol were unaffected by the irrigation regime. The tree water status had a marked effect on the concentration of volatile compounds, such as the C_6-saturated and unsaturated aldehydes, alcohols, and esters.

KEYWORDS: ***Olea europaea*; deficit irrigation; fatty acid composition; leaf water potential; oil; phenols; volatile compounds**

INTRODUCTION

One of the most significant changes that are currently occurring in olive tree cultivation is the expansion of irrigated orchards. Although it is well-known that the lack of water decreases yield and fruit size (*1*−*4*), there is evidence that controlling the degree of water deficit by supplying reduced volumes of irrigation water has limited negative effects on oil yield (*4*−*7*). Strategies using regulated deficit irrigation (RDI) have been proposed to optimize water use in olive growing (*5*, *7*). Under RDI conditions, water productivity is higher than when the trees are fully irrigated (*8*).

Recently, Gucci et al. (*9*) showed that DI, managed by imposing short stress-relief cycles, could maintain a yield within 20% of that of FI trees over two growing seasons, while it allowed one to save over 50% of the applied water, confirming results obtained using other DI strategies and cultivars (*4*, *7*).

Few studies investigated the effects of water deficit on olive oil quality, and, hence, many aspects are still controversial. Early studies on the effect of irrigation focused on the acidic composition and total concentration of phenolic compounds (*3*, *10*), and showed that irrigation decreased the concentration of phenolic compounds (*10*, *11*), but did not affect the fatty acid composition of the oil (*3*, *10*, *11*). Berenguer et al. (*12*) reported that mono-unsaturated fatty acid levels and oleic−linoleic ratios declined, while poly-unsaturated fatty acid levels increased with increasing irrigation; however, there was no difference in the ratio between mono- to poly-unsaturated fatty acids in the second year of their experiment.

Nowadays, the concept of quality is mainly based on the sensory and health-related properties of VOO, which are closely related to the concentration and composition of the phenolic and volatile fractions, respectively (*13*, *14*). Tovar et al. (*15*), using young trees of cv. Arbequina in a linear irrigation experiment, showed a negative correlation between the volume of water applied and the concentration of secoiridoid derivatives of VOO, such as 3,4-DHPEA-EDA, 3,4-DHPEA-EA, and *p*-HPEA-EDA. They also found that the concentration of lignans

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was lower in the oils extracted from fruits of the least irrigated treatment (*15*). In another study, Motilva et al. (*6*) concluded that hydrophilic phenols in VOO increased when the trees (cv. Arbequina) were grown under RDI conditions, whereas other authors (*16*) reported that the highest level of hydrophilic phenols in the oil was obtained from regularly irrigated olives.

The controlled use of water to improve the qualitative characteristics of horticultural products is becoming more and more important. For fruit trees and grapevines there is documented evidence that RDI can improve fruit quality (*17*, *18*), but there are few works on the "qualitative" effects of deficit irrigation in olive growing. To the best of our knowledge, only one very recent study investigated the effect of irrigation on volatile compound concentrations in VOO (*19*).

The objective of this study was to determine how the tree water status during fruit development affected the analytical parameters of VOOs obtained from trees of the cv. Leccino grown in a high-density orchard. In particular, we manipulated the PLWP, a plant-based indicator of water deficit, using different irrigation regimes to study the effects on fatty acid composition, concentrations of phenols and volatile compounds, and sensory attributes of VOO. Our experimental approach allowed us to improve the understanding of the relationship between VOO quality and the water relations of the olive tree.

MATERIALS AND METHODS

Plant Material and Climatic Conditions. Olive trees (*Olea europaea* L. cv. Leccino), planted in 1998 in a sandy-clay soil at a density of 420 trees ha^{-1} at Bibbona, Italy (43° 16′ N, 10° 35′ E), were used in 2003 and 2004. In 2003 eighteen trees from three adjacent rows (total 54 trees) were selected to be similar in potential yield, and then the fruits of 15 trees per row were hand-thinned between five and six weeks after full bloom (AFB) (*9*). In the following year, 14 trees per row were chosen from the same rows (consequently the same irrigation regime) used in 2003, and two crop loads (unthinned and 80% of the fruits removed) were established 2−3 weeks AFB. In both years, the final crop load was expressed on the basis of trunk cross-sectional area (TCSA) to account for differences in tree size.

The annual rainfall and temperature averaged over a 30-year period were 772 mm and 15.6 °C, respectively. In 2003, precipitation was virtually absent (5 mm) during the first part of the summer, but reached 88 mm from September 1 through harvest, whereas in 2004 they were 46 and 89 mm, respectively (*7*). The summer of 2003 was hotter than that of 2004 with mean temperatures of 26.1 and 23.1 °C and mean maximum temperatures of 36.3 and 31.2 °C, respectively. In 2004, rainfall and temperatures were similar to the long-10-year means for that site. Other characteristics of the plant material, soil, climatic conditions, and agricultural practices were reported in Gucci et al. (*9*).

Irrigation Treatments and Leaf Water Potential Measurements. The orchard was drip irrigated, and the details of irrigation management were previously reported (*9*).

In brief, irrigation was managed in order to achieve PLWP: (a) higher than −1.1 MPa (FI, fully irrigated); (b) between −1.0 and −3.3 MPa (DI, deficit irrigated); (c) higher than −4.2 MPa (SI, severe deficit irrigated). The amount of water supplied to the FI trees was estimated weekly by calculating reference evapotranspiration according to the Penman−FAO equation (*20*) and crop evapotranspiration (*21*) using a crop coefficient of 0.55. In any case, the volume of the irrigation water was adjusted depending on the weekly measurements of PLWP, which allowed us to guide irrigation more precisely than using crop evapotranspiration estimates only. The irrigation period lasted from 6 through 16 and from 4 through 16 weeks AFB in 2003 and 2004, respectively. In 2003, each of the FI, DI, and SI trees received an average of 2732, 1150, and 573 L of water, respectively. In 2004, larger volumes of water were applied to FI and DI trees than in 2003 due to the longer irrigation period (3899, 1927, and 566 L for FI, DI, and SI trees respectively). Each irrigation treatment included a range of crop loads resulting from the previously described hand-thinning.

Fully expanded leaves from fruiting shoots of all trees were used to measure PLWP at approximately weekly intervals, using a Scholander-type pressure chamber (Tecnogas, Pisa, Italy). The leaf was cut at the petiole, then immediately put in the chamber cylinder and pressurized with nitrogen gas at a flow rate of 0.02 MPa s^{-1} (*1*). To account for the fluctuations in tree water status, the PLWP values were integrated over the irrigation period (*9*).

Yield, Maturation Index. Immediately before harvest, 100 fruits were randomly sampled from around the canopy of each tree to determine the maturation index (MI) according to a 0 to 7 scale (*22*) and the fruit fresh weight. The oil yield at harvest was calculated as reported in Gucci et al. (*9*). Since irrigation delayed fruit maturation (*9*) and maturation affects oil quality, 5 kg of fruits at the same stage of maturation (MI = 4) for all irrigation treatments were selected at harvest from five trees with high yields per treatment and used for oil extraction.

Oil Mechanical Extraction Process. Oil extraction was performed using a lab scale system, whereby the fruits were crushed by a hammer mill, the resulting olive paste malaxed at 25 °C for 20 min, and the oil separated by centrifugation. Then the oil was filtered and stored in the dark at 8 °C until analysis.

Oil analyses. *Reference compounds.* (3,4-Dihydroxyphenyl)ethanol (3,4-DHPEA) was obtained from Cayman Chemicals LTD (USA), while the (*p*-hydroxyphenyl)ethanol (*p*-HPEA) was obtained from Janssen Chemical Co. (Beerse, Belgium). The dialdehydic form of elenolic acid linked to 3,4-DHPEA or *p*-HPEA (3,4-DHPEA-EDA and *p*-HPEA-EDA, respectively), the isomer of oleuropein aglycon (3,4-DHPEA-EA), the (+)-1-acetoxypinoresinol, and (+)-pinoresinol were extracted from VOO using a previously reported procedure (*23*). The purity of all the substances obtained from direct extraction was tested by high-performance liquid chromatography (HPLC), and their chemical structures were verified by NMR.

Pure analytical standards of volatile compounds were purchased from Fluka and Aldrich (Milan, Italy).

Marketable Parameters. The free acidity, peroxide value, and fatty acid composition were measured in accordance with the European Official Methods (UE 1989/2003 modifying the ECC 2568/91) (*24*).

Total Phenols and Orthodiphenols. The total phenols and *o*-diphenols were determined colorimetrically according to Montedoro et al. (*25*). The effect of the water status on phenolic composition was determined on oil samples produced in 2004 only.

Phenolic Compounds. The phenolic fractions were extracted by liquid−liquid extraction (*25*) and analyzed by HPLC. Before injection, the phenolic extract was solubilized with 1 mL of methanol and filtered through a PVDF syringe filter 0.2 μm. The HPLC analysis was conducted as reported by Selvaggini et al. (*26*), using an Agilent Technologies system model 1100 (Agilent Technologies, Palo Alto, CA), composed of a vacuum degasser, a quaternary pump, an autosampler, a thermostated column compartment, a DAD (diode array detector), and a FLD (fluorescence detector). The analysis of the oil extract was performed using C18 columns Spherisorb ODS-1 250 × 4.6 mm with a particle size of 5 μm (Phase Separation Ltd., Deeside, U.K.). The mobile phase was composed of 0.2% acetic acid (pH 3.1) in water (solvent A)/methanol (solvent B) at a flow rate of 1 mL/min, and the gradient changed as follows: 95% A/5% B for 2 min, 75% A/25% B in 8 min, 60% A/40% B in 10 min, 50% A/50% B in 16 min, 0% A/100% B in 14 min and this composition maintained for 10 min. Then, initial conditions were reset and equilibration was reached in 13 min; the total running time was 73 min.

Volatile Compounds. The volatile compounds were determined in duplicate by HS-SPME−GC/MS (head space solid phase microextraction−gas chromatography−mass spectrometry), as previously reported (*27*). For the solid phase microextraction (SPME) analysis, the oil (3 g) was put into a 10 mL vial and thermostated at 35 °C for 15 min. Then the 65 μm Carbowax/divinylbenzene fiber (Supelco, Inc., Bellefonte, PA) was exposed to the vapor phase for 30 min in order to sample the volatile compounds. Afterward, the fiber was inserted into the injector of the gas chromatograph (GC) and set in splitless mode using a splitless inlet liner of 0.75 mm i.d. for thermal desorption where it was left for 5 min. All of the SPME operations were automated using a Varian 8200 CX AutoSampler (Varian, Walnut Creek, CA).

Table 1. Main Experimental Conditions, Yield, and Fruit Fresh Weight of Unthinned Olive Trees (cv. Leccino) Grown under Full Irrigation (FI), Deficit Irrigation (DI), or Severe Deficit Irrigation (SI) in 2003 and 2004[a]

year	irrigation	cumulative PLWP[a] (–MPa d)	fresh fruit yield/TCSA (g dm^{-2})	oil yield/TCSA (g dm^{-2})	fruit fresh wt (g)
2003	FI	97	18257	3274	2.30
	DI	180	17294	3929	1.72
	SI	269	11187	2992	1.29
	LSD (0.05)		2820	761	0.25
2004	FI	112	26533	3383	1.99
	DI	189	24184	2424	1.52
	SI	291	19659	2051	1.38
	LSD (0.05)		3468	799	0.18

[a] The cumulative predawn leaf water potential (PLWP) was calculated over the 7–20 and 3–20 weeks AFB periods in 2003 and 2004. respectively. Significant differences between treatments were determined at the 0.05 level of probability by one-way analysis of variance. Values are means of six and eight replications in 2003 (includes 80% thinned trees) and 2004, respectively. Legend: TCSA, trunk cross-sectional area.

The volatile compounds were identified by comparison of their mass spectra and retention times against those of reference compounds. When standards were not available, identification of the volatile compounds was obtained by comparing their mass spectral data with those of the Wiley 6 mass spectra library. Integration of all of the chromatographic peaks was performed by choosing the three masses, among those specific for each compound, with the highest intensities as to selectively discriminate them from the nearest neighbors. The results of the peak areas were expressed as area counts. A quantitative determination of selected volatile compounds was performed as reported by Servili et al. (*27*).

Sensory Analysis. Quantitative descriptive analysis (QDA) was carried out by a total of 8 assessors, members of staff of the University of Perugia, using the procedure reported in a previous paper (*28*). The assessors had experience in QDA (*29*) and virgin olive oil sensory evaluation in accordance with the current UE Regulation (*24*). VOO samples (15 g) were served to the assessors in clear glass tumblers (100 mL), covered with watch glasses at room temperature (approximately 20 °C). The samples were presented in duplicate, in balanced order to each assessor.

For a description of the VOOs, the following terms were used: yellow, yellow-green, intense green, musk-green to describe the VOO color; fruity, cut-grass, floral, hay-like, almond, apple, and artichoke for the VOO olfactory profile; pungent, bitter, greasy, and sweet to define the VOO taste notes.

The intensity of those sensations was graded using a line scale and thus converted into a numerical score by measuring the position of the placed mark along a 10 cm line. The results were calculated as averages among assessor sensory scores.

The sensory profiles of VOOs were reported as spider plots to compare the samples' differences according to the irrigation regime.

Statistical Analysis. The means of irrigation treatments were compared by a one-way analysis of variance (ANOVA) using SIGMAPLOT (Jaendel Scientific, San Rafael, CA) software. Multivariate analyses such as a principal component analysis (PCA) and partial least-squares regression (PLS) were performed using SIMCA-P v. 8.0 chemometric package (Umetrics AB, Umeå, Sweden).

RESULTS AND DISCUSSION

Tree Water Status and Yield. The different irrigation regimes determined wide differences in the degree of water stress experienced by the trees. At the end of the 2003 growing season, the cumulative PLWP values of DI and SI trees were 1.96 and 2.77 times that of FI trees, respectively, and 1.69 and 2.60 in 2004 (**Table 1**). While the PLWP of SI trees was allowed to decrease below the turgor loss point with values as low as −4.2 MPa, the DI treatment was designed to maintain the PLWP between full hydration and turgor loss point (*9*, *30*), thus exposing trees to short stress-relief cycles within a physiologically significant range. The PLWP intervals selected for the FI and SI treatments were typical of nonstressed trees during fruit development and trees growing under rain-fed conditions in Central Italy (*1*), respectively.

Therefore, our DI treatment was different from most RDI experiments, whereby only a percentage of the volume applied to the fully irrigated control is supplied at certain phenological stages (in olive trees it is common to decrease irrigation from the beginning of massive pit hardening through the phase of final fruit swell) or linear irrigation experiments, where trees receive a fixed percentage of their evapotranspirative demand throughout the irrigation period. One of the advantages of our approach was that we could reach cumulated PLWP values similar for respective irrigation treatments in both years, despite differences in temperature, precipitation, and duration of the irrigation period between the two growing seasons (*9*).

The fruit yield of DI trees was over 90% that of FI trees in both years, whereas the fruit yield of SI trees was 61−74% that of FI trees (**Table 1**). The oil yield/TCSA of DI and SI treatments was 95 and 76% that of FI trees, respectively (means of both growing seasons). Hence, deficit irrigation allowed us to maintain a yield similar to that of fully irrigated trees while saving about 750 m^3 ha^{-1} of water per year (*9*), confirming results obtained with other olive cultivars (*4*, *6*, *8*). Irrigation had a marked influence on fruit size (**Table 1**), similar to what has been previously reported (*3*, *7*, *8*, *10*, *11*).

VOO quality. *Marketable Parameters, Phenols, and Fatty Acid Composition in VOO*. The free acidity and peroxide value of all samples were well below the limit established by EU regulation (*24*) for extra virgin olive oils (**Table 2**). The irrigation regime had minor effects on free acidity and peroxide values, although the responses were different between the two growing seasons. Patumi et al. (*11*) reported no effect of irrigation on free acidity and peroxide value of VOO of cv. Kalamata; Berenguer et al. (*12*) and Gomez-Rico et al. (*31*) also found inconsistencies in changes induced by irrigation between two consecutive years.

Full irrigation decreased the concentrations of total phenols and *o*-diphenols in the oil (**Table 2**). Interestingly, the VOO from DI and SI trees had the same concentrations of total phenols and *o*-diphenols in both years (**Table 2**), in agreement with published data (*32*, *33*). When the concentration of total phenols was expressed as a function of tree water status, there appeared a linear inverse relationship, which was tighter in 2003 than in 2004 (data not shown). Hence, trees that had experienced a certain degree of water deficit yielded oils with higher concentrations of phenols, which were presumably more abundant in the olive fruit, as reported by Patumi et al. (*11*) and Tovar et al. (*34*). In grapevines, it has been shown that periods of water deficit increased phenolics in the berry skins both by reducing their sizes and by effects independent of berry size (*35*). It has been hypothesized that the activity of phenylalanine ammonia-lyase (PAL), a key enzyme in the biosynthetic pathway of phenolic compounds, is directly involved in the accumulation of polyphenols and *o*-diphenol contents in the olive fruit and, hence, in VOO (*34*). Moreover, periods of water deficit appear to directly influence PAL activity in olive fruit (*10*, *34*), and this could be an explanation for the reduced level of phenolic compounds (*19*) at high irrigation regimes.

Table 2. Acidity, Peroxide Value, and Concentration of Phenolic Compounds of Virgin Olive Oils (VOOs) from Olive Trees (Cv. Leccino) Grown under Full Irrigation (FI), Deficit Irrigation (DI), or Severe Deficit Irrigation (SI) in 2003 and 2004[a]

	2003			2004		
	FI	DI	SI	FI	DI	SI
acidity (g of oleic acid/100 g of oil)	0.14 ± 0.01 a	0.15 ± 0.01 a	0.2 ± 0.04 b	0.21 ± 0.09 a	0.21 ± 0.02 a	0.25 ± 0.05 a
peroxide value (mequiv of O_2/kg of oil)	9.80 ± 1.0 a	9.38 ± 1.6 a	10.0 ± 1.0 a	4.62 ± 0.8 a	3.29 ± 0.4 b	5.58 ± 0.9 a
total phenols (mg/kg of oil)	249.6 ± 14.0 a	526.2 ± 151.0 b	584.6 ± 117.0 b	308.0 ± 14.0 a	479.8 ± 94.0 b	487.5 ± 121.0 b
o-diphenols (mg/kg of oil)	73.8 ± 14.0 a	165.2 ± 35.0 b	201.3 ± 48.0 b	150.7 ± 6.0 a	231.2 ± 50.0 b	239.3 ± 61.0 b

[a] Values are the means of five different VOO samples ($n = 5$) ± standard deviations. Different letters indicate significant differences ($P < 0.05$) between irrigation treatments within each year.

Table 3. Phenolic Composition (mg/kg of Oil) of Virgin Olive Oils (VOOs) from Trees (Cv. Leccino) Grown under Full Irrigation (FI), Deficit Irrigation (DI), or Severe Deficit Irrigation (SI) in 2004[a]

	FI	DI	SI
(3,4-dihdroxyphenyl) ethanol (3,4-DHPEA)	2.3 ± 0.3 a	2.4 ± 0.7 a	3.5 ± 0.9 a
(*p*-hydroxyphenyl) ethanol (*p*-HPEA)	7.7 ± 0.8 a	7.4 ± 1.4 a	3.1 ± 0.4 b
dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA)	130.1 ± 25.9 a	291.7 ± 40.3 b	318.5 ± 39.1 b
dialdehydic form of decarboxymethyl elenolic acid linked to *p*-HPEA (*p*-HPEA-EDA)	80.2 ± 11.8 a	128.2 ± 25.6 b	129.9 ± 24.1 b
(+)-1-acetoxypinoresinol	4.4 ± 0.8 a	3.8 ± 0.9 a	6.0 ± 2.6 a
(+)-pinoresinol	44.8 ± 6.7 a	49.7 ± 5.6 a	47.2 ± 7.9 a
oleuropein aglycon (3,4-DHPEA-EA)	114.1 ± 19.2 a	139.6 ± 20.1 a	185.5 ± 23.0 b

[a] Values are the means of five different VOO samples ($n = 5$) ± standard deviation. Different letters indicate significant differences ($P < 0.05$) between irrigation treatments within each row of data.

Table 4. Fatty Acid Composition (%) of Virgin Olive Oils (VOOs) from Olive Trees (Cv. Leccino) Grown under Full Irrigation (FI), Deficit Irrigation (DI), or Severe Deficit Irrigation (SI) in 2003 and 2004[a]

	2003			2004		
	FI	DI	SI	FI	DI	SI
myristic acid	0.01 ± 0.01 a	0.01 ± 0.001 b	0.01 ± 0.001 a	0.01 ± 0.001 a	0.01 ± 0.001 b	0.01 ± 0.001 a
palmitic acid	15.2 ± 0.6 a	15.6 ± 0.7 a	14.4 ± 0.8 a	14.0 ± 0.8 a	13.7 ± 1.5 [a]	14.6 ± 1.8 a
palmitoleic acid	1.5 ± 0.1 a	1.9 ± 0.2 b	1.2 ± 0.2 c	1.4 ± 0.2 a	0.8 ± 0.1 b	1.2 ± 0.3 a
margaric acid	1.4 ± 0.2 a	0.8 ± 0.1 b	1.2 ± 0.3 a	0.04 ± 0.01 a	0.03 ± 0.008 a	0.03 ± 0.005 a
eptadecenoic acid	0.08 ± 0.009 a	0.08 ± 0.004 a	0.1 ± 0.01 b	0.1 ± 0.02 a	0.04 ± 0.01 b	0.1 ± 0.01 ab
stearic acid	1.0 ± 0.4 a	1.5 ± 0.4 a	2.1 ± 0.2 b	1.0 ± 0.6 a	2.1 ± 1.1 a	1.0 ± 0.7 a
oleic acid	74.8 ± 0.4 a	74.0 ± 0.7 a	71.7 ± 0.8 b	77.3 ± 1.2 a	76.1 ± 2.7 a	76.1 ± 2.1 a
linoleic acid	4.8 ± 0.4 a	5.1 ± 0.3 a	7.9 ± 0.6 b	4.9 ± 0.3 a	5.9 ± 0.5 b	5.9 ± 0.5 b
linolenic acid	0.7 ± 0.04 a	0.5 ± 0.09 b	0.7 ± 0.1 ab	0.6 ± 0.07 a	0.7 ± 0.06 a	0.6 ± 0.1 a
arachic acid	0.2 ± 0.02 a	0.2 ± 0.02 a	0.3 ± 0.04 a	0.3 ± 0.02 a	0.3 ± 0.04 a	0.3 ± 0.04 a
eicosenoic acid	0.2 ± 0.02 a	0.2 ± 0.1 a	0.2 ± 0.04 a	0.2 ± 0.03 a	0.2 ± 0.02 a	0.2 ± 0.04 a
behenic acid	0.1 ± 0.04 a	0.1 ± 0.05 a	0.02 ± 0.02 b	0.1 ± 0.04 a	0.1 ± 0.05 a	0.02 ± 0.02 b
lignoceric acid	0.1 ± 0.01 a	0.05 ± 0.009 a	0.1 ± 0.01 a	0.1 ± 0.01 a	0.05 ± 0.009 a	0.1 ± 0.01 a

[a] Values are the means of five different VOO samples ($n = 5$) ± standard deviations. Different letters indicate significant differences ($P < 0.05$) between irrigation treatments within each year.

Irrigation affected the phenolic composition of VOO in 2004 (**Table 3**). Wide differences between the FI treatment and deficit irrigated treatments were measured in the concentration of the aglycon derivative of oleuropein 3,4-DHPEA-EDA. As for the concentrations of the derivative of demethyloleuropein (3,4-DHPEA-EA) and ligstroside (*p*-HPEA-EDA) in VOO, there were significant differences between SI trees and the other two treatments, whereas the lignans (+)-1-acetoxipinoresinol and (+)-1-pinoresinol were unaffected by the water supply. The oil from SI trees had a significantly higher concentration of 3,4-DHEPA-EA, whereas it had less *p*-HPEA than the other two treatments. The lower concentration of *p*-HPEA in SI VOO than in the other two treatments may be a consequence of decreased activity of the endogenous esterase in the olive fruit, that hydrolyzes the bond between *p*-HPEA and the elenolic acid of ligustroside.

The ratio in the VOO concentrations of 3,4-DHPEA-EDA, 3,4 DHPEA-EA, and *p*-HPEA-EDA between the SI and FI treatments of cv. Leccino was similar to those recently published for the respective treatments (FAO irrigation and rain-fed) of cv. Cornicabra at the same ripening stage (*19*). However, our results contradicted those by Patumi et al. (*10*), who concluded that irrigation only affected the concentration of the total phenols without altering the phenolic composition in VOO.

The fatty acid concentration of VOOs was only slightly influenced by irrigation, but the changes in the individual fractions depended on the growing season (**Table 4**). Significant differences in palmitoleic, oleic, linoleic, and linolenic acids in the VOO samples of 2003 appeared related to water availability, in contrast with results reported by other authors which showed an opposite behavior in cv. Arbequina (*12*). Conflicting results in fatty acid composition are, nevertheless, present in the

Table 5. Volatile Composition (μg/kg of Oil) of Virgin Olive Oils (VOOs) from Olive Trees (Cv. Leccino) Grown under Full Irrigation (FI), Deficit Irrigation (DI), or Severe Deficit Irrigation (SI) in 2003 and 2004[a]

	2003			2004		
	FI	DI	SI	FI	DI	SI
1-penten-3-one	544.9 ± 73.0 a	1003.8 ± 273.0 b	630.6 ± 108.0 a	855.8 ± 55.0 a	869.3 ± 43.0 a	786.0 ± 117.0 a
pentanal	186 ± 63.0 a	83.5 ± 27.0 a	237.9 ± 58.0 a	70.1 ± 32.0 a	117.2 ± 23.0 a	137.9 ± 49.2 a
2-pentenal	67.4 ± 13.0 a	82.0 ± 24.0 a	46.9 ± 2.2 a	88.3 ± 12.1 a	72.0 ± 5.4 a	57.5 ± 8.4 b
hexanal	1011.3 ± 160.0 a	674.3 ± 71.0 b	981.4 ± 232.2 ab	492.1 ± 94.0 a	500.1 ± 75 a	372.1 ± 52.0 a
(*E*)-2-hexenal	33850.0 ± 3499.0 a	35969.8 ± 4595.0 a	15535.9 ± 1363.0 b	33091 ± 3644.0 a	27676.3 ± 2286 b	22632.6 ± 2364.4 c
nonanal	625.1 ± 61.0 a	560.4 ± 69.0 a	1927.0 ± 113.0 b	65.3 ± 13.0 a	77.0 ± 23.3 a	71.1 ± 18.4 a
1-butanol	61.7 ± 11.0 a	71.2 ± 18.0 a	53.8 ± 9.0 a	51.8 ± 13.0 a	21.0 ± 3.6 a	20.1 ± 4.6 a
2-methyl-1-butanol	317.2 ± 31.0 a	390.8 ± 50.0 b	374.9 ± 36.0 ab	341.4 ± 43.0 a	308.9 ± 23.1 a	307.5 ± 21.6 a
1-pentanol	81.3 ± 22.0 a	36.2 ± 6.0 b	145.5 ± 3.6 c	33.9 ± 0.7 a	23.5 ± 2.4 b	27.8 ± 5.0 b
1-penten-3-ol	520.7 ± 107.0 a	728.3 ± 196.0 ab	793.3 ± 104.0 b	615.4 ± 136.1 a	769.0 ± 50.0 a	706.0 ± 124.8 a
1-hexanol	1940.2 ± 141.0 a	621.3 ± 74.0 b	800.7 ± 101.0 b	128.4 ± 18.3 a	126.7 ± 26.0 a	177.9 ± 38.3 b
(*E*)-2-hexen-1-ol	33.9 ± 5.0 a	43.3 ± 15.0 ab	53.5 ± 1.5 b	7915.7 ± 102.1 a	2519.2 ± 42.5 b	1834.0 ± 202.1 c
(*Z*)-2-hexen-1-ol	2560.2 ± 242.0 a	3204.2 ± 179.5 b	4350.2 ± 208.0 c	562.8 ± 65.2 a	648.2 ± 29.0 a	786.3 ± 114.0 b
(*E*)-3-hexen-1-ol	15.5 ± 5.0 a	14.5 ± 1.0 a	12.9 ± 0.9 a	13.4 ± 0.4 a	8.7 ± 0.4 b	9.4 ± 1.2 b
(*Z*)-3-hexen-1-ol	225.7 ± 63.0 a	341.1 ± 77.0 b	342.4 ± 34.0 b	164.9 ± 41.2 ab	202.4 ± 25.0 a	137.8 ± 21.8 b
1-hexen-3-ol	19.0 ± 7.0 a	15.2 ± 2.0 a	127.4 ± 12.0 b	11.1 ± 2.0 ab	7.7 ± 1.2 a	13.9 ± 3.5 b
hexyl acetate	13.2 ± 2.0 a	18.0 ± 1.5 a	61.8 ± 4.3 b	9.8 ± 0.6 a	9.7 ± 0.5 a	43.4 ± 1.6 b

[a] Values are the means of five different VOO samples ($n = 5$) ± standard deviation. Different letters indicate significant differences ($P < 0.05$) between irrigation treatments within each year.

literature, whereas other aspects are consistent with the data shown in **Table 4** (*10*, *11*, *33*, *36*, *37*). It should be noted that the small changes in fatty acid composition measured had neither nutritional nor marketable value.

Composition of Volatile Compounds and Sensory Analysis. **Table 5** reports the concentrations of several volatile compounds responsible for VOO flavor (*14*). The tree water status had a marked effect on the concentration of volatile compounds and, in particular, on C_6-saturated and unsaturated aldehydes, alcohols, and esters. These substances are produced via the lipoxygenase pathway during the oil mechanical extraction process, and represent the main classes of compounds responsible for "fruity", "cut-grass", and "floral" flavors. Although the response of the volatile compound concentrations depended mainly on the growing season, there were some consistent trends which could be attributed to water status. In particular, the concentrations of hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, and 1-hexen-3-ol were significantly affected by irrigation, similar to results reported by Gomez-Rico et al. (*19*). The large differences in C_6-saturated and unsaturated aldehydes and alcohols measured in our study may indicate that transient conditions of water deficit induced changes in the activities of hydroperoxide-lyase and alcohol-dehydrogenase, key enzymes in the LPO pathway.

Furthermore, the chemical composition of VOOs obtained from the DI treatment highlights an interesting combination of high phenol quantities and concentrations of volatile substances responsible for virgin olive oil aroma similar to those of VOO from FI trees.

The sensory analysis confirmed the findings with regard to volatile compounds and phenolic concentrations (**Figure 1**). In both years, DI and SI VOOs, which had higher phenolic concentrations, showed significant differences ($P < 0.05$) for "bitter" and "pungent" characteristics than FI VOOs, as also reported on other cultivars (*6*, *31*). On the other hand, differences among the three treatments for the "fruity" sensory note in VOO were significant ($P < 0.05$) only in 2003. In 2003, the "cut-grass" flavor in VOOs varied significantly ($P < 0.05$) between the SI treatment and the other two treatments. There were no significant differences for the "fruity" and the "cut-grass" attributes in 2004.

Multivariate Statistical Analysis. In **Table 6**, we reported the variables of VOO quality used for multivariate statistical analyses.

To highlight the effect of water availability, two PCA were performed on the dataset of the first and second year, separately. The PCA models therein reported explain the 62% total variance with three components, and the 64% total variance with four components respectively, and show a similar sample distribution according to the tree water status (data not shown). For both PCA models, the relative loading plots show that total phenols, *o*-diphenols, "bitter", and "pungent" are the parameters associated with VOOs obtained in SI; other substances deriving from the lypoxygenase pathway (in particular (*E*)-2-hexenal, hexanal, (*Z*)-2-hexen-1-ol, and (*E*)-3-hexen-1-ol, 1-hexen-3-ol) and sensory notes like "artichoke", "cut-grass", "floral", and "apple" are well correlated to VOOs belonging to the FI treatment. On the contrary, fatty acid compositions show lower loading values, thereby confirming the marginal effect of water applied on this chemical parameter (data not shown).

The PLS performed on the results of both years confirm the relationships between the chemical and sensory parameters of oil and the tree water status and are expressed as an average leaf water potential. The model reported in **Figure 2**, which explains 92% of the total variance with the two components, shows in the score plot a linear sample distribution and a discrimination in three clusters according to the available water level thus demonstrating a good correlation between the tree water status and the VOO analytical values.

The relative loading plot confirms a negative correlation between the phenolic concentration and the integrated water potential. At the same time, it shows that several volatile compounds, such as hexanal, (*E*)-2-hexenal, and other lipoxygenase derivative products, were positively correlated with the irrigation rate. The same loading confirms the correlation between some typical virgin olive oils' taste and olfactory sensations with their phenolic and volatile compositions (*13*, *14*). In fact, the "pungent" and "bitter" sensory notes are localized in the same loading area of the total phenols and the *o*-diphenols, whereas "cut-grass" is present near C_6 unsaturated aldehydes and alcohols. The relationship observed between the tree water status and volatile and phenolic substances confirms

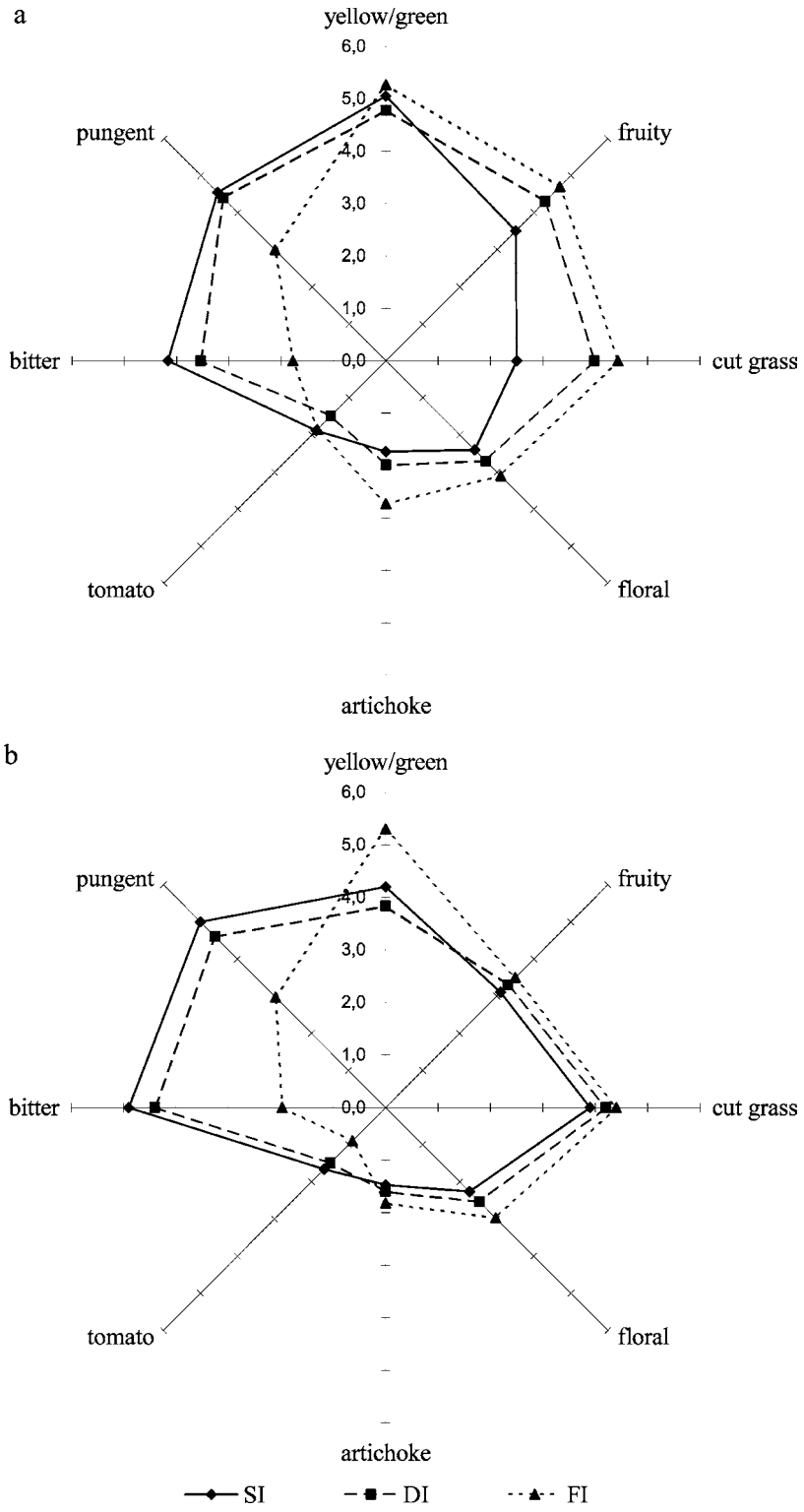

Figure 1. Sensory profile (spider plot) of virgin olive oils (VOOs) from olive trees (cv. Leccino) grown under full irrigation (FI), deficit irrigation (DI), or severe deficit irrigation (SI) in 2003 (**a**) and 2004 (**b**). Values are the means of five different VOO samples ($n = 5$).

previous results (*37*). Those authors, in fact, observed for the first time a correlation between the water stress condition through the years and several virgin olive oil minor components such as phenolic and volatile compounds of many olive growing areas (*37*).

In conclusion, deficit irrigation of olive trees appears to be beneficial not only for its well-known positive effects on water use efficiency, but also for optimizing VOO quality. The changes in the VOO phenolic and volatile compositions induced by manipulating tree water statuses during fruit development imply that irrigation can play a key role to improve the nutritional and sensory quality of VOO. Appropriate volumes of irrigation water can be used to optimize the phenolic and volatile concentrations in VOO. There is currently wide evidence documenting the importance of VOO secoridoid derivatives, like 3,4-DHPEA-EDA and *p*-HPEA-EDA, as natural antioxidants, in reducing the risks for various types of cancers (breast, oral cavity, pancreas, oesophagus, colon-rectum, prostate, and lung),

Table 6. List of Variables Used for the Multivariate Statistical Analysis

			Alcohols		
7	1-propanol[a]	32	(*Z*)-2-penten-1-ol[a]	49	2-hepten-1-ol[a]
12	2-methyl-1-propanol[a]	35	1-hexanol[a]	52	1-octanol[a]
14	3-pentanol[a]	36	(*E*)-3-hexen-1-ol[a]	54	2-butyl-1-octanol[a]
15	1-butanol[a]	37	(*Z*)-3-hexen-1-ol[a]	57	2-ethyl-1-decanol[b]
18	1-penten-3-ol[a]	39	(*E*)-2-hexen-1-ol[a]	60	2-hexyl-1-octanol[b]
20	2-methyl-1-butanol[a]	40	(*Z*)-2-hexen-1-ol[a]	63	benzyl alcohol[a]
21	3-methyl-1-butanol[a]	42	1-hexen-3-ol[a]	64	2-phenylethyl alcohol[a]
23	1-pentanol[a]	43	1-heptanol[a]	66	3-phenyl-2-propyn-1-ol[b]
30	(*E*)-2-penten-1-ol[a]	46	2-ethyl-1-hexanol[a]		
			Aldehydes		
3	pentanal[a]	26	octanal[a]	48	2,4-heptadienal (i)[b]
11	hexanal[a]	33	(*Z*)-2-heptenal[a]	51	benzaldehyde[a]
13	4-pentenal[b]	38	nonanal[a]	58	ethyl-benzaldehyde[b]
16	heptanal[a]	41	(*E*)-2-octenal[a]	59	2,6-dimethyl-benzaldehyde[b]
17	2-pentenal[a]	44	(*E*,*E*)-2,4-heptadienal[a]	62	3-phenyl-2-propenal[a]
22	(*E*)-2-hexenal[a]	47	decanal[a]		
			Ketones		
2	3-pentanone[a]	25	2-octanone[a]	50	(*E*,*E*)-3,5-octadien-2-one[b]
6	1-penten-3-one[a]	27	3-hydroxy-2-butanone[a]		
			Esters		
1	ethyl acetate[a]	31	(*Z*)-4-hexenyl acetate[b]	56	ethyl caprate[a]
24	hexyl acetate[a]	34	(*E*)-2-hexenyl acetate[a]	61	methyl salicylate[a]
29	3-hexenyl acetate[a]				
			Hydrocarbons		
4	3-ethyl-1,5-octadiene[b]			9	1,1-dimethyl-2-(2-methyl-2-propenyl)-cyclopropane[b]
5	3-ethyl-1,5-octadiene(i)[b]			10	1,1-dimethyl-2-(2-methyl-1-propenyl)-cyclopropane[b]
8	1,1-dimethyl-2-(1-methyl-2-propenyl)-cyclopropane[b]			19	limonene[a]
			Volatile Phenol		
65	phenol[a]				
			Nitrogen Compound		
28	geranyl nitrile[b]				
			Free Acids		
45	acetic acid[a]			55	formic acid[a]
53	propionic acid[a]				
			Fatty Acids		
67	myristic acid			74	linoleic acid
68	palmitic acid			75	arachic acid
69	palmitoleic acid			76	linolenic acid
70	margaric acid			77	eicosenoic acid
71	heptadecenoic acid			78	behenic acid
72	stearic acid			79	lignoceric acid
73	oleic acid				
			Merceological Indexes		
80	acidity			82	total phenols
81	peroxide value			83	*o*-diphenols
			Sensorial Notes		
84	yellow-green			89	floral
85	fruity			90	tomato
86	cut-grass			91	bitter
87	artichoke			92	pungent
88	apple				
			Dependent Variable		
93	integrated idric potential				

[a] Identified by comparison with standard compounds. [b] Tentatively identified by comparison with the Wiley 6 mass spectra library.

protecting against chronic degenerative diseases, inducing apoptosis in tumor cell-lines (*13*, *38*), and preventing cardiovascular diseases in humans (*39*).

In addition, optimization of the sensory characteristics of VOO appears critical for meeting consumers' demands and marketing strategies. Although responses of different cultivars (Leccino, Arbequina, Cornicabra, Kalamata) to irrigation were fairly consistent among studies, it is likely that specific RDI protocols will have to be developed or adjusted for different cultivars grown under certain climatic and soil conditions. Our results are further evidence of direct effects of deficit irrigation regimes on qualitative parameters of olive oil and, specifically, volatile compounds and the phenolic composition in VOO.

ABBREVIATIONS USED

VOO, virgin olive oil; PLWP predawn leaf water potential; FI, full irrigation; DI, deficit irrigation; SI, severe deficit

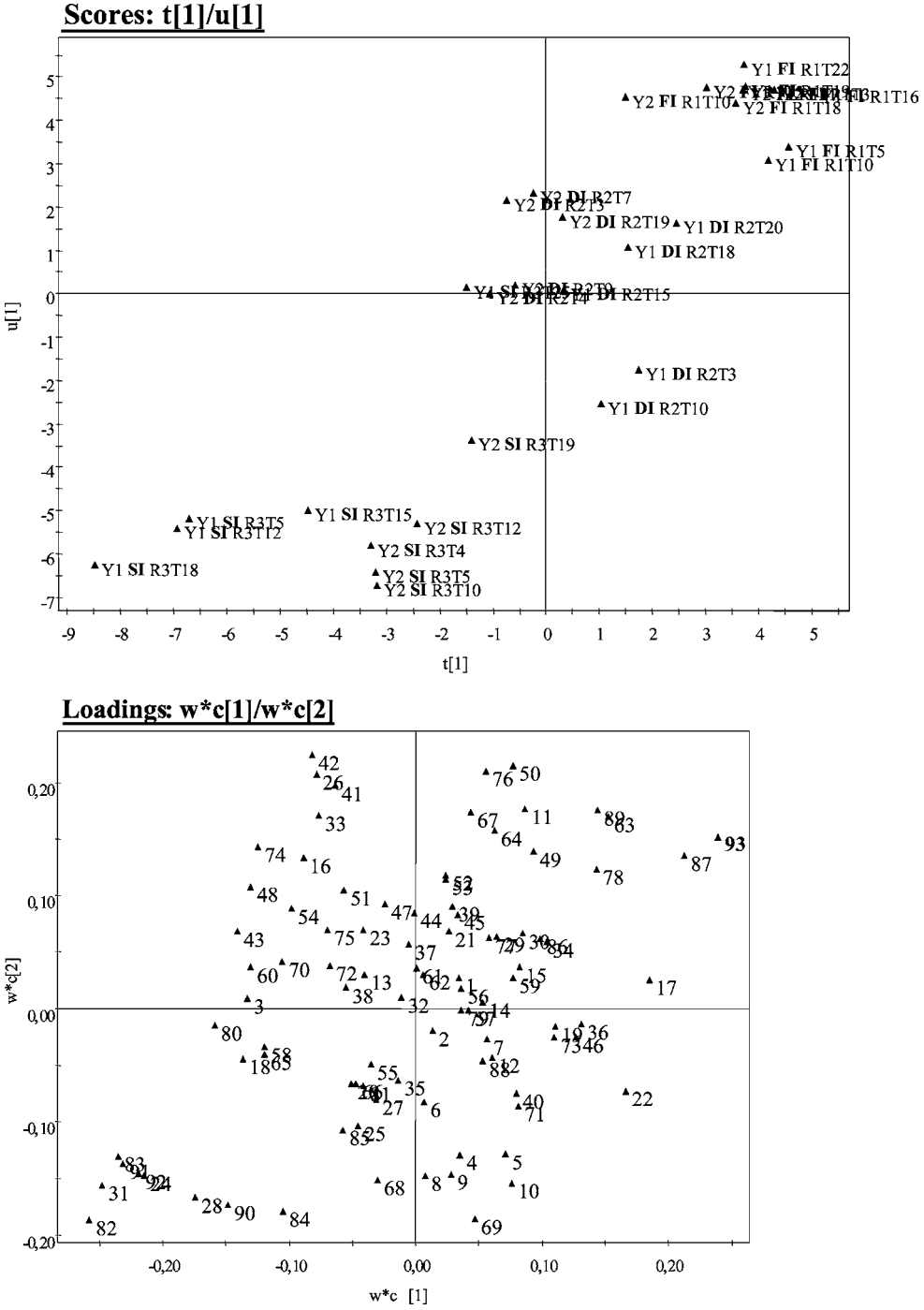

Figure 2. PLS model of virgin olive oils (VOOs) from olive trees (cv. Leccino) grown under full irrigation (FI), deficit irrigation (DI), or severe deficit irrigation (SI) during years (Y) 2003 (1) and 2004 (2). Total explained variance of $Y = 92\%$ after two components: PC 1 = 76%; PC 2 = 16%. Variance 65% with four components: PC 1 = 34%; PC 2 = 16%; PC 3 = 9%; PC 4 = 6%. Legend: R = row. T = tree.

irrigation; HPLC, high-performance liquid chromatography; 3,4-DHPEA-EDA, dialdehydic form of decarboxymethyl elenolic acid linked to (3,4-dihydroxyphenyl)ethanol; 3,4-DHPEA-EA, isomer of the oleuropein aglycon; *p*-HPEA-EDA, dialdehydic form of decarboxymethyl elenolic acid linked to (*p*-hydroxyphenyl)ethanol; 3,4-DHPEA, (3,4-dihydroxyphenyl)ethanol; *p*-HPEA, (*p*-hydroxyphenyl)ethanol; RDI, regulated deficit irrigation; AFB, after full bloom; TCSA, trunk cross sectional area; MI maturation index; DAD, diode array detector; FLD, fluorescence detector; HS-SPME, head space solid phase microextraction; EI, electron ionization; GC/MS, gas chromatography with mass spectrometer; LSD, least significant differences; PCA, principal component analysis; PLS, partial least squares regression.

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