Effect of different irrigation volumes during fruit development on quality of virgin olive oil of cv. Frantoio

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Abstract

An experiment was carried out in a high-density olive (*Olea europaea* L. cv. Frantoio) orchard to determine the effect of different irrigation regimes (full, deficit, complementary) on virgin olive oil (VOO) quality over three consecutive years. Irrigation had negligible effects on free acidity, peroxide value, and fatty acid composition of VOO, but strongly influenced its phenolic concentration. Trees with high water status yielded oils with lower concentrations of total phenols and O-diphenols with respect to oils from severely stressed trees. The concentrations of secoiridoids, like 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 fully-irrigated trees than in trees under deficit irrigation or those that received complementary irrigation only. The concentrations of lignans (+)-1-acetoxipinoresinol and (+)-1-pinoresinol were unaffected by the irrigation regime. Volatile compounds, evaluated on the VOO head space, seemed to be more consistently influenced by the year rather than soil water availability.

1. Introduction

Olive trees are usually grown in areas with limited water resources. Although trees can grow and produce adequate crops under naturally low annual water supply, irrigation is essential to achieve high yields. Effects due to irrigation in the olive orchard are the increase in fruit size, the number of fruits per tree and fruit oil content (Caruso et al., 2013; Grattan et al., 2006; Gucci et al., 2007; Lavee et al., 2007; Tognetti et al., 2006), as well as the reduction in fruit drop and alternate bearing (Moriana et al., 2003). These advantages explain why this practice is becoming more and more common despite the exceptional tolerance of olive to water deficit and its adaptation to climates with long periods of summer drought. It is estimated that about 28 and 26% of olive growing area is currently irrigated in Spain and Italy, respectively, the two leading oil producing countries in the world (EU, 2012).

While beneficial effects of irrigation on yield components have been elucidated (Caruso et al., 2013; Grattan et al., 2006; Gucci et al., 2007), those on olive oil quality are less clear, which explains why the convenience of irrigation for producing high quality olive oils is still controversial. Most studies have shown that tree water status has hardly any effect on free acidity, peroxide value (PV) and UV absorption parameters at 232 and 270 nm (Motilva et al., 2000; Patumi et al., 1999; Servili et al., 2007; Tognetti et al., 2007; Tovar et al. 2002a). Only in two studies free acidity was reportedly higher in oils from irrigated trees than those from rainfed treatment (Dag et al., 2008; Ben-Gal et al., 2011). In particular, Dag et al. (2008) reported that oils obtained from

trees that received the highest irrigation volumes (75, 100 and 125% of reference evapotranspiration) showed values of free acidity that exceeded the 0.8% limit established for extra-virgin category (EU 1989/2003 modifying the ECC 2568/91).

The fatty acid composition of VOO did not seem to be affected by soil moisture in trials conducted in Spain and in Italy (Inglese et al. 1996; Motilva et al., 2000; Patumi et al., 1999; Servili et al., 2007; Tovar et al., 2002a). In a study conducted in California on cv. Arbequina, Berenguer et al. (2006) reported that the oleic/linoleic ratio was higher in oils obtained from trees that received low volumes of water (341 L per tree) than those from trees that received greater amounts. However, these differences were found only in one out of two years. Gómez-Rico et al. (2009) found that the mono-unsaturated/poly-unsaturated (MUFA/PUFA) and saturated/unsaturated (SFA/UFA) ratios were virtually similar in oils of either fully-irrigated or deficit-irrigated trees of two cultivars (cvs. Cornicabra and Morisca). Crop load and cultivar may interfere with the effect of irrigation on fatty acids composition (Ben Gal et al., 2011). For instance, linolenic acid increased with the irrigation level in oils of cv. Souri in both “on” and “off” year, but in those of cv. Barnea only in the “off” year (Ben Gal et al., 2011).

Among the oil components the phenolic compounds are the most affected by irrigation. In general, the concentration of phenolic compounds in VOO decreases as water availability in the soil increases (Berenguer et al., 2006; Motilva et al., 2000; Patumi et al., 1999; Tovar et al., 2002a). Servili et al. (2007) showed that fully-irrigated trees of cv. Leccino yielded oils with a lower concentration of hydrophilic phenols and O-diphenols than either the deficit-irrigated (about 50% water of fully-irrigated trees) or complementary-irrigated ones. These findings agreed with results obtained on cv. Arbequina, in which oils from irrigated trees had a lower concentrations of phenolic compounds than non irrigated trees (Romero et al., 2002). The compounds with a phenolic structure affect organoleptic characteristics, the oxidative stability and the healthy properties of VOO (Servili et al., 2007; Visioli and Galli, 1998). Moreover, secoiridoids derivatives of oleuropein and dimethyloleuropein, such as 3,4-DHPEA-EDA and 3,4-DHPEA-EA, are the main contributors to

the VOO bitterness, whereas ligstroside derivates as *p*-HPEA-EDA are strongly correlated with both bitter and pungent sensory notes.

The volatile fraction includes many compounds produced during the mechanical processing of olive fruits via the lipoxygenase pathway (Angerosa et., al 2004). Their concentration is mainly dependent on the cultivar and the ripening stage (Angerosa et., al 2004), although some recent studies indicate that soil water availability is also important (Dabbou et al., 2011b; Gomez-Rico et al., 2006; Servili et al., 2007). Servili et al. (2007) reported that tree water status had a marked effect on the concentration of volatile compounds obtained by the head space of VOOs of cv. Leccino and, in particular, on C_6-saturated and unsaturated aldheydes, alcohols and esters. Gomez-Rico et al. (2006) also reported that major volatile compounds concentrations were higher in oils produced under irrigated conditions. Dabbou et al. (2011b) showed that the effect of irrigation was cultivar dependent, since the concentration of volatile compounds in VOOs of 'Arbequina', 'Koroneiki', and 'Coratina' responded differently to the level of water applied. However, the effect of water is far from being clear since it was shown that the concentration of some volatile compounds appeared markedly affected by the growing season (Fernandes-Silva et al., 2013; Servili et al., 2007).

The objective of this three-year study was to comprehensively determine the changes in oil quality induced by different irrigation regimes for the cv. Frantoio, widely cultivated in Italy and abroad for the high quality of its oil. The studies available in literature on the qualitative response of this cultivar to water deficit have been conducted in only one year (Tognetti et al., 2007) or on mature trees with little productivity (d'Andria et al., 2009; Magliulo et al., 2003) and did not include the volatile compounds of VOO. The parameters used for olive oil classification as well as fatty acid composition, phenolic compounds and volatile compounds were measured in oils obtained from trees that were either fully-irrigated, deficit-irrigated or severely water stressed during fruit development over three consecutive years in an attempt to partition irrigation effects from variability due to the growing season.

2. Materials and Methods

2.1. Plant material and site characteristics

Experiments were conducted in a fully-productive, irrigated olive (cv. Frantoio) orchard located at Venturina, Italy, in 2008, 2009 and 2010. The trees were planted at a spacing of 5 x 3.9 m (513 trees ha^{-1}) in April 2003. The soil was a deep (1.5 m), sandy-loam (ISSS classification), consisting of 60% sand, 15% clay and 25% silt; the climate at the study site was sub-humid Mediterranean (Nahal, 1981; Caruso et al., 2013). The climatic conditions over the study period were monitored using a weather station iMETOS IMT 300 (Pessl Instruments GmbH, Weiz, Austria) installed on site in May 2006. Reference evapotranspiration (ET_0), calculated according to the Penman-Monteith equation, was 993, 1101 and 1001 mm in 2008, 2009, and 2010 respectively. Annual and summer precipitations are reported in Table 1. Effective precipitation (EP), calculated as 75% of the daily rainfall (individual rains less than 4 mm were excluded), was 756, 528 and 785 mm in 2008, 2009, and 2010, respectively.

The orchard was divided into three blocks, each consisting of three irrigation treatments (three plots per treatment) randomly distributed. Each of the nine plots included 12 trees arranged in three rows of four trees. Only the inner trees of the central row were used for measurements and sampling, and only four of the six trees per treatment were used to evaluate the effect of irrigation on olive oil quality.

2.2. Irrigation regimes and tree water status

Subsurface drip irrigation lines (2.3 L h-1 pressure compensated drippers spaced at 0.6 m), placed at a depth of 0.35-0.40 m and 0.8 m distance from the tree row, were used to supply 100% (Full, FI), 46-48% (Deficit, DI) or 2-6% (Complementary, CI) of water requirements calculated from reference evapotranspiration using a crop coefficient of 0.55 (Caruso et al., 2013). Irrigation periods for all treatments were: 2 July-10 October, 1 July-9 October and 9 July-17 September in

2008, 2009 and 2010, respectively. Fully-irrigated trees received water 4-5 days a week (3 to 7 h per day) and the volumes applied were 1860, 2134 and 1025 m^3 ha^{-1}, in 2008, 2009 and 2010, respectively. Trees subjected to controlled deficit conditions received about half the volume distributed to fully-irrigated trees in 2008 (893 m^3 ha^{-1}), whereas in 2009 and 2010, due to summer precipitations, the water applied was 23% and 12% of FI trees. However, when EP was considered the amount of water received by DI trees during the 2009 irrigation period was 46% of FI trees. Severely-stressed trees received 117, 45 and 47 m^3 ha^{-1}, in three irrigation events in 2008 and two in 2009 and 2010, respectively. Therefore, the severe stress regime corresponded to an almost rainfed condition since the volume of water applied by irrigation ranged 2-6% of that of well irrigated trees. Irrigation volumes were calculated based on the effective evapotranspiration and tree water status determined by measuring pre-dawn leaf water potential (PLWP) during the dry season at 7-10 d intervals as reported in Caruso et al. (2013). To account for the fluctuations in tree water status of deficit treatments, measured PLWP values were cumulated over the irrigation period (CLWP), as previously reported (Caruso et al., 2013).

Fertigation was used to supplymineral nutrients. Every spring, before irrigation treatments were put into action, a total of approx. 25, 50 and 30 g per tree of N, P_2O_5 and K_2O were supplied in 2008, 2009, and 2010, respectively.

2.3. Harvest and oil extraction

Each of the six trees per treatment was harvested individually by hand on 21 October 2008, 19 October 2009 and 25 October 2010.. Detailed results on the effect of irrigation on vegetative growthm flowering, fruit and oil yields, yield components, yield efficiency and fruit maturation were previously published (Caruso et al., 2013). The maturation index of either 50 (2008 and 2010) or 100 (2009) fruits per tree was determined based on skin and flesh colors, as previously reported (Caruso et al., 2013): values were 2.9, 3.6 and 3.5 for FI, DI, and CI treatments respectively in 2008, 2.3, 3.4 and 3.9 in 2009, and 1.1, 1.2 and 1.0 in 2010. A sample of approx. 3.5 kg of fruits per

tree was taken from four of the six trees and was used for oil extraction. About 250 cc of oil were obtained by a mechanical process using a laboratory scale system. Fruits were crushed by a hammer mill within 24 h from harvest, the resulting olive paste malaxed at 25 °C for 30 min, and the oil separated by centrifugation (Servili et al., 2007). The oils were then filtered and stored in the dark at 13 °C for about three months until analysis.

2.4. Oil analyses

Free acidity, peroxide value, fatty acid composition and UV absorption characteristics at 232 and 270 nm of oils obtained were measured in accordance with the European Official Methods (EU 1989/2003 modifying the ECC 2568/91). The total phenols and O-diphenols were determined by the Folin-Ciocalteau method according to Montedoro et al. (1992), whereas individual phenolic fractions were extracted by liquid-liquid extraction (Montedoro et al., 1992) and analyzed by high performance liquid chromatography (HPLC) according to Selvaggini et al. (2006). Standards were obtained from different sources: 3,4-Dihydroxyphenyl ethanol (3,4-DHPEA) was obtained from Cayman Chemicals LTD (USA), while the (p-hydroxyphenyl)ethanol (*p*-HPEA) 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 and separated by semipreparative HPLC according to the procedure reported previously (Montedoro et al., 1993). The nuclear magnetic resonance (NMR) data of 3,4-DHPEA-EDA, p-HPEA-EDA, and 3,4-DHPEA-EA were consistent with those reported by Montedoro et al., (1993) while the NMR data relative to (+)-1-acetoxypinoresinol and (+)-pinoresinol were in accordance with those obtained by Owen et al. (2000).

The evaluation and the quantification of volatile compounds in VOOs were performed by headspace Solid Phase Microextraction (SPME) followed by gas chromatography–mass spectrometry analysis (HS-SPME-GC/MS) according to Servili et al. (2011). For sampling the

headspace volatile compounds SPME was applied as follows: 3 g of VOO were placed into a 10 mL vial and thermostated at 35 °C, then the SPME fibre (a 50/30 μm divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) with a length of 10 mm, StableFlex - Supelco, Inc., Bellefonte, PA, USA) was exposed to the vapour phase for 30 min to sample the volatile compounds. Afterward, the fibre was inserted into the GC injector, set in splitless mode, using a splitless inlet liner of 0.75 mm i.d. for thermal desorption, where it was held for 10 min. All of the SPME operations were automated by using a Varian CP 8410 AutoInjector (Varian, Walnut Creek, CA, USA).

GC/MS analysis was performed using a Varian 4000 GC/MS equipped with a 1079 Universal Capillary Injector (Varian, Walnut Creek, CA, USA). A fused-silica capillary column was employed (DB-WAXetr, 50 m, 0.32 mm i.d., 1 μm film thickness; Agilent J&W Scientific, Folsom, CA). The column was operated with helium at a constant flow rate of 1.7 mL min^{-1} maintained with an electronic flow controller (EFC). The GC oven heating program started at 35 °C. This temperature was maintained for 8 min, then increased to 45 °C at a rate of 1.5 °C min^{-1}, increased to 150 °C at a rate of 3 °C min^{-1}, increased to 180 °C at a rate of 4 °C min^{-1} and finally increased to 210 °C at a rate of 3.6 °C min^{-1}; then this temperature was held for 14.5 min. The total time of analysis was 80 min. The injector temperature was maintained at 250 °C; the temperature of the transfer line was fixed at 170 °C. The mass spectrometer was operated in the electron ionization (EI) mode at an ionization energy of 70 eV, with scanning in the mass range of m/z 25 – 350 a.m.u. at a scan rate of 0.79 s/scan and a trap setpoint temperature of 150 °C. The GC-MS was operated with the Varian MS Workstation Software, Version 6.6 (Varian, Walnut Creek, CA, USA). The volatile compounds were identified by comparison of their mass spectra and retention times with those of authentic reference compounds purchased from Fluka (Milan, Italy) and Aldrich (Milan, Italy). Integration of all the chromatographic peaks was performed by choosing the three masses with the highest intensities among those specific for each compound, to selectively discriminate them from their nearest neighbours. The results of the peak areas were calculated on the basis of the

relative calibration curve for each compound and expressed in micrograms per kilogram of oil or micrograms per gram of fresh weight (Servili et al., 2011).

2.5. Statistical analyses

Means of irrigation treatments were separated by least significant differences (LSD) at $p \leq 0.05$ after analysis of variance (ANOVA). Fatty acids composition data were transformed by arcsine transformation before ANOVA. Where applicable, data were analyzed by regression (Pearson's correlation coefficient) using Costat (CoHort Software, Monterey, USA).

3. Results

Differences in summer precipitations were evident during the three years of study (Tab. 1). In particular, rains were evenly distributed during the summer of 2010 and totalled 189% and 161% the summer rainfall of 2008 and 2009, respectively. In 2010, due to abundant precipitations, differences in tree water status between treatments were observed only for approx. 20 days in July. As a result, differences in CLWP and minimum PLWP between irrigation treatments were marked in 2008 and 2009, but small in 2010. Summer temperatures were similar in the three years of study (Tab. 1). Fully-irrigated trees yielded 20.029, 19.514, and 30.509 kg of fruits per tree in 2008, 2009, and 2010, respectively. Fruit yield of fully-irrigated trees was higher than that of deficit-irrigated trees by 31, 32 and 16% in 2008, 2009 and 2010, respectively. However, differences were significant only in 2008 and 2009, and yield, expressed on a TCSA (trunk cross sectional area) basis, was similar for FI and DI treatments over the three-year period (data not shown). Yield of CI trees was 53, 58, and 84% of that of FI trees in 2008, 2009, and 2010, respectively.

Free acidity was unaffected by irrigation, although slightly lower values were measured in oils obtained from severely stressed trees (Tab. 2). In 2008 PV and K_{232} decreased as the degree of water deficit increased and were significantly lower in CI than FI treatment, but no differences were found in 2009 and 2010 (Tab. 2). In all three years free acidity and PV were low and within the

limits of VOO classification (EU 1989/2003 modifying the EU 2568/91). Significant differences in K_{270} emerged between treatments in 2008, but not in 2009 and 2010. ΔK was never significantly different between irrigation treatments (Tab. 2).

The fatty acid composition was only slightly affected by irrigation. Differences between treatments appeared dependent on the growing season and no consistent pattern of change was observed in response to tree water status (Tab. 3). Some significant differences between irrigation regimes were evident for palmitic, palmitoleic and stearic acid in 2008 and for palmitoleic, oleic, linoleic, and arachic acid in 2009 (Tab. 3), but there were no differences between treatments in 2010. Oils from FI trees showed higher percentage of linolenic acid in 2008 and 2009, but not in 2010 (Tab. 3). When fatty acids were grouped according to their degree of unsaturation some differences between irrigation treatments emerged in the ratio between unsaturated/saturated (UFA/SFA) in 2008, and mono/poly-unsaturated (MUFA/PUFA) fatty acids in 2009 (Tab. 3). The MUFA/PUFA ratio was higher in 2010 than in 2008 or 2009. However, there was no clear response of fatty acids composition to irrigation treatments across the years.

Total phenols and O-diphenols were lower in oils obtained from fully-irrigated trees than deficit-irrigated or severely stressed trees. These differences were also observed in 2010 when, due to the precipitations that occurred during the summer, the concentrations of total phenols and O-diphenols in the oil were lower than those measured in 2008 and 2009 (Tab. 4). The phenolic concentration of oils from CI trees was 198, 170 and 146% that of FI in 2008, 2009 and 2010, respectively. As for the O-diphenols the differences between FI and CI were even more evident with values of CI equal to 2.7, 2.4 and 1.4 times those of FI oils. In 2008 oils from DI trees had lower concentrations in total phenols and O-diphenols than CI oils (79 and 71%, respectively), whereas in 2009 and 2010 there were no differences between these two treatments (Tab. 4).

The relationship between the sum of phenolic fractions and tree water status, expressed as daily integrated PLWP, is illustrated in Fig. 1. In all three years total phenolic concentration increased as the degree of water deficit increased. Similarly, the individual fraction of secoiridoids,

such as the dialdehydic form of elenolic acid linked to 3,4-DHPEA or p-HPEA (3,4-DHPEA-EDA, p-HPEA-EDA) and the 3,4-dihydroxyphenyl ethanol (3,4-DHPEA-EA), increased as water deficit increased (Fig. 1). In all years tyrosol (p-HPEA) concentrations were similar across irrigation treatments (Fig. 2). The response of hydroxytyrosol (3,4-DHPEA) concentrations to irrigation was variable depending on the year. In 2008 irrigation increased 3,4-DHPEA, but the opposite was true in 2009; In 2010 3,4-DHPEA concentrations were very low regardless of the irrigation regime (Fig. 2)The concentrations of lignans ((+)-1-acetoxypinoresinol and (+)-1-pinoresinol) in the oils were not significantly affected by the irrigation regime and no changes were measured in response to tree water status with the exception in 2008 when acetoxypinoresinol decreased as the degree of water deficit increased (Fig. 3).

A total of 30 compounds of the volatile fraction were detected and quantified. The concentrations of the major compounds responsible for VOO flavor (Angerosa et., 2004), averaged over irrigation treatments and years, are reported in Table 5. The ANOVA showed that the effect of the year was significant for all compounds except 3-hexen-1-ol, whereas that of irrigation was significant only for 2-hexen-1-ol and hexyl ester acetate. Moreover, there was a significant interaction between irrigation regime and year for 2-hexenal (E) and hexyl ester acetate (Table 5). The concentrations of volatile substances during the three years were highly variable and this appeared prevalently attributable to the growing season rather than the irrigation regime. Total aldehydes (expressed as sum of concentrations) in VOOs of FI, DI and CI trees increased to 739, 360 and 386% respectively from 2008 to 2009, and to 524, 388 and 469% from 2008 to 2010 (Table 6). Esters concentrations were high in 2009 regardless of the irrigation regime, and reached values much higher than in 2008 or 2010; for instance, esters concentrations in 2009 were 917, 785, 715% those of 2008 for full, deficit and severely stressed trees, respectively. Ketone concentrations also varied according to the growing season, whereas alcohols concentrations were stable in 2008 and 2010 for respective irrigation regimes, but lower in 2009 (Table 6).

4. Discussion

Free acidity, peroxide value and spectrophotometric (K_{232}, K_{270} and ΔK) indexes that are used for the trade classification of olive oils were not affected by the irrigation regime. During the three years of study the significant differences in peroxide value, K_{232} or K_{270} were inconsistent and they did not allow to identify a clear pattern of variations in response to soil water availability. A slightly lower value of free acidity or K_{232} was apparent in oils from CI trees, but differences were either not significant or inconsistent between years. Berenguer et al. (2006) reported that oils of cv. Arbequina had higher values of free acidity as irrigation increased, but this effect was present only in one of the two years of study. Stefanoudaki et al. (2009) found that oils from rainfed trees had lower free acidity and peroxide value than those from trees that received 261 mm water by irrigation; however, this effect was found in only one of the two years of study. Inconsistent effects of irrigation on free acidity and peroxide value of oils between growing seasons were also reported for cv. Koroneiki (Dabbou et al., 2011a). Tovar et al. (2002a) never found any effect of irrigation on free acidity and spectrophotometric indexes of oils (cv. Arbequina) obtained from different irrigation treatments over three years. In that same study the peroxide value was greater in fully-irrigated trees than those that had received 75, 50 and 25% water of the control only in one out of the three years. In brief, the increase in free acidity due to irrigations reported by Dag et al. (2008) and Ramos and Santos (2010) may be due to particular growing and processing conditions which also determined high values (> 0.5%) across all treatments.

Irrigation also had negligible effects on fatty acid composition. The only change that consistently appeared for two years was the increase in linolenic acid of oils from trees that had received more water. However, in the third year of the study linolenic acid was similar across irrigation treatments. Linolenic acid has been shown to increase in response to irrigation level for cv. Souri, but not for cv. Barnea (Ben Gal et al., 2011). Other changes in fatty acid composition were present only in one out of the three years and thus the results were inconsistent between years. It should be noted that changes in the concentrations of fatty acids, expressed as percentage of total,

result in adjustments in other fractions, and this can be sometimes deceptive. Fatty acid biosynthesis starts from Acetyl-CoA and proceeds by increasing the length of the carbon chain. Saturated fatty acids are synthesized first, then they are modified by desaturases to yield MUFA and poly-UFA (Salas et al. 2000). An increase in stearic acid concentrations of oils produced from severely stressed trees (15-25% water of control) was reported for cv. Arbequina (Berenguer et al., 2006), but most studies agreed that irrigation did not significantly affect fatty acids composition (Inglese et al., 1996; Motilva et al., 2000; Patumi et al, 1999). In our study there was no clear pattern of changes when tree water status was different in agreement with previous results reported by Patumi et al. (1999) and Berenguer et al. (2006). On the other hand, Stefanoudaki et al. (2009) found that trees (cv. Koroneki) irrigated with 261 mm of water per year produced oils with a higher mono-unsaturated/poly-unsaturated ratio than rainfed trees in both years. Hence, the effect of irrigation on fatty acids composition appears to be dependent on climatic conditions and cultivar. We hypothesize that in relatively cool climates water stress has little effect on fatty acids composition, but in hot areas or years, an abundant water supply increases linoleic acid and decreases the oleic acid percentage of some olive cultivars. Mannina et al. (2001) observed that oils produced in Italy from several cultivars (including Frantoio) had a higher concentration (119%) in oleic acid and a lower concentration (57%) in linoleic acid than oils produced by the same cultivars in the hot climate of the Catamarca province of Argentina. An increase in linoleic acid has been measured in oils from irrigated trees of cv. Arbequina due to increased activity of Fatty Acid Desaturase 2 (FAD2), which has been related to gene expression (Hernandez et al., 2009). Under water deficit conditions the small increase of linoleic acid correlated well with the increase detected for the FAD2-2 expression level. However, this effect was cultivar dependent as it was not found for cv. Picual (Hernandez et al., 2009), in agreement with results reported for cv. Koroneiki in which the linoleic acid percentage also did not change (Stefanoudaki et al., 2001).

Soil water availability strongly influenced phenolic concentration of olive oil. Trees with high water status yielded oils with lower concentrations of total phenols and O-diphenols with

respect to oils obtained from trees that only received complementary irrigation. In 2010, characterized by a humid summer, oils from all treatments had low phenolic concentration and trees subjected to regulated deficit irrigation produced oils with similar concentrations of O-diphenols of oils from severely stressed trees. Over the 2008 and 2009 growing seasons a deficit irrigation strategy allowed to produce oils with 86% of the maximum concentration of phenolic compounds yielded by stressed trees of cv. Frantoio. These results confirm what previously found in a two-year experiment on cv. Leccino in a high-density orchard in Tuscany (Servili et al., 2007) and over three years on cv. Souri (Ben Gal et al., 2011). Tovar et al. (2002a) reported that total biophenols of fully-irrigated oils of cv. Arbequina were 93 and 74% of oils obtained from trees irrigated with 50 and 25% of that water volume, respectively. Similar responses were found in a study conducted on cv. Arbequina where total phenolic concentration of oils from deficit irrigated trees (65% of ETc) was 147% of that from fully-irrigated trees (Garcia et al., 2013) and in cvs Frantoio and Leccino (Magliulo et al., 2003). Tovar et al. (2002b) attributed the differences in total phenols induced by irrigation to the dilution of hydrosoluble compounds in the water phase during oil extraction and also to the increased synthesis of phenols induced by water deficit due to increased activity of L-phenilalanine ammonia lyase (PAL), a key enzyme directly responsable for the accumulation of hydrophilic phenolic compounds in olive fruit and, hence, in oils (Patumi et al., 1999).

Irrigation also changed the relative composition in phenolic compounds. The 3,4-DHPEA-EDA, 3,4-DHPEA-EA, p-HPEA-EDA, originating from oleuropein and ligstroside during the process of mechanical extraction of oil from the fruit, account for about 90% of the biophenols. These compounds were mainly responsible for the decrease in total phenols measured in oils from fully-irrigated trees. These three compounds are synthesized through the same biosynthetic pathway and consequently their concentrations respond similarly to water deficit conditions (Tovar et al., 2001). Romero et al. (2002) reported that fully-irrigated six-year-old olive (cv. Arbequina) trees yielded oils with similar concentrations in 3,4-DHPEA-EDA, p-HPEA-EDA and 3,4-DHPEA-EA than those obtained from trees that received 51% of water applied to control. In the same study the

concentrations of these compounds in oils from trees that received 24% of water applied to control were 253, 159 and 158% than those of fully-irrigated trees. Servili et al. (2007) determined that concentrations in oils from trees irrigated with only either 49 or 15% of water applied to the control trees were similar and both higher than respective concentrations in oils from fully-irrigated trees. Gómez-Rico et al. (2009) reported that the phenolic compounds most affected by the irrigation regime were 3,4-DHPEA-EDA, 3,4-DHPEA-EA and p-HPEA-EDA, the concentrations of which were significantly higher in the VOO of cvs. Cornicabra and Morisca from the most stressed treatment.

The volatile fraction of olive oil is a complex mixture of more than 100 compounds (Angerosa et al., 2004). In our study we detected the main classes of compounds responsible for "fruity", "cut grass", "green", "flower", and "pungent" sensory notes typical of cv. Frantoio. The concentrations of volatile compounds, measured in the head space of VOOs, were highly variable across the three years: aldehydes were low in 2008, whereas esters were high in 2009 (Tab. 5). The effect of the growing season prevailed over that of irrigation and there was often a clear interaction between these two factors. Although there were few significant changes which could be attributed to tree water status, such as the higher concentration of 2-hexen-1-ol (E) (responsible for "fruity" and "cut grass" flavor) in VOOs from fully-irrigated trees than water stressed oils, oils from irrigated treatments (FI and DI) showed higher concentrations of aldehydes (Tab. 5), in agreement with previous studies on other cultivars (Gomez-Rico et al. 2006; Gomez-Rico et al. 2009; Servili et al., 2007). However, the effect of the irrigation regime was usually not significant for most volatile compounds due to the high variability of data and it was, therefore, difficult to identify a consistent trend across years. For example, concentrations of aldehydes, alcohols and esters in VOO of the fully-irrigated treatment varied markedly despite the comparable values of CLWP across years. The response of volatile compounds concentrations to irrigation is complex and variable as it has been shown to be dependent on cultivar (Dabbou et al., 2011b) and environmental conditions (Fernandes-Silva et al., 2013) and this may explain some discrepancies existing in the literature and

importance of long-term studies.

In conclusion, irrigation has no effects on qualitative parameters used to classify VOO (UE 1989/2003 modifying the ECC 2568/91), whereas it can only occasionally affect fatty acid composition under our climatic conditions, but may increase oleic acid percentage in very hot climates. On the contrary, it is clearcut that soil water availability determines changes in the phenolic fraction and that the VOO from fully-irrigated have lower concentrations of phenolic compounds, secoiridoids and derivates of secoiridoids than VOOs from trees experiencing severe water deficit or under deficit irrigation regime. These changes in phenolic compounds have also consequences on sensory and healthy properties of VOO as oils of irrigated orchards tend to be less bitter and pungent than those from rainfed olive orchards. Volatile compounds, evaluated on the VOO head space, seemed to be more consistently influenced by the year rather than soil water availability. The results of irrigation experiments and oil quality are useful for interpreting the year-to-year variation in organoleptic and analytical profiles of olive oils, namely the pungency and bitter notes that are mainly attributed to phenolic compounds. In 2010, when summer precipitations were abundant, there was no effect of irrigation on fruit and oil yields and VOOs had low concentrations of phenolic compounds. In normally dry years deficit irrigation appears an optimal compromise between maximizing yield and qualitative parameters of VOO. Deficit irrigation allowed to produce -84.5% (average of 2008 and 2009) of the maximum oil production of the fully-irrigated trees while increasing the oil phenolic concentration to 120-154% of that of FI treatment. Complementary irrigation slightly increased the oil phenolic concentration, but significantly decreased oil yield, which resulted 74% that of deficit irrigated trees.

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Captions for figures

Figure 1. Total phenolic compounds (A, B, C), dialdehydic form of decarboxymethyl elenolic acid linked to 3.4-DHPEA (3.4-DHPEA-EDA) (D, E, F), oleuropein aglycon (3.4-DHPEA-EA) (G, H, I), the dialdehydic form of decarboxymethyl elenolic acid linked to p-HPEA (p-HPEA-EDA) (L, M, N) concentrations of virgin olive oil from trees with different water status, expressed as daily integrated PLWP, in 2008 (A, D, G, L), 2009 (B, E, H, M) and 2010 (C, F, I, N). Each symbol represents one olive oil sample from one tree. Regression equations: (A) $y=429.9x+71.7$. $R^2=0.74$; (B) $y=290.4x+302.1$. $R^2=0.89$; (C) $y=107.6+277.9x$. $R^2=0.65$; (D) $y=217.3x+31.6$. $R^2=0.85$; (E) $y=-81.9x^2+374.5x-56.8$. $R^2=0.88$; (F) $y= 38.3+99.4x$. $R^2=0.57$; (G) $y=176.7x+36.4$. $R^2=0.91$; (H) $y= 111.3x+82.9$. $R^2=0.76$; (I) $y=94.0+134.1x$. $R^2=0.79$; (L) $y=45.9x+78.9$. $R^2=0.66$; (M) $y=48.5x+50.3$. $R^2=0.82$. All regression equations are significant ($p < 0.05$) except that for p-HPEA-EDA in 2010 (N).

Figure 2. Tyrosol (p-DHPEA) (A, B, C) and hydroxytyrosol (3,4-DHPEA) (D, E, F) concentrations of virgin olive oil from olive trees with different water status expressed as daily integrated PLWP in 2008 (A, D), 2009 (B, E) and 2010 (C, F). Each symbol represents one olive oil sample from one tree.

Figure 3. (+)-1-acetoxypinoresinol (A, B, C) and (+)-1-pinoresinol (D, E, F) concentrations of virgin olive oil from olive trees with different water status expressed as daily integrated PLWP in 2008 (A, D), 2009 (B, E) and 2010 (C, F). Each symbol represents one olive oil sample from one tree.

Tab. 1. Annual and summer precipitation, summer mean temperature, cumulated leaf water potential (CLWP) and pre-dawn leaf water potential (PLWP) at the experimental site in 2008, 2009 and 2010. Days after full bloom when minimum PLWP was measured are reported in brackets. Values of CLWP and PLWP are means of 6 replicate trees. Summer indicates the period from June 21 through September 22. Legend: FI, full irrigation; DI, deficit irrigation; CI, complementary irrigation;

Year	Annual precipitation (mm)	Summer precipitation (mm)	Summer mean temperature (°C)	Irrigation	CLWP (MPa)	Minimum PLWP value (MPa)
2008	1107	74	23.1	FI	-97.7	-0.97 (100)
				DI	-146.3	-2.18 (75)
				CI	-193.8	-3.61 (109)
2009	771	87	23.3	FI	-77.2	-1.13 (110)
				DI	-138.3	-2.65 (110)
				CI	-187.0	-5.24 (110)
2010	1140	140	22.7	FI	-72.0	-0.80 (75)
				DI	-81.0	-1.53 (75)
				CI	-86.7	-1.88 (75)

Tab. 2. Free acidity (g of oleic ac./100 g), peroxide value (meq O_2/kg of oil), K_{232}, K_{270} and ΔK of virgin olive oils (VOOs) from olive trees (cv. Frantoio) grown under full (FI), deficit (DI), or complementary (CI) irrigation in 2008, 2009 and 2010. Values are means ± standard deviations of four different VOO replicates (*n=4*). Different letters indicate least significant differences (LSD) between irrigation treatments after analysis of variance (ANOVA) within each year ($p \leq 0.05$).

	2008			2009			2010		
	FI	DI	CI	FI	DI	CI	FI	DI	CI
Free acidity	0.36 ± 0.03	0.37 ± 0.03	0.30 ± 0.07	0.34 ± 0.02	0.34 ± 0.09	0.31 ± 0.05	0.25 ± 0.04	0.23 ± 0.03	0.22 ± 0.02
Peroxide value	10.5 ± 1.04 a	9.7 ± 0.47 ab	8.7 ± 0.66 b	6.0 ± 0.13	7.2 ± 1.46	6.7 ± 1.28	8.9 ± 0.74	9.3 ± 0.57	7.7 ± 0.42
K_{232}	1.78 ± 0.07 a	1.73 ± 0.12 ab	1.53 ± 0.20 b	1.93 ± 0.03	2.00 ± 0.09	1.88 ± 0.11	1.85 ± 0.041	1.88 ± 0.024	1.79 + 0.026
K_{270}	N.A.	N.A.	N.A.	0.13 ± 0.01 b	0.16 ± 0.02 a	0.15 ± 0.01 ab	0.11 ± 0.012	0.11 ± 0.015	0.10 ± 0.015
ΔK	0.005±0.004	0.04±0.062	0.003±0.004	0.0002±0.00004	0.0002±0.0001	0.003±0.001	-0.003±0.0005	-0.003±0.0010	-0.003±0.0012

N.A., not available

Tab. 3. Fatty acids composition (%) of virgin olive oils (VOOs) from olive trees (cv. Frantoio) grown under full (FI), deficit (DI) or complementary (CI) irrigation in 2008, 2009 and 2010. Values are means ± standard deviations of four different VOO replicates (*n=4*). Different letters indicate least significant differences (LSD) between irrigation treatments after analysis of variance (ANOVA) within each year ($p \leq 0.05$). Data were transformed by arcsine transformation prior to ANOVA. Legend: UFA, unsaturated fatty acids; SFA, saturated fatty acids; MUFA, mono-unsatured fatty acids; PUFA, poly-unsaturated fatty acids.

	2008			2009			2010		
	FI	DI	CI	FI	DI	CI	FI	DI	CI
Palmitic	12.8 ± 0.21 b	13.3 ± 0.33 a	13.3 ± 0.37 a	14.2 ± 0.2	14.0 ± 0.32	14.2 ± 0.12	15.9 ± 1.28	15.5 ± 1.86	15.9 ± 1.18
Palmitoleic	1.1 ± 0.05 b	1.2 ± 0.08 ab	1.3 ± 0.17 a	1.4 ± 0.21 a	1.3 ± 0.12 ab	1.1 ± 0.08 b	1.1 ± 0.34	0.9 ± 0.13	1.0 ± 0.13
Eptadecenoic	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.04	0.00	0.03 + 0.06	0.03+0.06
Stearic	2.0 ± 0.05 b	2.1 ± 0.15 b	2.4 ± 0.18 a	2.1 ± 0.29	2.4 ± 0.30	2.2 ± 0.14	1.8 ± 0.20	2.0 ± 0.15	2.0 ± 0.26
Oleic	74.7 ± 0.80	73.5 ± 0.88	73.7 ± 1.32	72.5 ± 0.67 b	73.4 ± 0.18 a	73.6 ± 0.18 a	74.9 ± 1.35	74.8 ± 1.47	74.7 ± 0.65
Linoleic	7.8 ± 0.61	8.4 ± 0.39	7.9 ± 0.81	8.6 ± 0.33 a	7.9 ± 0.37 b	7.9 ± 0.19 b	5.2 ± 0.25	5.5 ± 0.36	5.2 ± 0.78
Linolenic	0.7 ± 0.05 a	0.6 ± 0.00 b	0.6 ± 0.00 b	0.8 ± 0.00 a	0.6 ± 0.06 b	0.7 ± 0.04 b	0.8 ± 0.07	0.8 ± 0.06	0.8 ± 0.06
Arachic	0.3 ± 0.05	0.3 ± 0.00	0.3 ± 0.05	0.1 ± 0.00 b	0.2 ± 0.05 a	0.1 ± 0.00 b	0.1 ± 0.16	0.2 ± 0.17	0.2 ± 0.10
UFA/ SFA	5.5 ± 0.09 a	5.3 ± 0.18 b	5.2 ± 0.17 b	5.1 ± 0.04	5.0 ± 0.12	5.0 ± 0.08	4.6 ± 0.40	4.7 ± 0.57	4.5 ± 0.30
MUFA/ PUFA	9.6 ± 0.72	8.2 ± 0.45	8.9 ± 1.05	7.9 ± 0.33 b	8.8 ± 0.34 a	8.8 ± 0.20 a	12.8 ± 0.60	12.1 ± 0.61	12.8 ± 1.45

Tab. 4. Total phenols and O-diphenols of virgin olive oils (VOO) from olive trees (cv. Frantoio) grown under full (FI), deficit (DI) or complementary (CI) irrigation in 2008, 2009 and 2010. Values are means ± standard deviations of four different VOO replicates (*n=4*). Different letters indicate least significant differences (LSD) between irrigation treatments after analysis of variance (ANOVA) within each year ($p \leq 0.05$).

Year	Irrigation	Total phenols (mg kg^{-1})	Ortho-diphenols (mg kg^{-1})
2008	FI	386 ± 6.0 c	107 ± 8.0 c
	DI	462 ± 63.0 b	205 ± 29.4 b
	CI	504 ± 76.6 a	289 ± 41.1 a
2009	FI	457 ± 42.2 b	141 ± 33.0 b
	DI	702 ± 98.8 a	326 ± 44.2 a
	CI	779 ± 81.3 a	340 ± 39.8 a
2010	FI	106 ± 25.7 b	56 ± 16.5 b
	DI	130 ± 25.5 ab	65 ± 14.1 ab
	CI	155 ± 17.3 a	77 ± 8.7 a

Table 5. Analysis of variance of volatile compounds concentrations (μg kg^{-1}) of virgin olive oils (VOOs) from olive trees (cv. Frantoio) grown under full, deficit or complementary irrigation over three consecutive years. Values are means of four different VOO replicates (*n=4*). The significance of irrigation regime, and year and their interaction is also reported.

Variable	Pentanal	Hexanal	2-Pentenal, (E)-	2-Hexenal, (E)-	*Sum of aldehydes*	1-Penten-3-ol	1-Pentanol	2-Penten-1-ol, (E)-	1-Hexanol	3-Hexen-1-ol, (Z)-	2-Hexen-1-ol, (E)-	*Sum of alcohols*	Acetic acid, hexyl ester	3-Hexen-1-ol, acetate, (Z)-	*Sum of esters*
Irrigation (I)															
Full	235	934	261	103995	105426	1021	96	161	1076	278	1537	4169	286	300	586
Deficit	234	941	273	100279	101728	1051	95	160	823	282	1378	3787	279	286	565
Complementary	247	849	292	87602	88990	1009	65	138	984	227	1271	3694	215	334	549
Year (Y)															
2008	328	312	158	28243	29042	834	135	167	1044	246	1939	4366	72	92	164
2009	199	1522	503	134109	136332	1247	44	189	609	232	782	3103	652	662	1314
2010	189	891	165	129525	130771	999	77	102	1230	309	1465	4181	56	167	223
Significance															
I	0.8984	0.5679	0.5985	0.3150	0.3162	0.7464	0.2049	0.2399	0.1835	0.3508	0.0015	0.0971	0.0079	0.3966	0.4810
Y	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0002	0.1872	0.0000	0.0000	0.0000	0.0000	0.0000
I x Y	0.9456	0.0669	0.5673	0.0209	0.0204	0.6867	0.3974	0.3105	0.5058	0.7247	0.0673	0.9866	0.0002	0.9325	0.6462

Table 6. Volatile compounds concentrations (μg kg^{-1}) of virgin olive oils (VOOs) from olive trees (cv. Frantoio) grown under full (FI), deficit (DI) or complementary (CI) irrigation in 2008, 2009 and 2010. Values are means ± standard deviations of four different VOO replicates (n=4). Different letters indicate least significant differences (LSD) between irrigation treatments after analysis of variance (ANOVA) within each year ($p < 0.05$).

	2008			2009			2010		
	FI	DI	CI	FI	DI	CI	FI	DI	CI
Aldehydes									
Pentanal	322±66	317±70	346±160	205±10	205±46	187±47	178±56	181±31	208±36
Hexanal	285±9	306±9	345±137	1652±26	1652±345	1262±354	866±194	866±213	941±239
(E)-2-Pentenal	123±11	147±22	205±158	524±12	501±71	505±19	160±26	172±39	164±26
(E)-2-Hexenal	22453±4276b	35242±808a	27033±3098b	169275±41044a	127115±39712ab	105936±22384b	120258±16987	138481±8850	129838±16607
(E)-2-Heptenal	40±28b	78±35ab	134±69a	414±65a	364±52ab	261±80b	175±14	157±29	193±52
(E)-2-Octenal	4±8	6±12	71±141	58±9a	42±4b	35±3b	178±30	158±49	132±34
(E,E)-2,4-Hexadienal	n.d.	57±114	188±252	531±120	487±99	384±103	398±92	464±85	493±100
Alcohols									
1-Penten-3-ol	895±119	796±145	812±232	1208±90	1297±153	1237±132	961±59	1058±123	977±118
1-Pentanol	165±31	146±19	95±89	49±7a	41±1b	41±5b	74±22	97±64	61±28
(E)-2-Penten-1-ol	190±33	176±54	135±18	190±22	191±28	187±6	103±14	112±21	91±17
1-Hexanol	1113±142	954±72	1065±695	751±183	571±239	505±90	1364±241a	944±121b	1382±212a
(Z)-3-Hexen-1-ol	292±86a	265±10 a	181±7b	235±51	224±104	237±25	307±114	357±191	263±131
(E)-2-Hexen-1-ol	2045±49a	1949±27a	1824±122b	925±170a	635±164b	786±144ab	1641±293a	1549±34a	1204±12b
Esters									
Ethyl acetate	72±95	211±278	n.d.	n.d.	n.d.	n.d.	62±22	61±27	58±33
Acetic acid, hexyl ester	71±4	74±3	72±3	725±41a	703±51a	528±79b	62±22	61±27	46±8
(Z)-3-Hexen-1-ol acetate	79±3	94±48	102±3	652±145	617±166	717±128	170±56	146±70	183±132
Ketones									
3-Pentanone	2814±438a	2094±1224ab	1123 ± 1037b	139± 26b	152±26ab	176±16a	794±641	1023±842	581±579
1-Penten-3-one	27±54b	491± 539ab	834±604a	2125±397	2285±256	2453±152	495±107	495±220	566±143
6-Methyl-5-hepten-2-one	17±5a	17±2a	11± 3b	20±3a	15±3b	12±3b	22±6	19±3	16±2

FIG. 1

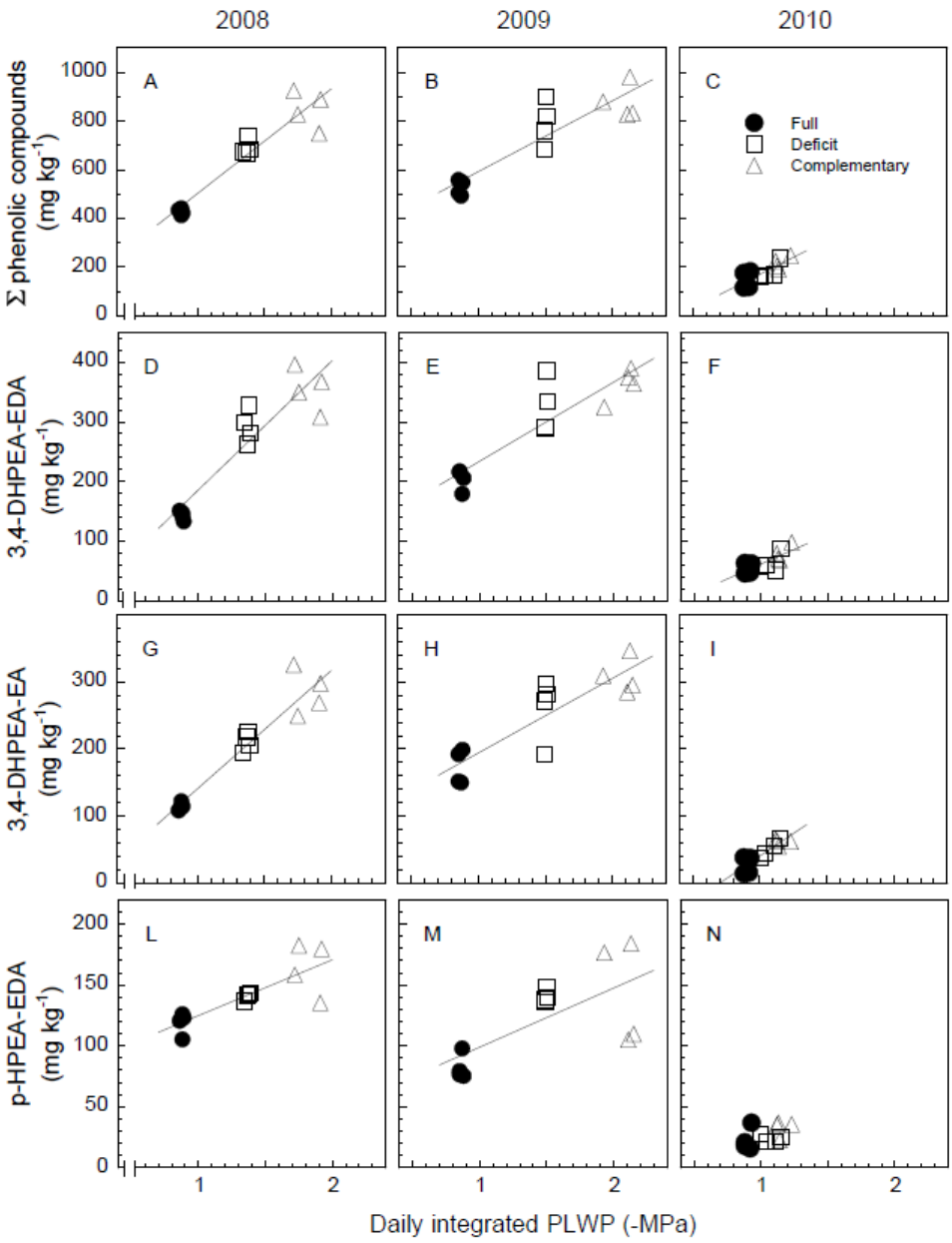

2008
2009
2010
A
B
C
D
E
F
G
H
I
L
M
N
Σ phenolic compounds (mg kg-1)
3,4-DHPEA-EDA (mg kg-1)
3,4-DHPEA-EA (mg kg-1)
p-HPEA-EDA (mg kg-1)
Full
Deficit
Complementary
Daily integrated PLWP (-MPa)

FIG. 2

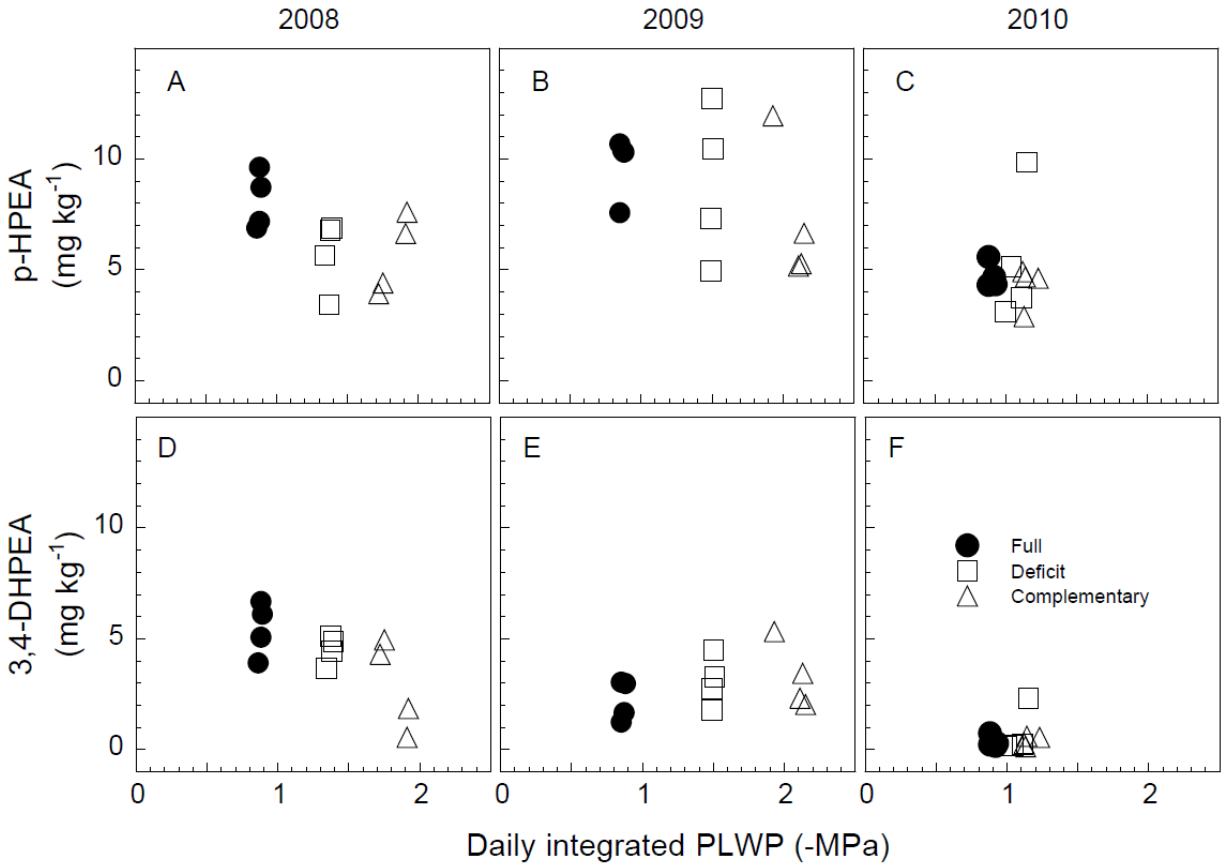
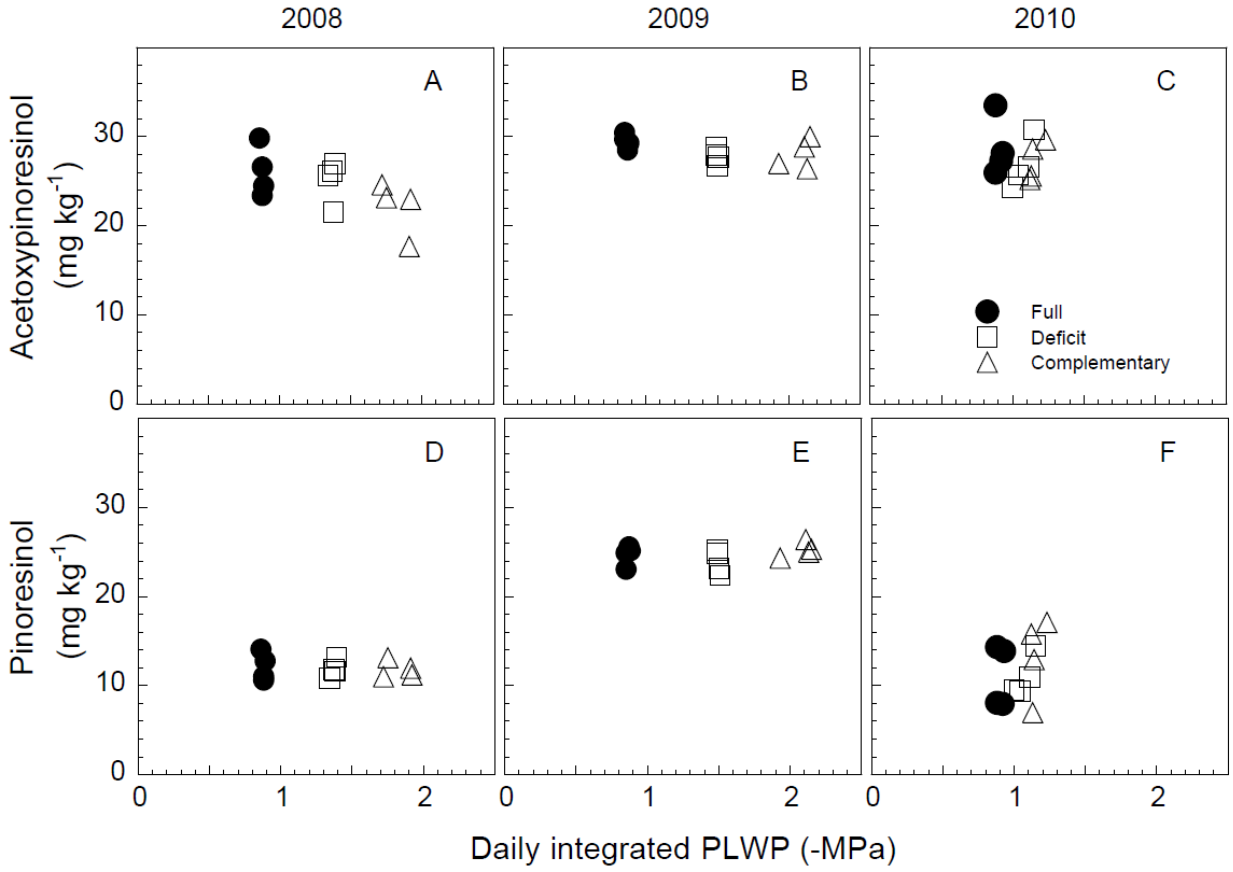

2008
2009
2010
A
B
C
D
E
F
p-HPEA (mg kg-1)
3,4-DHPEA (mg kg-1)
0
5
10
0
1
2
Full
Deficit
Complementary
Daily integrated PLWP (-MPa)

FIG. 3


2008
2009
2010
A
B
C
D
E
F
Acetoxypinoresinol (mg kg-1)
Pinoresinol (mg kg-1)
0
10
20
30
0
1
2
Full
Deficit
Complementary
Daily integrated PLWP (-MPa)