Virgin olive oil quality of hedgerow 'Arbequina' olive trees under deficit irrigation

Running title: Oil quality from deficit irrigated olive hedgerow

José M. García^{a,*}, Ana Morales-Sillero^b, Ana G. Pérez-Rubio^a, Antonio Díaz-Espejo^c, Antonio Montero^c, José E. Fernández^c

^a*Instituto de la Grasa (IG-CSIC) Campus Universidad Pablo de Olavide, Edificio 46, Carretera Sevilla-Utrera km 1, 41013-Sevilla, Spain*

^b*Departamento de Ciencias Agroforestales, ETSIA, Universidad de Sevilla, Carretera de Utrera km 1, 41013 Sevilla, Spain*

^c*Instituto de Recursos Naturales y Agrobiología (IRNAS, CSIC), Avenida de Reina Mercedes, n.º 10, 41012-Sevilla, Spain*

* Corresponding author. Tel.: +34 954 611550 (ext. 226); fax: +34 954 616790.

E-mail address: jmgarcia@cica.es

Abstract

BACKGROUND: Regulated deficit irrigation (RDI) is used in hedgerow olive orchards to achieve a sustainable balance between water savings, tree vigor and oil production. Its effects on the presence of compounds responsible for the taste of the oil and its nutritional value are controversial. This three years'-work was made in an 'Arbequina' orchard (1667 trees ha⁻¹) under a full irrigation (FI) treatment (470.1 mm year⁻¹ of water) and two RDI treatments scaled to replacing 60% and 30%, respectively, of FI. The quality parameters, antioxidant contents and volatiles of the virgin olive oils (VOO) extracted were analyzed.

RESULTS: In general, 30RDI oils had greater contents in pigments and phenolic compounds, a higher oleic/linoleic ratio and the highest oxidative stability, in spite of their lower tocopherol contents. FI oils showed higher (E)-2-hexenal, 1-penten-3-one, Ocimene, E-2-pentenol and pentene dimer contents than 30RDI oils, but lower contents in (E)-2-pentenol, and volatile esters.

CONCLUSIONS: The results suggest that a RDI strategy supplying 30% of the total irrigation needs induces an increase in natural antioxidants in VOO. **Neither yield nor** the rest of quality parameters were affected by the reduced irrigation. However, abundant autumn precipitation can override these effects of 30RDI on oil quality.

Keywords: Acidity, natural antioxidants, oxidative alterations, sensory quality, stability, super-high density.

INTRODUCTION

The olive tree is drought-resistant and well adapted to rain-fed farming. Still, well managed irrigation supplies have a significant impact on fruit production¹. The effect of irrigation on the production of virgin olive oil (VOO), however, is not clear. Ramos and Santos² found no significant differences in fruit and VOO production between fully irrigated (FI) and rain-fed ‘Cordovil’ trees. The physical oil extractability decreases with water content and the benefit of the increase in fruit production obtained with irrigation could be lost due to a decrease in oil extractability³. The effect of irrigation level on VOO quality is even more controversial. Most studies have shown that tree water status has no effect on the commercial quality of VOOs⁴⁻⁵. Nevertheless, Ben-Gal et al.⁶ observed, in ‘Souri’ and ‘Barnea’ olives, that the oil acidity increased as a function of increasing irrigation. While Motilva et al.⁷ reported no effect of the irrigation level on the fatty acid composition of the VOO extracted from ‘Arbequina’ olives, Berenguer et al.⁸ found that reduced irrigation led to an increase in the monounsaturated / polyunsaturated ratio in the VOO obtained from trees of the same variety. Concerning the effect of irrigation on phenolic compounds, most findings show a reduction in phenolic compounds with irrigation for different olive varieties.^{1,9} However, Greven et al.¹⁰ reported that drought induced a decrease in the phenol content of the ‘Australian Verdale’ oil.

The lack of consistent results on the effect of irrigation on the production and quality of VOO is particularly evident for hedgerow orchards with plant densities ≥ 1500 trees ha⁻¹, also called super-high-density (SHD) orchards. SHD is considered among the most profitable orchard management systems for olives.¹¹

Regulated deficit irrigation (RDI) seems to be the most advantageous for SHD olive orchards. RDI strategies are designed for replacing a crop’s water needs during the

phases of the growing cycle when the crop is more sensitive to water stress, and reducing or even withholding irrigation for the rest of the cycle. Thus, events of excessive water stress could be better avoided with RDI than with another deficit irrigation (DI) strategy such as sustained deficit irrigation (SDI), which supplies a fixed fraction of the crop's water needs throughout the irrigation season⁸. The success of RDI in SHD olive orchards depends both on the suitability of the chosen RDI strategy to the orchard conditions and on a precise monitoring of water stress. In addition, RDI seems to favour VOO quality in SHD orchards. It has been reported that the oil extracted from RDI 'Arbequina' olives of SHD orchards had higher oxidative stability and phenol contents than oils from FI, without a detrimental effect on oil production.¹² Fernandez et al¹³ tested a FI treatment and two RDI treatments with the same RDI strategy but with different irrigation levels, replacing 60% and 30%, respectively, of the water supplied in FI. The RDI strategy consisted of keeping the trees close to FI conditions at bloom, at the beginning of pit hardening and in the 2-3 week period prior to ripening, moments identified as highly sensitive for water stress¹⁴. Fernandez et al¹³ evaluated the effect of the three irrigation treatments on water status and gas exchange, leaf area development and tree production. They did not report, however, any data on VOO quality. The aim of this work was to evaluate the effect of the RDI strategy and levels of irrigation used by Fernández et al.¹² on the quality of the obtained VOO.

EXPERIMENTAL

Orchard characteristics

The experiments were carried out in a SHD olive orchard (*Olea europaea* L., cv. Arbequina) in south-west Spain (37° 15' N, -5° 48' W). Trees were planted in 2006, in rows N-NE to S-SW oriented, and at 4 m × 1.5 m (1667 trees ha⁻¹). The study was made

for three seasons (2010 to 2012). Values of FAO-56 Penman-Monteith potential evapotranspiration (ET_o) were calculated from data provided by a nearby standard weather station belonging to the Agroclimatic Information Network of the Junta de Andalucía. for the three year experimental years and irrigation periods, respectively, ET_o values were: 1465 mm and 997.5 mm for 2010; 1473.6 and 796.4 mm for 201; and 1598.2 and 914.8 mm for 2012. The values of precipitation were: 912 and 119 mm for 2010; 477.0 and 55.0 for 201; and 404.6 and 86.0 mm for 2012. A randomized block design with four 12 m $\times$ 16 m plots per treatment was used for the treatments. Each plot contained 8 central trees surrounded by 24 border trees. All samples were taken from the central trees only. This applied to all years and treatments, except for 2010, when we had one single plot for the FI treatment, as detailed in Fernández et al.¹³

Irrigation treatments

A FI treatment and two RDI treatments were applied. The irrigation needs (IN) for the FI treatment were calculated as $IN = ET_c - P_e$, with ET_c as the maximum potential crop evapo-transpiration calculated with the crop coefficient approach and P_e the effective precipitation calculated as 75% of the precipitation recorded in the orchard. The data shown in Fernández et al.¹³ reveal that the irrigation amounts (IA) actually supplied to the FI trees were very close to IN and that the water status and gas exchange of those trees corresponded to those normally recorded in the area for olive trees growing under non-limiting soil water conditions throughout the dry season. The applied RDI strategy considered three periods in which the trees are more sensitive to drought. Period 1 lasts from the end of floral development to full bloom. In our area this occurs in mid-April, when rainfall is usually sufficient to replace the crop water needs. Period 2 is the 2-3 weeks prior to massive pit hardening, which normally occurs from

ca. 6 to 10 weeks after full bloom. Period 3 corresponds to the period of the fastest oil synthesis, just before that the most of fruit achieves its final size and ripening begins (green-mature stage). In our zone this happens from late August to mid-September, just before the beginning of the rainy season. From here to harvesting (end of October) irrigation supplies vary greatly, depending on rainfall. This RDI strategy was applied with two levels of irrigation to replace 60% (60RDI) and 30% (30RDI) of the IN calculated for the FI treatment. Irrigation was not needed in Period 1 for any of the three tested seasons because rainfall was sufficient to replace the crop's water needs. In Period 2 irrigation was applied daily to replace 80% of IN in 60RDI and 60% of IN in 30RDI. Between Periods 2 and 3 we irrigated twice a week in 60RDI and once a week in 30RDI. In Period 3 the 60RDI trees were irrigated daily to replace IN and the 30RDI trees were irrigated twice a week only, receiving a total of 30% of IN. A drip line per tree row with a 2 L h^{-1} dripper every 0.5 m was used. Every Monday of the irrigation season, IN was calculated with the crop coefficient approach and the time and frequency of irrigation were input in an irrigation controller (Agronic 2000, SistemesElectrònics PROGRÉS, S.A., Lleida, Spain), which activated the pump and electro-valves. An adequate amount of fertilizer was injected into the irrigation system once a week to cover the plant's need.¹³ The IA supplied to the FI, 60RDI and 30RDI treatments, in that order, were: 502.0, 308.0 and 148.0 mm in 2010; 477.2, 288.3 and 127.3 mm in 2011; and 478.7, 291.3 and 134.6 mm in 2012.

Oil extraction parameters

Harvesting was done by hand on 2 November, 2010, 26 October, 2011 and 13 November, 2012. About 2 kg fruits were harvested from each experimental plot and transported the same day to the laboratory, where the oil was extracted with an Abencor

analyzer (ComercialAbengoa S.A., Seville, Spain).³ Samples of 2000 g were individually milled from each replicate, malaxated for 30 min and the oil was separated by centrifugation. The VOO amount physically extracted from the olive fresh weight was evaluated in g kg⁻¹, measuring the resulting volume of oil in a graduated cylinder (VOO density 0.915 kg L⁻¹). The VOO was then filtered and stored at -20 °C under N₂.

From the paste of each replicate a 60 g sample was dried at 105 °C up to constant weight to measure the fruit water content. Subsequently, the total oil content of the dried paste was calculated, using the Soxhlet method with hexane and expressed as g kg⁻¹ of fresh weight. The total synthesized oil was estimated considering the g kg⁻¹ of dry weight.

Oil quality

The legally established quality parameters of the VOO were analyzed following the European Union Standard Methods.¹⁵ A panel of eight trained tasters evaluated the intensities of the VOO sensory attributes, using a 10 cm line scale and thus converted into a continuous value by measuring the position of the placed mark along the line. Final results were the medians among the tasters' sensory scores. The overall sensory quality of each oil sample was also graded by the same panel from 1 (poorest possible) to 9 (best possible). The oils that presented at least one negative attribute (rancid, fusty, winey, musty, etc.) could not be assessed above 6.5, the limit established for the best commercial category ("Extra").

The oxidative stability was estimated according to Laubli and Bruttel¹⁶. The content of carotenoids and chlorophylls in the oil samples was measured by their absorbance at 470 and 670 nm, respectively.¹⁷ The tocopherol content of the oil samples was measured according to IUPAC.¹⁸ The phenolic fraction was isolated and its

composition analyzed according to Mateos et al.¹⁹ The composition of fatty acids was evaluated following the method described by García et al.³ The data found for the acids myristic, margaric, margaroleic, and gadoleic were too small ($\leq 1 \text{ g kg}^{-1}$) to have any significant effect on the oil quality and are not shown.

Aroma analysis

The aroma of the oils extracted in 2012 was also studied following the same method previously described by Morales-Sillero et al.²⁰

Statistical analysis

All the statistical analyses were carried out with the SPSS Statistics 17.0 software. Analyses of variance (one way-ANOVA $p \leq 0.05$) were performed on the data after evaluating the homogeneity of variance with the test of Levene ($p \leq 0.05$). In the case of non-homogeneity, the non-parametric test of Kruskal-Wallis was used ($p \leq 0.05$). When an effect due to the irrigation treatments was detected by ANOVA, Tukey's test was used to discriminate among mean values ($p \leq 0.05$).

RESULTS

Oil synthesis and extraction

As expected, fruit water content increased with IA (Table 1). Abnormally high rainfall was recorded in the autumn of 2012 (218.8 mm from 26 September to 12 November; in 2010 and 2011 the collected amounts for that period were 66.0 and 57.0 mm, respectively), which explains the greater water contents in the fruits of all treatments. As compared with previous years, the increase was greater in the 30RDI fruits. Both in 2010 and 2011, greater oil contents, on a fresh weight basis, were

recorded in the less-irrigated treatments. In 2012 we found low oil contents in all treatments, with no differences among them.

Different trends were found when the total oil content in the fruits was calculated as dry weight (Table 1), which depends on the actual oil synthesis in the fruit. No differences between treatments for that parameter were observed in 2010. In 2011 the influence of irrigation was not clear since the highest total oil contents were found in the fruits from 30RDI, followed by FI and 60RDI, respectively. In 2012, no differences were found between the two RDI treatments, and the greatest values were presented by the FI fruits.

The VOO yield indicates the amount of oil physically extracted in relation to the fruit fresh weight. VOO yield values showed a similar trend to those for total oil content (Table 1). Both in 2010 and 2011, the greatest VOO yields were obtained from 30RDI fruits. In 2012 no differences between treatments were observed, and the VOO yield values were lower than in the two previous years, with the decrease being especially notable for the 30RDI treatment.

Parameters of VOO commercial quality

The commercial quality of the extracted oils was hardly affected by the level of irrigation (Table 2). All parameters showed values within the limits established for the top “Extra” category. In 2010 and 2011 contradictory differences between treatments on peroxide value (PV) were found, whereas in 2012 differences among treatments were not significant. Concerning the K_{232} results, only differences between treatments were observed in 2011. This year K_{232} values were higher in the oils extracted from FI fruits than in the 30RDI oils. The same occurred with the PVs. This fact is not surprising since both parameters are related to the initial oxidation of the oil. For the rest of the

evaluated parameters, i.e. advanced oxidation (K_{270}), hydrolytic alteration (acidity) and sensory deterioration (panel test) no influence of irrigation was found.

Natural antioxidant content and oxidative stability

The oils extracted from 30RDI fruits showed higher carotenoid contents than the FI and 60RDI oils in the three tested seasons (Table 3). It means that the content of this antioxidant in VOO is associated with the lowest level of irrigation. Differences between FI and 60RDI, however, were not appreciable.

Chlorophyll contents presented a similar behaviour than those of carotenoids in the two last seasons tested. However, in 2010 FI oils showed the highest value of this parameter.

The tocopherol content of the oils showed an inverse response to irrigation compared to that of the pigments both in 2010 and 2011, when the contents of tocopherols in VOO increased with irrigation (Table 3). However, in 2012 the lowest contents were found in FI oils, perhaps as a consequence of the autumn rainfall.

The 30RDI oils showed the highest amounts of orthodiphenols, secoiridoid derivatives and total phenols in comparison with FI and 60RDI in the three years tested (table 3). On the other hand, the comparison between the contents in the main phenolic groups of the 60 RDI and FI oils offered different results for each season. While in 2010 60 RDI oils showed higher phenolic content than those from FI, in 2011 there were not observed significant differences between the contents of any of these phenolic groups of both oils and in 2012 FI oils presented, even, higher content of total phenols than the 60RDI ones.

The response to irrigation in the oxidative stability of the oils was more akin to the rather consistent behaviour displayed by the main phenolic derivatives and

carotenoids than to the variable results displayed by tocopherols (Table 3). Thus, the 30RDI and FI VOOs had the highest and the lowest values of this parameter, respectively, for the three tested seasons; whereas the 60RDI oils showed values progressively closer to those of the FI oils.

Phenolic composition

In 2010, most of the phenols decreased with irrigation (Table 4). The concentrations in vanillic acid, hydroxytyrosol-acetate, the dialdehydic form of the decarboxymethyl-oleuropein-aglycone (3,4-DHPEA-EDA), tyrosol-acetate, the dialdehydic form of the decarboxymethyl-ligstroside-aglycone (*p*-HPEA-EDA), hydroxyl-tyrosyl-elenolate (3,4-DHPEA-EA), tyrosyl-elenolate (*p*-HPEA-EA) and ferulic acid were greater in the 30RDI oils than in the FI ones; whereas the 60 RDI oils showed intermediate values. In contrast, this year the concentrations in tyrosol increased with the increase in the irrigation level; whereas the highest concentration in acetoxypinoresinol was found in the 60RDI oils. In 2011 the concentrations of the different phenolic molecules in the oils decreased regardless of the irrigation treatment. Besides, the concentrations of several molecules were also affected by the different irrigation levels. Thus, only the concentrations in the secoiridoid derivatives (3,4-DHPEA-EDA, *p*-HPEA-EDA, 3,4-DHPEA-EA and *p*-HPEA-EA) and of the *p*-coumaric acid in the 30RDI oils were significantly higher than those of the FI oils. Finally, in 2012 the majority of the phenols presented again a reduction and the response to different irrigation levels was less uniform than those of the previous seasons. Whereas 3,4-DHPEA-EDA, *p*-HPEA-EDA and 3,4-DHPEA-EA concentrations in the 30RDI oils continued to be significantly higher than those of the FI and 60RDI oils, the contents in

acetoxypinoresinol and ferulic acid exhibited an inverse effect, showing the lowest values in 30RDI oils.

Fatty acid composition

The response of the fatty acid composition in the oils from the irrigation treatments varied with the season (Table 5). While in 2010 and 2012 irrigation led to an increase in linoleic acid and a reduction in the oleic concentration; in 2011 the concentrations of these compounds were not affected by irrigation. Thus, compared to the FI oil, both in 2010 and 2012 the 30RDI oil showed a higher oleic/linoleic ratio, a greater concentration in total monounsaturated fatty acids and a lower concentration in total polyunsaturated; whereas 60RDI oils showed intermediate values.

Volatile composition

Table 6 shows the effect of the irrigation treatments on the main volatile components of the aroma profile of VOO. The presence of most volatile compounds depended on the treatment. (E)-2-hexenal was the predominant volatile component in the analyzed oils, representing 70% of the total aroma. This volatile compound, formed from linolenic acid (LNA) through the lipoxygenase (LOX) pathway, has been associated with the green-grassy smell. The content of (E)-2-hexenal was significantly higher in the FI oils than in the RDI oils, and no differences between 30RDI and 60RDI were found. Furthermore, the FI oils developed higher contents in the different volatiles related to this flavor (1-penten-3-one, Ocimene and E-2-pentenal) and systematically presented the highest content of pentene dimers. In contrast, 30RDI oils exhibited significantly higher contents in (E)-2-pentenol, and volatile esters. Volatile esters, which are important constituents of the aroma of many fruits, are minor components of

the aroma of oils from most olive cultivars. The VOO ester fraction is formed mainly by acetate esters with alcohol moieties synthesized through the LOX pathway, such as hexan-1-ol, (E)-hex-2-enol, and (Z)-hex-3-enol.

DISCUSSION

The total oil content in the fruit refers to the oil amount that is expected to be extracted in the industry, which includes the VOO extracted physically and the oil chemically extracted from the olive pomace. Ben Gal et al.⁶ reported that oil contents in ‘Barnea’ and ‘Souri’ fruits decreased when IA increased. Here, this factor **also** outweighs the greater fruit production obtained by the increase in IA, decreasing the differences in total oil yield between the treatments.¹³

The variability among seasons (Table 1) suggests that the oil synthesis was influenced by variables other than the level of irrigation. Stefanoudaki et al.²¹ also reported a lack of consistency between two consecutive seasons in oil synthesis versus water supply for ‘Koroneiki’ olives. It is known that the availability of water in the soil negatively affects virgin oil extraction.³ The peculiar results on VOO yield obtained in 2012 were likely due to high fruit water contents. These results confirm that the high water content in the fruit reduces oil extraction and discourages harvesting immediately after abundant watering or rain.

Many authors found a lack of influence of irrigation supplies on VOO quality.^{3,4,5,7} However, an increase in PV or K₂₃₂ with increasing irrigation has been widely reported.^{9,21,22} This effect was not observed in all the studied seasons. The milled olive paste from the most watered fruits is likely to have developed a higher lipoxigenase activity during the process of VOO extraction that would have led to an

increase in hydroperoxides in the extracted oil and, consequently, to higher PV and K₂₃₂ values.

A reduction in the positive attributes of the VOO such as its bitter or pungent characteristic is frequently associated with increasing irrigation.^{8,21,22} Nevertheless, these results agree with those of Ramos and Santos² for ‘Cordovil’ and García et al.³ for ‘Arbequina’, who did not find significant differences in the evaluation of the VOO sensory quality due to irrigation. Gómez-del-Campo and García¹² found that the oils extracted from hedgerow olives grown under DI conditions (30% ET_c) in July were more bitter than those extracted from FI olives or from olives grown under the same DI conditions but applied in August, or fruits grown under DI conditions (50% ET_c) in July and August. It must be noted that the two latest treatments received the same and less amount of water, respectively, than the first one. It seems, therefore, that the greater presence of positive sensory attributes in the oil occurs after certain threshold values of stress at precise stages of fruit development are achieved. Both timing and intensity of the stress events could be conditioned by environmental or genetic factors that make it difficult for repetitive results.

Tovar et al.²³ found that pigments were negatively associated with the amount of water supplied to ‘Arbequina’ trees. More recently, García et al.³ found that reduced irrigation increased the content of these pigments in VOO as compared to FI. This agrees with the results found in the 30RDI oils. The lack of this effect in the 60RDI trees could have been due to those trees not having reached the threshold value of water stress required to induce an increase in those parameters.

The literature shows contrasting results for the response of the tocopherol content in VOO to irrigation. Thus, Stefanoudakiet al.²¹ found higher contents of this antioxidant in the oils extracted from rain-fed ‘Koroneiki’ olives than in those extracted

from irrigated trees. In contrast, Baccouri et al.²⁴ had previously observed a significant increase in these contents associated with high irrigation in ‘Marsalina’ olives, whereas Palese et al.²⁵ did not find any effect in the oils from ‘Coratina’ trees under different irrigation systems and Tovar et al.²³ reported opposite results between two consecutive seasons. The results obtained here suggest that the increasing of irrigation and of the consequent fruit water content would induce the increase of these compounds in VOO, but an excess of humidity, such as the one caused by the precipitation in autumn of 2012, could induce its reduction, especially in the fruits that had previously been more watered.

Decreasing contents in the main phenolic molecules in VOO with increasing water supplies have been widely reported, regardless of the variety, location, tree density, or irrigation strategy.^{1,9} However, some authors have reported different or even opposite results. Baccouri et al.²⁴ and Dabbou et al.²⁶ found higher phenol contents in the VOO extracted from the most irrigated fruits. Others reported no effect,⁷ variable effects among years²⁵, or a dependency of the observed effect on the growing area.²⁷ Therefore, it must be assumed that an increase in irrigation does not always decrease the phenolic content in VOO, although in our paper it did. The high precipitation recorded in the autumn of 2012 was especially negative for the RDI treatments, in addition to reducing the extraction of VOO, coincided with a substantial loss of the different main groups of phenolic compounds. An elevated water content of the fruit would induce that the released phenolic molecules dissolve preferentially in the vegetative water and, to a lesser extent, in the VOO during the process of oil extraction.

It is known that phenols are the minor compounds which are most directly correlated with the oxidative stability of VOO.²³ Berenguer et al.⁸ observed that both of them decreased with an increase in water supply. The differences were greater in the

first season and for trees with the lowest irrigation. In the following season, when all trees were irrigated more heavily, few differences between treatments were found. The 2011 results show that the oxidative stability of VOO decreased in all the treatments compared to 2010, in agreement with decreases in all the different main phenolic groups observed. In 2012, however, and despite lower phenolic contents, the values of oxidative stability increased up to similar levels to those observed in 2010. According to Aparicio et al.²⁸, the VOO oxidative stability might depend on some synergistic effects among fatty acid composition, phenol, tocopherol, carotenoid and chlorophyll contents, although such effects have not been identified.

The reduction in the secoiridoids derived from oleuropein and ligstroside in VOO is considered a main effect induced by increasing irrigation.^{1,4,9,29} However, this effect is not linear and must be associated with other factors of cultivation. Romero et al.³⁰ found no significant differences between the content in these derivatives of the VOOs extracted from ‘Arbequina’ olives irrigated with 471 or 239 mm/yr of water, but the ones extracted from olives irrigated with 114 mm/yr showed increases in these compounds. This fact supports our hypothesis of a threshold of water stress being required for inducing an increase in these molecules, strongly related with the sensory quality and the oxidative stability of the VOO.²⁸ The rest of phenolic molecules usually showed a more variable response to irrigation. Thus, Tovar et al.²⁹ observed that hydroxytyrosol, tyrosol, vanillic acid and *p*-coumaric acid contents in the oils were not affected by increasing irrigation. In addition, the concentrations of lignans (acetoxypinoresinol and pinoresinol) and vanillin were even lower in the oils from the least irrigated treatment. Servili et al.⁴, however, did not find any effect of irrigation on the lignan contents in ‘Leccino’ oils. More recently, García et al.³ found that the contents in vanillic acid, vanillin, *p*-coumaric acid, tyrosol-acetate, pinoresinol, cinamic acid,

acetoxypinoresinol, luteolin and apigenin were not affected by the level of irrigation in ‘Arbequina’ oils and Caruso et al.⁹ observed that the concentrations of lignans did not significantly change in ‘Frantoio’ oils due to the different irrigation regimes applied.

The high ratio found in the 30RDI oils explains their high oxidative stability in 2012, a year in which the phenol contents decreased. A variable response of the fatty acid composition to irrigation depending on the season is often reported. Berenguer et al.⁸ and Gomez-Rico et al.¹ only reported higher oleic/linoleic ratio in the oils from trees that received low volumes of water than in those from FI trees in the first of the two seasons. Ben Gal et al.⁶ explained the alternate response of the fatty acid composition of the oil to irrigation as a consequence of the biannual cycle of the olive, which alternates high and low crop loads. They found that the monounsaturated/polyunsaturated ratio decreased with irrigation in 'Barnea' oil in the “off” year and in 'Souri' oil in the “on” year. The results of the present paper, however, show an alternate behavior of the fatty acid composition despite an increasing crop load year after year.¹³ Issaoui et al.²⁷ found different responses to irrigation in the fatty acid composition of ‘Chétoui’ depending on location. Caruso et al.⁹ hypothesized that in relatively cool climates water stress has little effect on fatty acid composition, but in hot areas or hot years, abundant water supply increases linoleic acid and decreases the oleic acid percentage. The fatty acid composition of the olive oil depends on the fatty acid and triacylglycerol biosynthesis pathways, which includes multiple types of enzymes (transacylases, dehydrases, reductases, synthetases, elongases and desaturases). The enzymes usually act from the beginning of pit hardening to the end of fruit maturation.³¹ Thus, the fatty acid composition of the oil is not the result of the activity of some determined enzymes exclusively during the VOO extraction, but of the continuous work of multiple enzymes during fruit maturation. As the level of irrigation has a great impact on the fruit

physiology, we could assume that water stress can affect the fatty acid desaturation. Consequently, reduced irrigation will lead to oils with an elevated oleic/linoleic ratio. Nevertheless, factors such as the timing and level of water stress, air temperature and radiation interception during oil biosynthesis could interfere in these metabolic pathways, making the assessment of final values for this parameter difficult.

The presence of (E)-2-hexenal indicates that the oil was extracted from olives at a low ripening stage.³² The alcohols synthesized through the LOX pathway, especially the unsaturated alcohols and their corresponding esters, are grouped among those VOO aroma compounds responsible for green-fruity perceptions and in the boundaries of the sweet perception according to canonical correlations reported by Aparicio et al.³³ Given that some of these compounds are among the most potent odorants in VOO, characterized by having high odor activity values,³⁴ the increase in LOX-esters may have a great impact on the aroma of 30RDI virgin olive oils. The higher presence of these compounds in 30RDI oils offsets the higher content in E-2-hexenal of the FI oils. The fact that both types of compounds are responsible for positive sensory attributes could explain why the test panel did not appreciate differences in the overall grading of the sensory quality of both VOOs.

CONCLUSIONS

The 30RDI strategy improved physical oil extraction and led to VOO with higher contents in phenolic compounds, i.e. increased oxidative stability, more photosynthetic pigments, and a greater oleic / linoleic ratio. In any case, the results suggest that a deficit irrigation supplying less than 60% of irrigation needs induced an increase in natural antioxidant in VOO, without deteriorating the parameters used to evaluate the commercial quality level. However, an excess of autumn precipitation can

override these effects, as it occurred in 2012. By comparing our results with those concerning production and water productivity reported by Fernández et al.¹³ from the same experimental set up, the hedgerow olive orchard farmers will have useful information to decide on a suitable irrigation approach to achieve their production target, aimed either at increasing VOO quantity or VOO quality, or to achieve the best balance between them.

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REFERENCES

1. Gomez-Rico A, Salvador MD, Moriana A, Pérez D, Olmedilla N, Ribas F and Fregapane G. Influence of different irrigation strategies in a traditional Cornicabra cv. olive orchard on virgin olive oil composition and quality. *Food Chem***100**, 568–578. (2007).
2. Ramos AF and Santos FL. Yield and olive oil characteristics of a low-density orchard (cv. Cordovil) subjected to different irrigation regimes. *Agr Water Manage***97**, 363–373. (2010).

3. García JM, Cuevas MV and Fernández JE. Production and oil quality in ‘Arbequina’ olive (*Olea europaea*, L.) trees under two deficit irrigation strategies. *Irrigation Sci***31**, 359–370.(2013).
4. Servili M, Esposto S, Lodolini E, Selvaggini R, Taticchi A, Urbani S, Montedoro G, Serravalle M, and Gucci R. Irrigation effects on quality, phenolic composition, and selected volatiles of virgin olive oils cv. Leccino. *J Agr Food Chem***55**, 6609-6618.(2007).
5. Tognetti R, D’Andria R, Sacchi R, Lavini A, Morelli G, and Alvino A. Deficit irrigation affects seasonal changes in leaf physiology and oil quality of *Olea europaea* (cultivars Frantoio and Leccino). *Ann Appl Biol* **150**, 169–186. (2007).
6. Ben-Gal A, Yermiyahu U, Zipori I, Presnov E, Hanoch E and Dag A. The influence of bearing cycles on olive oil production response to irrigation. *Irrigation Sci* **29**, 253-256. (2011).
7. Motilva MJ, Tovar MJ, Romero MP, Alegre S and Girona J. Influence of regulated deficit irrigation strategies applied to olive trees (Arbequina cultivar) on oil yield and oil composition during the fruit ripening period. *J Sci Food Agr***80**, 2037-2043. (2000).
8. Berenguer MJ, Vossen PM, Grattan SR, Connell JH, and Polito VS. Tree irrigation levels for optimum chemical and sensory properties of olive oil. *HorticSci***41**, 427-432. (2006).
9. Caruso G, Gucci R, Urbani S., Esposto S, Taticchi A., Di Maio I., Selvaggini R, and Servili M. Effect of different irrigation volumes during fruit development on quality of virgin olive oil of cv. Frantoio. *Agr Water Manage***134**, 94–103. (2014).

10. Greven M, Neal S, Green S, Dichio B and Clothier B. The effects of drought on the water use, fruit development and oil yield from young olive trees. *Agr Water Manage* **96**, 1525–1531. (2009).
11. Connor DJ. Towards optimal designs for hedgerow olive orchards. *Aust J Agr Res* **57**, 1067–1072. (2006).
12. Gómez del Campo M and García JM. Summer deficit-irrigation strategies in a hedgerow olive cv. Arbequina orchard: Effect on oil quality. *J Agr Food Chem* **61**, 8899–8905. (2013).
13. Fernández JE, Perez-Martin A, Torres-Ruiz JM, Cuevas MV, Rodriguez-Dominguez CM, Elsayed-Farag S, Morales-Sillero A, García-Martos JM, Hernández-Santana V and Díaz-Espejo A. A regulated deficit irrigation strategy for hedgerow olive orchards with high plant density. *Plant Soil*, **372**, 279–295. (2013).
14. Moriana A, Orgaz F, Pastor M, and Fereres E. Yield responses of a mature olive orchard to water deficits. *J Am Soc Hort Sci* **128**, 425–431. (2003).
15. European Union Standard Methods. Annexes II and IX in Official Journal European Communities n. L. 248 of 5 September, Regulation EEC/2568/91. (1991).
16. Laübli W and Bruttel PA. Determination of the oxidative stability of fats and oils by the ‘Rancimat’ method. *J Am Oil Chem Soc* **63**, 792–794. (1986).
17. Mínguez-Mosquera MI, Rejano-Navarro L, Gándul-Rojas B, Sánchez-Gómez AH and Garrido-Fernández J. Color pigment correlation in virgin olive oils. *J Am Oil Chem Soc* **68**, 332–336. (1991).
18. IUPAC. Standard method for the analysis of oils, fats and derivatives. Method 2432, 7th edn. Pergamon Press, Oxford. (1992).
19. Mateos R, Espartero JL, Trujillo M, Ríos JJ, León-Camacho M, Alcudia F and Cert A. Determination of phenols, flavones and lignans in virgin olive oil by solid-phase

- extraction and high-performance liquid chromatography with diodearray ultraviolet detection. *J Agr Food Chem***49**, 2185– 219. (2001).
20. Morales-Sillero A, García JM, Torres-Ruiz JM, Montero A and Fernández, J.E. Does the wetting of a fraction or the whole root zone influence olive yield and oil quality? *Agr Water Manage* **123**, 79–92.(2013).
 21. Stefanoudaki E, Williams M, Chartzoulakis Kand Harwood J. Effect of irrigation on quality attributes of olive oil. *J Agr Food Chem***57**, 7048-7055.(2009).
 22. Fernandes-Silva AA, Gouveia JB, Vasconcelos P, Ferreira TC, and Villalobos FJ. Effect of different irrigation regimes on the quality attributes of monovarietal virgin olive oil from cv. “Cobrançosa”. *Grasas Aceites*, **64**, 41-49.(2013).
 - 23.Tovar MJ, Motilva MJ, Luna M, Girona Jand Romero MP. Analytical characteristics of virgin olive oil from young trees (Arbequina cultivar) growing under linear irrigation strategies.*J Am Oil ChemSoc***78**, 843-849.(2001).
 - 24.Baccouri O, Guerfel M, Bonoli-Carbognin M, Cerretani L, BendiniA, Zarrouk M and Daoud, D. Influence of irrigation and site of cultivation on qualitative and sensory characteristics of a Tunisian minor olive variety (cv. Marsaline). *Riv Ital Sostanze Gr* **86**, 173–180.(2009).
 - 25.Palese, AM, Nuzzo V, Favati F, Pietrafesa A, Celano G and Xiloyannis C. Effects of water deficit on the vegetative response, yield and olive oil quality of trees (*Olea europaea* L., cvCoratina) grown under intensive cultivation. *SciHortic***125**, 222-229. (2010).
 - 26.Dabbou S, Dabbou S, Chehab H, Brahmi F, Taticchi A, Servili M and 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 SciTechnol* **46**, 577-585. (2011).

27. Issaoui M, Flamini G, Chehab H, Cioni PL, Braham M, and Hammami, M. Irrigation regimes and growing area effects on ‘Chétoui’ olive oil quality. *J Food Biochem* **37**, 185-192. (2013).
28. Aparicio R, Roda L, Albi MA and Gutierrez F. Effect of various compounds on virgin olive oil stability measured by rancimat. *J Agr Food Chem*, **47**, 4150–4155. (1999).
29. Tovar MJ, Motilva MJ, and Romero MP. Changes in the phenolic composition of virgin olive oil from young trees (*Olea europaea* L. cv. Arbequina) grown under linear irrigation strategies. *J Agr Food Chem*, **49**, 5502-5508. (2001)
30. Romero MP, Tovar MJ, Girona J and Motilva MJ. Changes in the HPLC phenolic profile of virgin olive oil from young trees (*Olea europaea* L. cv. Arbequina) grown under different deficit irrigation strategies. *J Agr Food Chem* **50**, 5349-5354. (2002).
31. García JM and Mancha M. Evolution of the lipids biosynthesis during the maturation of the olive varieties ‘Picual’ and ‘Gordal. *Grasas Aceites* **43**, 277-280. (1992).
32. Reboredo-Rodríguez P, González-Barreiro C, Cancho-Grande B and Simal-Gándara J. Dynamic headspace/GCMS to control the aroma fingerprint of extra-virgin olive oil from the same and different olive varieties. *Food Control*. **25**, 684-695. (2012).
33. Aparicio R, Morales MT, and Alonso MV. Relationship between volatile compounds and sensory attributes of olive oils by the sensory wheel. *J Am Oil Chem Soc* **73**, 1253–1264. (1996).
34. Reinert J and Grosch W. Odorants of virgin olive oils with different flavor profiles. *J Agr Food Chem* **46**, 2754–2763. (1998).

Table 1. Parameters related to the extraction of oil from fruit samples of the experimental orchard.

Season	Treatment	Water content (g kg ⁻¹)	Total oil extracted (g kg ⁻¹)		Virgin oil extracted (g kg ⁻¹)
			Oil content (Fresh weight)	Synthesized oil (Dry weight)	
2010	30RDI ^a	535b	162a	321	94a
	60RDI ^b	639a	98b	307	72b
	FI ^c	652	116	335	64
2011	30RDI	554b	183a	404a	98a
	60RDI	641a	120b	336c	58b
	FI	661a	125b	370b	64b
2012	30RDI	612c	117	301b	51
	60RDI	654b	111	324b	40
	FI	683a	123	387a	54

Each value represents the mean values from four replicates. Values with different letters within each season are significantly different according to Tukey's test ($p \leq 0.05$). Absence of letter means that no significant effect has been found for this variable in this season by ANOVA ($p \leq 0.05$).

^a30RDI: Regulated deficit irrigation that replaced 30% of the irrigation needs calculated for the treatment of full irrigation.

^b 60RDI: Regulated deficit irrigation that replaced 60% of the irrigation needs calculated for the treatment of full irrigation.

^c FI: Full irrigation

Table 2. Legally established parameters for evaluating the level of commercial quality of virgin olive oils extracted from fruit samples of the experimental orchard.

Season	Treatment	Acidity (% oleic acid)	Peroxide value (meq O ₂ kg ⁻¹)	K ₂₃₂	K ₂₇₀	Panel test (1 worst – 9 best)
2010	30RDI ^a	0.2	11.1a	1.46	0.11	7.6
	60RDI ^b	0.2	6.0b	1.45	0.09	7.5
	FI ^c	0.2	8.8	1.61	0.08	7.4
2011	30RDI	0.4	9.2b	1.73b	0.10	7.4
	60RDI	0.3	10.4b	2.32a	0.08	7.2
	FI	0.4	13.3a	2.33a	0.09	7.1
2012	30RDI	0.2	2.2	1.16	0.07	7.1
	60RDI	0.2	2.8	1.13	0.06	7.0
	FI	0.3	4.2	1.27	0.06	7.1

Each value represents the mean values from four replicates. Values with different letters within each season are significantly different according to Tukey's test ($p \leq 0.05$). Absence of letter means that no significant effect has been found for this variable in this season by ANOVA ($p \leq 0.05$).

^a30RDI: Regulated deficit irrigation that replaced 30% of the irrigation needs calculated for the treatment of full irrigation.

^b 60RDI: Regulated deficit irrigation that replaced 60% of the irrigation needs calculated for the treatment of full irrigation.

^c FI: Full irrigation

Table 3. Natural antioxidants and oxidative stability of the virgin olive oils extracted from fruit samples of the experimental orchard.

Season	Treatment	Carotenoids (mg kg ⁻¹)	Chlorophylls (mg kg ⁻¹)	Tocopherols (mg kg ⁻¹)	<i>O</i> -diphenol (mg kg ⁻¹)	Secoiridoids (mg kg ⁻¹)	Total phenols (mg kg ⁻¹)	Oxidative stability (h)
2010	30RDI ^a	9.5a	9.2a	352.4b	318.9a	367.2a	538.8a	38.2a
	60RDI ^b	4.9b	6.1b	385.2a	215.4b	259.3b	445.6b	36.2a
	FI ^c	6.1	10.5	399.5	33.0	46.5	131.5	23.4
2011	30RDI	6.9a	8.2a	327.5b	160.2a	141.1a	256.5a	24.3a
	60RDI	2.5b	2.5c	337.1b	79.8b	88.2b	161.5b	20.5ab
	FI	3.0b	4.1b	370.4a	75.7b	69.0b	158.6b	17.5b
2012	30RDI	8.7a	7.8a	321.1a	56.1a	61.5a	101.9a	37.7a
	60RDI	4.7b	6.8a	327.2a	31.4b	34.0b	71.8b	30.6b
	FI	4.2b	4.9b	255.4b	31.6b	27.5b	94.3a	29.8b

Each value represents the mean values from four replicates. Values with different letters within each season are significantly different according to Tukey's test ($p \leq 0.05$). Absence of letter means that no significant effect has been found for this variable in this season by ANOVA ($p \leq 0.05$).

^a30RDI: Regulated deficit irrigation that replaced 30% of the irrigation needs calculated for the treatment of full irrigation.

^b 60RDI: Regulated deficit irrigation that replaced 60% of the irrigation needs calculated for the treatment of full irrigation.

^c FI: Full irrigation.

Table 4. Phenolic composition (mg kg⁻¹) of virgin olive oil extracted from fruit samples of the experimental orchard.

Season	2010			2011			2012		
Treatment	30RDI ^a	60RDI ^b	FI ^c	30RDI	60RDI	FI	30RDI	60RDI	FI
Phenolics									
Hydroxytyrosol	4.5	4.6	3.8	1.6	1.2	2.8	2.1	1.2	0.8
Tyrosol	7.4b	13.4a	16.8	1.9	1.7	2.4	1.9	2.2	1.6
Vanillic acid	2.9a	1.5b	1.1	1.2	0.6	1.1	0.6	0.8	0.9
Vanillin	0.8	0.6	0.0	0.2	0.2	1.1	0.1	0.1	0.2
p-Coumaric acid	0.5	0.6	0.7	0.7a	0.2b	0.2b	0.1	0.1	0.1
Hydroxytyrosolacetate	66.9a	56.6a	8.1	33.6	33.8	30.3	14.8	12.2	15.2
3,4-DHPEA-EDA ^d	189.5a	110.8b	11.1	82.1a	44.1b	31.6b	34.3a	16.0b	13.0b
Tyrosolacetate	22.2a	10.9b	2.4	7.6a	1.4b	1.5b	0.0	0.0	0.0
P-HPEA-EDA ^e	97.2a	81.7a	17.6	44.6a	17.7b	21.2b	16.8a	11.6ab	7.4b
Pinoresinol	7.4	7.4	2.6	2.4	2.2	2.0	2.3	1.7	1.9
Cinamicacid	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Acetoxypinoresinol	46.0b	73.6a	37.2	22.3	21.0	28.2	15.2b	15.5b	24.7a
3,4-DHPEA-EA ^f	54.1a	39.5b	7.7	19.7a	7.8b	7.4b	2.8a	0.7b	0.7b
p-HPEA-EA ^g	26.3a	27.4a	10.1	13.9a	10.0ab	8.8b	7.6	5.8	6.5
Ferulicacid	17.3a	11.5b	10.1	19.1	15.0	16.1	0.0c	1.9b	18.8a
Luteolin	3.8	3.9	2.3	4.2	3.4	3.5	2.1	1.4	2.0
Apigenin	1.4	1.7	1.1	1.4	1.2	1.1	1.2	0.7	0.6

Each value represents the mean values from four replicates. Values with different letters within each season are significantly different according to Tukey's test ($p \leq 0.05$). Absence of letter means that no significant effect has been found for this variable in this season by ANOVA ($p \leq 0.05$).

^a30RDI: Regulated deficit irrigation that replaced 30% of the irrigation needs calculated for the treatment of full irrigation.

^b60RDI: Regulated deficit irrigation that replaced 60% of the irrigation needs calculated for the treatment of full irrigation.

^cFI: Full irrigation.

^d3,4-DHPEA-EDA: Dialdehydic form of the decarboxymethyloleuropeinaglycone.

^eP-HPEA-EDA: Dialdehydic form of the decarboxymethylgistrosideaglycone.

^f3,4-DHPEA-EA: Hydroxytyrosylelenolate.

^gp-HPEA-EA: Tyrosylelenolate.

Table 5. Fatty acid composition (g kg⁻¹) of virgin olive oil extracted from fruit samples of the experimental orchard.

Season	2010			2011			2012		
Treatment	30RDI ^a	60RDI ^b	FI ^c	30RDI	60RDI	FI	30RDI	60RDI	FI
Fatty acid									
Palmitic	164	174	190	199	203	205	171	185	181
Palmitoleic	23	20	24	23	28	26	16	26	20
Stearic	19	16	16	20	18	19	16	16	16
Oleic	655a	629b	592	561	550	555	653a	606b	609b
Linoleic	125b	148a	167	184	187	180	133b	156a	162a
Linolenic	8	8	5	9	9	1	7	7	8
Arachidic	5	5	6	5	5	5	4	4	4
Oleic/linoleic	5.2a	4.2b	3.5	3.0	2.9	3.1	4.9	3.9	3.8
Monounsaturated	678a	649b	616	584	578	581	669a	632b	629b
Polyunsaturated	134b	156a	172	193	197	190	140b	163a	170a

Each value represents the mean values from four replicates. Values with different letters within each season are significantly different according to Tukey's test ($p \leq 0.05$). Absence of letter means that no significant effect has been found for this variable in this season by ANOVA ($p \leq 0.05$).

^a30RDI: Regulated deficit irrigation that replaced 30% of the irrigation needs calculated for the treatment of full irrigation.

^b 60RDI: Regulated deficit irrigation that replaced 60% of the irrigation needs calculated for the treatment of full irrigation.

^c FI: Full irrigation.

Table 6. Individual volatile compounds of virgin olive oil extracted from fruit samples of the experimental orchard in the 2012 season.

Volatile (ng/g of oil)	Irrigation treatments		
	30RDI ^a	60RDI ^b	FI ^c
(<i>E</i>)-3-hexenal	91.61	64.70	134.46
(<i>Z</i>)-3-hexenal	143.60	148.86	157.51
(<i>Z</i>)-2-hexenal	283.92b	286.30b	409.17a
(<i>E</i>)-2-hexenal	10725.40b	11678.03b	17148.18a
(<i>E</i>)-3-hexenol	17.43	8.57	10.35
(<i>Z</i>)-3-hexenol	464.11	299.97	424.98
(<i>E</i>)-2-hexenol	451.03	369.88	516.72
hexanal	656.59	595.15	761.39
hexanol	105.01	86.38	147.28
1-penten-3-one	54.54b	68.60b	98.24a
(<i>Z</i>)-2-pentenol	3.13	2.22	2.56
(<i>E</i>)-2-pentenol	15.12c	22.70b	33.76a
1-penten-3-ol	98.24	79.60	76.95
(<i>Z</i>)-2-pentenol	154.12	138.22	147.08
(<i>E</i>)-2-pentenol	347.27a	204.65b	105.37c
2+3-pentanone	26.20	27.86	31.22
pentanal	36.13	40.71	25.03
pentanol	10.83	10.36	10.77
Hexylacetate	26.74	22.50	22.08
(<i>E</i>)-2-hexenyl acetate	99.24a	19.04b	20.69b
(<i>Z</i>)-3-hexenyl acetate	247.71	251.59	267.13
Methylacetate	18.69	18.85	15.27
Ethylacetate	209.06a	200.69a	167.02b
Methylhexanoate	13.43	5.34	7.21
Ethylhexanoate	13.88	18.92	13.93
2-Methyl-butanal	272.16a	252.76a	145.74b
3-Methyl-butanal	75.15	87.98	56.03
2+3-Methyl-butanol	52.65	52.86	57.21
Limonene	10.16	11.11	11.97
Ocimene	91.07c	232.51b	310.08a
Pentenedimer 1	64.84b	55.13b	100.21a
Pentenedimer 2	84.77	70.75	111.43
Pentenedimer 3	419.70b	267.97c	562.34a
Pentenedimer 4	347.71b	230.48c	507.64a
Pentenedimer 5	399.17b	289.29c	548.75a
Pentenedimer 6	283.25a	186.64b	286.23a
Pentenedimer 7	281.97b	242.16b	474.91a
Total volatiles	16635.76b	16649.31b	23927.21a

Each value represents the mean values from four replicates. Values with different letters within each season are significantly different according to Tukey's test ($p \leq 0.05$). Absence of letter means that no significant effect has been found for this variable in this season by ANOVA ($p \leq 0.05$).

^a30RDI: Regulated deficit irrigation that replaced 30% of the irrigation needs calculated for the treatment of full irrigation.

^b60RDI: Regulated deficit irrigation that replaced 60% of the irrigation needs calculated for the treatment of full irrigation.

^cFI: Full irrigation.