Fruit growth, yield and oil quality changes induced by deficit irrigation at different stages of olive fruit development

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Abstract

Experiments were performed in a high-density olive orchard to compare the effect of regulated deficit irrigation (RDI) at two different phenological stages with fully-irrigated trees (FI) over two years. Stress was imposed either prior to pit hardening (RDI 1) or after endocarp sclerification during the initial phase of oil accumulation (RDI 2). Fully irrigated trees received 2277 and 1648 m^3 ha^{-1} in 2012 and 2013, respectively, RDI 1 ones 76 and 53% of those volumes in 2012 and 2013, respectively (RDI 2 trees 48 and 67%). There were no differences in fruit set or return bloom due to the irrigation regime. At harvest differences in fruit size between FI and RDI treatments were significant only in the first year. The fruit yields of RDI 1 and RDI 2 trees were 70 and 81% of FI ones, respectively (means of two years), but the yield efficiency was similar across all treatments. The phenolic concentration in RDI 1 fruits was higher than that in fruits from trees subjected to the other water regimes. Verbascoside, 3-4 DHPEA-EDA, and oleuropein of RDI 1 fruits were higher in 2012 (only verbascoside in 2013). Oleuropein and 3-4 DHPEA-EDA of RDI treatments were higher than those of FI in 2013. Higher concentrations of biophenols were measured in oils from RDI 1 trees in both years, whereas FI and RDI 2 showed similar values. An early water stress was more effective to increase the phenolic concentration of olive oil compared with a late deficit or full irrigation.

Keywords: deficit irrigation, fatty acids, leaf water potential, lignans, *Olea europaea* L., ortho-diphenols.

INTRODUCTION

Deficit irrigation (DI), that is supplying less water than the volume actually required to compensate for evapotranspirative losses during the irrigation season, is a common practice in orchards (Behboudian and Mills, 1997; Fereres et al., 2012). While early studies focused on the control of tree vigour induced by deficit irrigation (Chalmers et al., 1981), the saving of water and beneficial effects on fruit quality have been more recently emphasized (Caruso et al., 2014; Fereres et al., 2012; Gelly et al., 2003; Roccuzzo et al., 2014). Several effects on fruit quality have been described. In peach moderate water deficits applied during stage II of fruit development improved fruit colour, firmness and total soluble solids (Gelly et al., 2003; 2004). Intrigliolo and Castel (2010) reported that some degree of water stress imposed during early stages of fruit growth increased soluble solids and firmness of plum fruits as long as the stem water potential was maintained above -1.4 MPa and stress was relieved at least one month before harvest. In almond there were no differences in the chemical composition of kernels between fully- and deficit-irrigated trees, but kernel dry weight was decreased by the most stressed treatments (Egea et al., 2009).

Deficit irrigation usually improves water use efficiency (Behboudian and Mills, 1997; Cui et al., 2009; Iniesta et al., 2009; Roccuzzo et al., 2014). Unlike annual crops, a decrease in biomass production for many fruit trees does not necessarily lead to a parallel reduction in fruit yield because of changes in biomass partitioning between the different organs (Behboudian and Mills, 1997; Cui et al., 2009; Roccuzzo et al., 2014). As a result, no reductions in yield have been reported for peach (Gelly et al., 2003), plum (Intrigliolo and Castel, 2010), almond (Stewart et al., 2011), pear-jujube (Cui et al., 2009), apricot (Perez-Pastor et al., 2014), and olive (Lavee et al., 2007), when the stress applied during the irrigation season was moderate.

On the other hand, one of the problems in deficit irrigation of perennial crops may be the prolonged effects of stress that last longer than the current season and often become

detrimental in the following years. For instance, Goldhamer et al. (2006) showed that the yield of almond trees declined most if a post-harvest water deficit was imposed, whereas a sustained DI was the most productive strategy. In sweet cherry fruit growth is short and sensitive to water deficit; when post-harvest DI was used it did cause reductions in fruit set and crop load the following year unless the post-harvest stress was maintained at values of stem water potential above -1.5 MPa (Marsal et al., 2010). In peach it has also been shown that post-harvest DI affected fruit set the following year (Girona et al., 2004).

Different strategies of deficit irrigation can be developed for fruit crops depending on environmental and cultural conditions. Sustained DI consists in applying a constant volume of water that is less than the evapotranspirative demand during the entire irrigation season. In this case trees usually uptake water from the soil reservoir, which is then gradually depleted as the growing season progresses (Fereres et al., 2012). Regulated DI, instead, imposes stress at definite phenological stages while fully supplying water during the rest of the irrigation season (Fereres et al., 2012); this latter strategy is particularly useful in areas where water is drastically restricted during the summer because of severe drought or priorities for urban uses.

In olive trees the water volume can be reduced well below the level of full satisfaction of water needs with limited or no effects on fruit yield and oil yield (Gomez del Campo, 2013; Gucci et al., 2007; Lavee et al., 2007; Moriana et al., 2003). Moderate restrictions of irrigation accelerated fruit maturation, increased pulp-to-pit ratio, and maintained oil yield of olive trees over 80% that of fully-irrigated trees (Caruso et al., 2013; Gomez del Campo, 2013; Gucci et al., 2009). In a previous paper we reported that the oil yield of deficit irrigated olive trees of cv Frantoio was 82% that of well irrigated ones over four years, while the saving of water applied was about 50% (Caruso et al., 2013). In a hedgerow olive orchard of cv. Arbequina the drastic cut of irrigation by 70% in July allowed to save 16% of the total irrigation water and decrease oil production by only 8% compared to fully-irrigated trees (Gomez del Campo, 2013).

Changes in oil quality due to water deficit have also been reported for many olive cultivars (Caruso et al., 2014; Caruso et al., 2017; Gomez del Campo and Garcia 2013; Gomez-Rico et al., 2007; Servili et al., 2007; Tovar et al., 2001). Most of these studies have shown that phenolic concentrations in the oil were inversely correlated with the amount of water applied, whereas the irrigation regime had negligible or no effects on other

parameters (free acidity, peroxide values, spectrophotometric indices and fatty acid composition). There is evidence that the increase in the oil phenolic concentrations of trees subjected to water deficit is due to enhanced synthesis of these compounds in the fruit (Alagna et al., 2012; Artajo et al., 2006), but recent findings suggest that the catabolism of phenolic substances in the fruit is likely influenced by water stress too (Cirilli et al., 2017). The sensory profile of oils has also been reported to be affected by soil water availability during fruit development (Benelli et al., 2015; Servili et al., 2007; Tovar et al., 2002).

Optimizing DI implies reaching the best balance between yield, oil quality and water saving issues. In particular, the period when stress is applied appears crucial to achieve the best compromise. Given the strong effect of tree water status on oil phenolic concentrations (Caruso et al., 2014; Servili et al., 2007) and the fact that the transcriptional regulation of phenolic biosynthesis in olive fruits appears to be time dependent (Alagna et al., 2012), we hypothesize that the timing of RDI would affect phenolic concentrations in the fruit and the oil.

The objective of the present work was to compare the effect of RDI at two stages of fruit development with the performance of fully-irrigated trees (FI). Stress was imposed either prior to pit hardening (RDI 1), or after endocarp sclerification during the initial phase of oil accumulation (RDI 2). In both cases the level of maximum stress was moderate to severe since stem water potential reached minima of -3.3 -3.8 and -3.2 -4.6 MPa, respectively. We investigated the effects on fruit set, growth of the mesocarp and endocarp, yield components, and oil quality parameters at harvest over two consecutive growing seasons in a high-density olive orchard.

MATERIALS AND METHODS

Plant material and climatic conditions

Experiments were conducted using mature trees, planted at a 5 x 3.9 m distance and trained to a free vase system, in an olive (*Olea europaea* L., cv. Frantoio) orchard at the experimental farm of the Department of Agriculture, Food and Environment of the University of Pisa at Venturina, Italy, over two consecutive years. The soil was a sandy-loam, consisting of 60% sand, 15% clay and 25% silt (Caruso et al., 2013). The orchard was divided into three blocks, each consisting of three randomly distributed irrigation

treatments (three plots per treatment). Each of the nine plots included 12 trees arranged in three rows of four trees. To avoid border effects only the central rows were used and all measurements and samplings were carried out on the inner two trees of the central row. The same trees were used throughout the experiment. The canopy volume and tree height were about 23 m^3 and 3.4 m, respectively.

Fertilizers (55 and 45 g of N, P_2O_5, and K_2O per tree) were supplied via the irrigation system in spring, before irrigation treatments were put into action. Pesticides were sprayed at standard concentrations to protect the crop against the olive fruit fly (*Bactrocera oleae* Rossi) and diseases.

The climatic conditions over the study period were monitored using a weather station installed on site. Annual precipitation was 820 and 915 mm in 2012 and 2013, respectively (Fig. 1). Effective precipitation (EP), calculated as 75% of the daily rainfall (individual rains less than 4 mm were excluded), was 576 and 635 mm in 2012 and 2013, respectively. Summer precipitation was 45 and 23 mm in 2012 and 2013, respectively; temperatures were similar in both years (22.4 and 22.3 °C, respectively). The mean maximum temperature reached 27.0 (28 August) and 28.6°C (8 August) in 2012 and 2013, respectively. Potential evapotranspiration (ET_0), calculated according to the Penman-Monteith equation, was 931 and 909 mm in 2012 and 2013, respectively (Fig. 1).

Irrigation and tree water status

Water was supplied using subsurface drip lines (2.3 l h^{-1} pressure-compensated drippers spaced at 0.6 m) running on the South side of the tree row at a 0.8 m distance from the trunk.

The following irrigation treatments were established and maintained in both years: i) fully irrigated trees (FI), that received 100% of water needs (2277 and 1648 m^3 ha^{-1} in 2012 and 2013, respectively) during the entire irrigation period (from 23 to 100 and from 28 to 103 days after full bloom in 2012 and 2013, respectively); ii) deficit irrigated trees (RDI 1), that were not irrigated from 23 to 41 and from 28 to 60 DAFB in 2012 and 2013, respectively; iii) deficit irrigated trees (RDI 2), that did not receive irrigation water from 41 to 71 and from 60 to 85 DAFB, respectively. Both RDI 1 and RDI 2 were fully irrigated during the rest of the irrigation period. Trees subjected to the RDI 1 treatment received 76 and 53% the volume distributed to fully-irrigated trees in 2012 and 2013, respectively,

whereas the water applied to RDI 2 trees in the same years was 48 and 67% that of FI trees (Table 1). In the three years before the beginning of this experiment FI trees had always been fully irrigated, whereas RDI 1 and RDI 2 trees had received 43-63% of the water (irrigation + precipitation) of FI trees, except in 2011 when they received only 29%. As a result of irrigation regimes in previous years, the average trunk cross sectional area (TCSA) in December 2011, before the beginning of the experiments here described, was 2.20 ± 0.29, 1.82 ± 0.21, and 1.98 ± 0.25 dm^2 for FI, RDI 1 and RDI 2 trees, respectively.

Tree water status was assessed by measuring pre-dawn leaf water potential (PLWP) by a pressure chamber at 7-10 d intervals (Caruso et al., 2013). To account for the fluctuations in the water potential of deficit treatments and compare levels of water stress across the two years, measured PLWP values were cumulated over the irrigation period (CLWP), as previously reported (Caruso et al., 2013).

Fruit set, fruit growth and yield

In spring, at the time of complete inflorescence elongation, the number of one-year old shoots, the number of flowering shoots bearing at least one inflorescence and the number of inflorescences were measured on three selected branches per tree of six trees per treatment (Caruso et al., 2013). Full bloom, estimated when 70% of inflorescences showed at least 50% of flowers open, occurred for all treatments on June 3 and June 4 in 2012 and 2013, respectively. Fruitlets present on each selected branch were counted about 30 days after full bloom (DAFB) and fruit set expressed as the number of fruits per inflorescence.

Five fruits per tree in the South-East sector of the canopy were identified at 32 and 36 DAFB, in 2012 and 2013, respectively, and their volume measured non destructively by water displacement every week until final harvest. In addition, starting from 32 through 136 DAFB in 2012 and from 58 through 135 DAFB in 2013, 10 fruits per tree were sampled and their fresh weight (FW) determined destructively. The same fruits were also used for the determination of the maturation index and mesocarp oil content. The oil content in the mesocarp was measured, after oven-drying at 70ºC, by nuclear magnetic resonance using an Oxford MQC-23 analyzer (Oxford Analytical Instruments Ltd., Oxford, UK). The maturation index was calculated according to a standard methodology, whereby the skin and flesh colors were scored according to a 0–7 scale (Caruso et al., 2013).

At 41, 71, 82, 95 and 136 DAFB in 2012, and at 58, 85, 122 and 135 DAFB in 2013,

five fruits similar to those measured for fruit volume were also sampled and their mesocarp and endocarp weight (FW and DW) determined. The mesocarp was separated from the endocarp using a sharp blade, the fresh weight (FW) of both tissues was measured and then the dry weight (DW) determined after oven drying to constant weight.

Each tree was harvested individually by hand on 5 November 2012 and 23 October 2013 (155 and 141 DAFB in those respective years). At harvest, 100 fruits were randomly sampled to measure average fruit weight and the total number of fruits per tree was calculated by dividing the crop yield by the average fruit weight (Caruso et al., 2013). The oil yield of individual trees was calculated after measuring the mesocarp oil content on a dry weight basis, the fruit fresh yield, the pulp-to-fruit ratio, and the ratio between DW and FW (Gucci et al., 2007). Final crop yield was also expressed on the basis of TCSA to calculate crop efficiency and to take into account differences in tree size.

Oil extraction and analysis

At harvest a sample of about 3.5 kg of fruits per tree was taken from four of the six trees and used for oil extraction. About 250 cc of oil were obtained by a mechanical process using a laboratory scale system within 24 h from harvesting. Fruits were crushed by a hammer mill, 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 until analysis. Free acidity, peroxide value, fatty acid composition and UV absorption characteristics at 232 and 270 nm of oils obtained were determined according to the European Official Methods (EU 1989/2003 modifying the ECC 2568/91). Total phenols and O-diphenols were determined by the Folin-Ciocalteau method (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) as reported by Selvaggini et al. (2006). Individual phenolic fractions were identified using nuclear magnetic resonance (NMR) techniques (Cirilli et al., 2017; Servili et al., 2007).

Experimental design and statistical analysis

The trees were arranged according to a completely randomized block design with three blocks, each consisting of three irrigation treatments. Means of irrigation treatments

were separated by least significant differences (LSD) after analysis of variance (ANOVA). Percentage values were arcsin transformed prior to ANOVA.

RESULTS

In both years the PLWP of FI trees was generally above -1 MPa and resulted in CLWP values of -92 and -94 MPa by the end of the irrigation season in 2012 and 2013, respectively (Fig. 2). The PLWP of RDI 1 trees reached minima of -3.8 and -3.3 MPa in 2012 and 2013, respectively; the CLWP course was similar in both years. On the other hand, the CLWP of RDI 2 reached lower values in 2012 than in 2013 (-164 and -131 MPa, respectively), with PLWP minima of -4.6 and -3.2 MPa, respectively at the end of the stress period. As a result, CLWP values of RDI 1 and RDI 2 were similar at the end of the experimental period in 2013, whereas those of RDI 2 were about 30 MPa lower than RDI 1 in 2012 (Fig. 2). The PLWP of previously stressed trees returned to values of FI ones in less than a week after irrigation was resumed in both years (Fig. 2).

The number of fruits per branch were lower in 2013 than in 2012 for all treatments and reflected the lower number of inflorescences per branch, which was not compensated by the higher initial fruit set (Table 2). There were no differences between irrigation treatments in either the number of inflorescences or that of fruits per branch within each year. In 2014 the number of inflorescences or fruits was similar across all treatments (Table 2).

Fruit growth reflected tree water status (Fig. 3). While fruit growth of FI trees proceeded at a generally high rate, periods of water deficit slowed down fruit volume and FW. Significant differences between FI and deficit irrigated trees were evident at the end of the first and second period of stress in 2012 (Fig. 3a, c). In 2013, although tree water deficit also slowed down fruit growth, fruit volume of RDI 2 was not significantly different from that of FI trees (Fig. 3b). However, when growth was expressed as FW, fruits of RDI 2 trees were smaller than FI ones at the end of the second period of water deficit. At harvest differences in fruit volume and FW between FI and RDI treatments were significant in 2012, but not in 2013 (Fig. 3 a, b, c, d). In 2012 the mesocarp oil content was initially affected by water deficit, but it turned out to be similar across all irrigation treatments at the last seven sampling dates including harvest (Fig. 3e). In 2013 the sigmoidal pattern of oil content showed little differences among treatments (Fig. 3f, Table 3).

The destructive measurements of fruit weight and that of fruit parts confirmed the differences in fruit growth between irrigation regimes (Figs. 4 and 5). In 2012 the mesocarp and fruit FWs of deficit irrigated trees were more affected by water status than when expressed on a DW basis. As a result, differences in mesocarp to endocarp ratio between FI and RDI trees were greater when expressed as FW rather than DW. At harvest the FI and RDI 1 trees had the highest mesocarp-to-endocarp ratio (both FW and DW) in 2013. The DW/FW of fruits reflected tree water status at each date of measurement (Fig. 4i). In 2013 the mesocarp FW was initially affected by water deficit (Fig. 5). However, at the last three sampling dates after stress had been relieved, mesocarp growth promptly recovered to values of FI trees. By the time of harvesting (141 DAFB) differences in mesocarp FW were maintained between FI and RDI trees, but they neither increased further at later sampling dates nor were evident on a DW basis.

Yield of FI trees was high and stable in both years (Table 3). In 2012 yield of RDI 1 and RDI 2 was 94.2% and 96.1% that of FI trees, respectively, but it was only 45.7% and 65.9% that of FI trees in 2013 (Table 3). The yield of RDI treatments was reduced in 2013 mainly because of a lower number of fruits, which was only 47 and 66% that of FI trees. Significant differences between irrigation treatments and between years for RDI 1 and RDI 2 were still apparent, albeit smaller, when the oil yield was calculated (Table 3). Summing up the fruit yields of both years, RDI 1 and RDI 2 trees produced 70 and 81% of FI ones, respectively. The yield efficiency was similar for all treatments despite the large differences in crop load. In both years fruits were harvested at the same stage of ripening, when the epicarp (skin) turned dark for less than half of its surface. Both maturation index and DW/FW ratios were similar across all irrigation treatments.

Periods of water deficit modified the qualitative characteristics of olive oil. Differences appeared in free acidity with oils of RDI 1 showing higher values than those of the RDI 2 treatment (Table 4). Acidity values of oils from FI trees were similar to RDI 2 samples in 2012 and to RDI 1 in 2013. The K_{232} and K_{270} indexes of RDI 1 trees were higher than those of RDI 2 in both years; K_{232} and K_{270} of fully-irrigated trees showed intermediate values (Table 4). The peroxide value and the ΔK were unaffected by the irrigation regime in both years. The fatty acid composition of VOOs did not reveal any clear effect of the irrigation regime (Table 5). Linoleic, stearic, and linolenic acids were significantly different between FI and RDI trees in 2012, but not in 2013 (Table 5).

In both years the sum of phenolic fractions, determined by HPLC techniques, in fruits sampled at harvest from RDI 1 trees was higher than that in fruits from trees subjected to the other water regimes (Table 6). Verbascoside, 3-4 DHPEA-EDA, and oleuropein of RDI 1 fruits were higher in 2012, but only verbascoside in 2013. In any case, oleuropein and 3-4 DHPEA-EDA of both RDI treatments were higher than those of fully-irrigated trees in 2013 (Tab. 6). (+)-1-acetoxypinoresinol and (+)-pinoresinol (either in the fruit or the oil) did not change consistently in response to irrigation treatments (Tabs. 6 and 7).

Significantly higher values of total polyphenols were measured in oils from RDI 1 trees in both years (Table 4), whereas FI and RDI 2 trees showed similar values (Table 4). In 2013 oils had higher concentrations of total polyphenols and those of ortho-diphenols than in 2012. Differences among irrigation treatments were apparent for individual polyphenolic fractions in olive oil. Higher concentrations in the sum of phenolic fractions was measured in oils from RDI 1 in both years, whereas RDI 2 and FI showed similar concentrations. 3-4 DHPEA-EDA and 3-4 DHPEA-EA were higher in oils obtained from RDI 1 trees in both years, whereas p-HPEA-EA only in 2013 (Tab. 7).

DISCUSSION

Irrigation is one of the key practices to increase yield and reduce production costs in olive orchards. Many studies have shown that irrigating olive trees increases yield and improves fruit characteristics compared with rainfed cultivation (Gucci et al., 2007; Gucci et al., 2009; Lavee et al., 2007; Moriana et al., 2003). Despite the variability in cultivars, soil types and environmental conditions of the different trials, there is currently a general consensus about the advantages of using deficit irrigation to save water and increase water use efficiency in the arid and semi-arid areas where olive trees are grown (Caruso et al., 2013, Iniesta et al. 2009; Moriana et al., 2003; Gispert et al., 2013; Rosecrance et al., 2015). Moriana et al. (2003) estimated a water productivity of 5 and 0.2 kg oil ha^{-1} mm^{-1} under low (450-550 mm range) and high evapotranspiration (750-850 mm range) conditions, respectively. Iniesta et al. (2009) calculated that water productivity was 4.5-5.0 kg oil ha^{-1} mm^{-1} of applied water for well irrigated trees, whereas it was about three times greater for deficit irrigated trees. Tognetti et al. (2006) reported that water use productivity decreased as the volume of water supplied to mature trees of cv. Frantoio increased from 33 to 100%

of evapotranspiration. Different strategies of deficit irrigation (sustained, regulated, alternate cycle, partial root drying) use stress to decrease water consumption but, despite the many investigations under different environmental conditions, cultivars and planting densities, there is yet no scientific evidence of the supremacy of one strategy over others for olive orchards (Caruso et al., 2013; Fereres, et al., 2012; Gomez del Campo, 2013; Iniesta et al., 2009; Moriana et al., 2003). In our work the saving of water for the RDI 1 and RDI 2 treatments was considerable, as the respective trees received 64 and 59% of water supplied to FI trees, corresponding to 1400-1600 m^3 less per hectare. Thus, limiting water at certain stages of fruit growth allowed to save relevant volumes of water compared with fully-irrigated trees over the irrigation season and, therefore, determined a more efficient use of water.

Since growers' revenues largely depend on productivity, any improvement in water use efficiency should not be at the expense of yield. A survey of the recent literature shows that deficit irrigation allows to maintain oil yield above 80% of that of fully-irrigated trees while water saving ranges from about 15 to 50% of the volume applied (Caruso et al., 2013; Gispert et al., 2013; Gomez del Campo, 2013; Iniesta et al., 2009; Gucci et al., 2007). In our work the yield of trees subjected to a late deficit averaged 81% that of FI ones, hence within the expected range of response of olive trees to RDI (Caruso et al., 2013; Gucci et al., 2007; Iniesta et al., 2009). On the other hand, when an early water deficit was imposed trees yielded only 70% of the oil of FI trees, but in subsequent years their performance returned to above 80% (Gucci R. and Caruso G., unpublished results). The irrigation regime did not alter appreciably the oil accumulation process, which confirms results from previous investigations (Gucci et al., 2007; Moriana et al., 2003). In general, the relationship between oil content and added water is rather weak (Gucci et al., 2007). In addition, this lack of response in oil content may be partially explained by the relatively low fruit density (ranging from about 180 to 400 fruits per m^3 of canopy) in our study, below the threshold beyond which oil content seems to respond to crop load in well irrigated olive trees of cv. Arbequina (Trentacoste et al., 2010). We also confirmed the absence of any effect of RDI and irrigation scheduling on flowering, fruit set, and return bloom of olive trees (Lavee et al., 2007; Gucci et al., 2007; Caruso et al., 2013).

The question remains about the time when a moderate water deficit should be imposed to achieve the best compromise between yield, quality and water saving. Deficit

irrigation can be better tolerated by the tree during periods when processes determining productivity (fruit set, fruit growth, oil accumulation) are the least sensitive to water stress. In stonefruit trees the central phase of fruit development, when fruit growth rate slows down and massive endocarp sclerification takes place (oftern referred to as pit hardening), is apparently little sensitive to water deficit (Chalmers et al., 1981; Gucci et al., 2012). Hence, many RDI studies have used pit hardening as a reference phenological stage to restrict irrigation in olive trees (Gispert et al., 2013; Gomez del Campo, 2013; Lavee et al., 2007; Tognetti et al., 2006). In Israel Lavee et al. (2007) reported that irrigation influenced fruit characteristics and recommended to supply all the water volume after pit hardening. In hedgerow planting systems restricting water before pit hardening was considered a better strategy than imposing a deficit later during fruit development in Central Spain (Gomez del Campo, 2013). Gomez-Rico et al. (2007) reported that RDI from the beginning of the oil accumulation phase did not decrease yield compared with controls of cv. Cornicabra. Decreasing irrigation volumes by 50% between pit hardening and the onset of ripening resulted in oil yields similar to control trees (Gispert et al., 2013). Deficit treatments at 66% of evapotranspiration from the beginning of pit hardening to the onset of colour change decreased fruit dry yield per hectare by 19% in cv. Frantoio (Tognetti et al., 2006). Our objective was to test whether a water deficit before pit hardening (RDI 1) was beneficial or detrimental compared with a later water deficit (RDI 2).

There is evidence that the timing of water restriction affects the development of fruit tissues and cellular processes involved in mesocarp growth (Gucci et al., 2009; Rapoport et al., 2004). In potted olive plants water stress applied from 28 through 56 DAFB causes a reduction in fruit size and a marked delay in time of endocarp growth (Rapoport et al., 2004). In the present work the endocarp growth of RDI trees did not appreciably lag behind that of FI trees probably because the stress was not severe and long enough to induce, in mature field-grown trees, the response previously measured in young, potted ones (Rapoport et al., 2004). Mesocarp FW and fruit FW readily responded to conditions of water limitations, but the increase in DW over time progressed virtually unaffected by the irrigation regime. Fruits of RDI treatments reached similar sizes at harvest in both years. However, differences in fruit volume and FW between FI and RDI trees emerged in 2012, when the crop load was similar across treatments, but not in 2013 when fruits from all treatments reached the same final size. The significantly higher crop load borne by FI trees

with respect to RDI trees (both treatments) in the second year might have masked the effect on fruit size since it has been shown that crop load can significantly decrease fruit size in well irrigated or deficit irrigated trees (Gucci et al., 2007). Full irrigation was not needed to reach maximum mesocarp-to-endocarp ratio, presumably because RDI treatments received sufficient water and their daily PLWP exceeded the threshold value of -2.5 MPa beyond which the pulp-to-pit ratio is decreased (Gucci et al., 2009). The pulp-to-pit ratio is one of the most important qualitative parameters of fruits not only for table production, but also for oil extraction since the oil accumulates in the mesocarp (Gucci et al., 2009; Gucci et al., 2012; Rapoport et al., 2017). As far as cellular processes involved in fruit development, cell size has been shown to be more severely decreased by water shortage than cell number in the mesocarp, suggesting than cell expansion is a more sensitive process than cell division even at early stages of fruit growth when cells are actively dividing (Rapoport et al., 2004; Gucci et al., 2009). Yet, both cell number and cell size were decreased by long periods of water deficit (between 56 and 120 DAFB) in field-grown Japanese plum trees (Gennai et al., 2017).

Beneficial effects of deficit irrigation on oil quality have been widely documented in the literature (Caruso et al., 2014; 2017; Gomez del Campo and Garcia, 2013; Gomez-Rico et al., 2007; Servili et al., 2007; Tovar et al., 2001). The main changes occur in the phenolic fraction, as also shown in our work, whereas other qualitative components of the oil were either unaffected or did not change consistently across treatments and over the years, in agreement with previous studies (Caruso et al., 2014; 2017; Gomez-Rico et al., 2007; Servili et al., 2007; Tovar et al. 2001). In particular, the scheduling of water restriction during the regulated deficit experiment did not alter consistently free acidity, peroxide value, spectrophometric indices or the fatty acid composition of the oil. Many studies have shown the inverse relationship between oil biophenols and tree water status. Less water supplied to the trees results in higher concentrations of biophenols in the olive fruit and the oil (Artajo et al., 2006; Caruso et al., 2014; Cirilli et al., 2017; Servili et al., 2007), because tree water status influences the phenolic composition and phenolic metabolism in the fruit (Cirilli et al., 2017; Tovar et al., 2002). In our study the oil biophenols responded to water deficit similarly to previously reported results (Caruso et al., 2014; Servili et al., 2007), but we also showed that the period when stress was applied could modify the phenolic concentration both in the fruit and the oil. An early water deficit was

more effective in increasing those concentrations than a late deficit, despite the similar degree of stress experienced by RDI treatments in both years. This finding confirms that early stages of fruit growth are not only important for cell division processes and final fruit size in stonefruits (Gennai et al., 2017; Gucci et al., 2009; Rapoport et al., 2004; Rapoport et al., 2017), but also influence the phenolic composition and concentration at harvest. Alagna et al. (2012) associated different concentrations of phenolics in the fruit of several cultivars with transcripts putatively involved in secoiridoid biosynthesis. These authors hypothesized a regulatory role of these transcripts on secoiridoid accumulation during fruit development as they were almost exclusively present at early stages of fruit development (Alagna et al., 2012). There is also evidence that during early phases of fruit growth (35-45 DAFB) the phenolic composition is related to activities of β-glucosidase and peroxidase probably through their effects on oleuropein catabolism (Cirilli et al., 2017). It is likely that water deficit modifies both transcription and translation processes although it remains to be established whether it is more effective on either biosynthetic or degradative pathways of biophenols.

Secoiridoids (oleuropein and its derivatives, ligstroside and its derivatives), simple phenols (tyrosol, hydroxytyrosol), and flavonoids (luteolin and derivatives, apigenin and derivatives, rutin, and dismetin) are the most abundant phenolic fractions in the fruit and oil of olive trees (Alagna et al., 2012; Cirilli et al., 2017; Talahoui et al., 2016), although their content and proportion vary greatly with the cultivar (Talahoui et al., 2016). Other factors being equal, the oil phenolic concentration may differ up to one order of magnitude depending on the cultivar (Alagna et al., 2012; Talahoui et al., 2016). Since the oil concentration of phenolic compounds is tightly related to the initial concentration in the fruit (Alagna et al., 2012; Talahoui et al., 2016), the transfer rate between matrices during the oil extraction process may play a key role in determining the phenolic concentration and composition in the oil (Talahoui et al., 2016). Secoiridoids, the most lipophilic group of the phenolic fraction, have the largest transfer rate, followed by flavonoids and simple phenols. The transfer largely depends on the cultivar and fruit moisture content (Talahoui et al., 2016), but we can exclude both cultivar (we used the same cultivar) and moisture effects because the range of fruit water content was not wide enough (59-57, 60-53, 57-57% per FI, RDI 1 and RDI 2 respectively in 2012 and 2013) to justify the differences in phenolic concentrations between RDI 1 and RDI 2 treatments. Artajo et al. (2006) reported

that the partitioning of phenolic compounds between olive paste, pomace, olive oil and wastewater was affected by irrigation practices that determined marked differences in fruit water content that we did not measure in our work. In any case, the same authors concluded that the tree water status affected phenol synthesis in the fruit and thus the phenolic content of the olive paste more than the partitioning of phenolic compounds during the olive oil extraction process (Artajo et al, 2006).

Besides the key role that the genotype and the stage of fruit development play on the phenolic profile in the fruit and the oil, soil water availability strongly influences phenols (Alagna et al., 2012; Cirilli et al., 2017; Servili et al., 2007). Therefore, irrigation is the most effective practice growers can use to manipulate the phenolic profile of their product in the field. In particular, it appears interesting to impose short periods of water deficit at early stages of fruit development to those cultivars that are genetically low in phenolic concentrations. Although biophenolic concentrations are not used to classify oils (EU, 2003) these compounds play a key role in olive oil quality (Servili and Montedoro, 2002). Biophenols are perceived sensorially as bitter and pungent. They are also strong antioxidants and thus high phenolic concentrations prolong storage life and shelf life of oils. Maintaining high levels of poliphenols is beneficial for oil stability and human health (Servili and Montedoro, 2002; Servili et al., 2004). In both years biophenolic concentrations were high and well above the 250 mg Kg^{-1} oil value that is usually considered the threshold beyond which virgin olive oil exerts health benefits assuming a daily intake of 20 g of oil (4 mg biophenols a day).

In conclusion, reducing the water supply well below full irrigation is commercially feasible in olive orchards regardless of the training system and planting density. The degree of deficit imposed and the environmental conditions determine the amount of water saved and the reduction in yield compared with the fully irrigated condition. Local constraints in water distribution may become relevant in specific cases and they should be taken into account when developing protocols of deficit irrigation. An additional advantage of deficit irrigation lies in slowing down vegetative growth, which is useful to control tree size in very high density, hedgerow olive orchards. A moderate level of water deficit early in the summer, before pit hardening, induces a higher concentration of biophenols in the fruit and the oil than the same stress applied after pit hardening. The two RDI regimes were equivalent in terms of supplied water but the timing of stress imposition determined

different results in oil quality.

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Caption for figures

Fig. 1. Daily values of mean air temperature (+) (°C), evapotranspiration (•) (ET_0, mm x 10) and precipitation (histograms) (mm) at the experimental site in 2012 and 2013.

Fig. 2. Seasonal course of pre-dawn leaf (PLWP) (a, b) and cumulative (CLWP) (c, d) water potential of olive trees grown in the field grown under Full (FI), Deficit 1 (RDI 1) or Deficit 2 (RDI 2) irrigation in 2012 (a, c) and 2013 (b, d). Symbols are means of six trees. Vertical bars represent least significant differences between irrigation treatments after analysis of variance (ANOVA) ($p \leq 0.05$) within each date of measurement.

Fig. 3. Seasonal courses of volume (a, b), fresh weight (c, d) and mesocarp oil content (e, f) of fruits sampled from olive trees grown in the field under grown under Full (FI), Deficit 1 (RDI 1) or Deficit 2 (RDI 2) irrigation in 2012 (a, c, e) and 2013 (b, d, f). Values are means of four trees for each irrigation treatment (5 and 10 fruits per tree for fruit volume and fruit fresh weight measurements, respectively), except for the last three dates when three (FI) and two (RDI 1 and RDI 2) trees were sampled. Vertical bars represent least significant differences between irrigation treatments after analysis of variance (ANOVA) ($p \leq 0.05$) within each date of measurement.

Fig. 4. Seasonal courses of fruit (a, b), mesocarp (c, d), endocarp (e, f) weight, mesocarp to endocarp ratio (f, g) and fruit DW/fruit FW (i), expressed on FW (a, c, e, f) and DW (b, d, f, g) basis, sampled from olive trees grown in the field under Full (FI), Deficit 1 (RDI 1) or Deficit 2 (RDI 2) irrigation in 2012. Values are means of four trees for each irrigation treatment (five fruit per tree). Vertical bars represent least significant differences between irrigation treatments after analysis of variance (ANOVA) ($p \leq 0.05$) within each date of measurement.

Fig. 5. Seasonal courses of fruit (a, b), mesocarp (c, d), endocarp (e, f) weight, mesocarp to endocarp ratio (f, g) and fruit DW/fruit FW (i), expressed on FW (a, c, e, f) and DW (b, d, f, g) basis, sampled from olive trees grown in the field under Full (FI), Deficit 1 (RDI 1) or Deficit 2 (RDI 2) irrigation in 2013. Values are means of four trees for each irrigation treatment (five fruit per tree). Vertical bars represent least significant differences between irrigation treatments after analysis of variance (ANOVA) ($p \leq 0.05$) within each date of measurement.

Table 1. Irrigation period and water volume applied anually to olive trees in 2012 and 2013.

Irrigation	Year	Irrigation period	Volume		
			(m^3 ha^{-1})	(L/tree)	% of Full
Full	2012	25 Jun – 11 Sept	2277	4958	100
Deficit 1		14 Jul – 11 Sept	1724	3916	76
Deficit 2		25 Jun – 14 Jul and 13 Aug – 11 Sept	1215	2967	53
Full	2013	2 Jul – 15 Sept	1648	3212	100
Deficit 1		3 Aug – 15 Sept	799	1558	48
Deficit 2		2 Jul – 3 Aug and 28 Aug – 15 Sept	1108	2160	67

Tab. 2. Flowering and fruit set of olive trees (cv. Frantoio) grown in the field under Full (FI), Deficit 1 (RDI 1) or Deficit 2 (RDI 2) irrigation in 2012 and 2013. Fruit set was expressed as percentage of fruits per inflorescence 30 days after full bloom. Measurements were carried out in spring, before the beginning of irrigation. Values are means of 6 replicate trees ± standard error.

Irrigation treatment	Year	Shoots per branch	Flowering shoots per branch	Inflorescences per branch	Fruit per branch	Initial fruit set (%)
FI	2012	110 ± 15	98 ± 14	744 ± 85	198 ± 20	26.9 ± 2
RDI 1 *		105 ± 9	97 ± 9	719 ± 139	185 ± 28	26.4 ± 2
RDI 2 *		119 ± 11	107 ± 11	890 ± 122	230 ± 29	26.3 ± 1
FI	2013	137 ± 24	48 ± 16	196 ± 64	140 ± 48	70.4 ± 1
RDI 1		109 ± 37	29 ± 9	128 ± 42	83 ± 24	68.6 ± 3
RDI 2		127 ± 20	29 ± 6	104 ± 21	72 ± 13	69.3 ± 1
FI	2014	126 ± 19	116 ± 17	805 ± 126	329 ± 51	41.7 ± 2
RDI 1		106 ± 22	96 ± 20	696 ± 127	274 ± 58	39.9 ± 3
RDI 2		120 ± 10	109 ± 10	764 ± 66	306 ± 32	40.8 ± 1

(*)Trees received only 5% of full irrigation in 2011.

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Tab. 3. Yield and yield efficiency (Fruit yield/TCSA, Oil yield/TCSA) of olive trees (cv. Frantoio) grown under Full (FI), Deficit 1 (RDI 1) or Deficit 2 (RDI 2) irrigation in 2012 and 2013. Values are means ± standard error of four trees per irrigation treatment (*n=4*). Different letters indicate significant differences between irrigation treatments after analysis of variance within each year ($p \leq 0.05$). Legend: TCSA, trunk cross sectional area.

	2012			2013		
	FI	RDI 1	RDI 2	FI	RDI 1	RDI 2
Fruit yield (g)	24261 ± 1712	22858 ± 619	23319 ± 2133	25122 ± 3840 a	11494 ± 1823 b	16560 ± 2216 ab
Fruit yield/TCSA (g dm^{-2})	11994 ± 1370	14566 ± 2066	11332 ± 2057	10351 ± 1538	5946 ± 1356	6781 ± 960
Oil yield (g)	4113 ± 278	3486 ± 105	3950 ± 418	4760 ± 703 a	2250 ± 258 b	2706 ± 412 b
Oil yield/TCSA (g dm^{-2})	2031 ± 217	2214 ± 304	1930 ± 390	1989 ± 364	1154 ± 215	1103 ± 165
Fruits per tree	8093 ± 589	9164 ± 350	8685 ± 811	8579 ± 1197 a	4056 ± 591 b	5685 ± 792 b
Fruits/dm^{-2} TCSA	4003 ± 468	5826 ± 791	4208 ± 757	3545 ± 503	2096 ± 468	2332 ± 351
Mesocarp oil (% D.W.)	69 ± 0.16	66 ± 0.31	67 ± 0.99	65 ± 0.57	65 ± 0.62	64 ± 1.13
Maturation index	2.2 ± 0.07	2.4 ± 0.06	2.3 ± 0.08	1.9 ± 0.17	2.2 ± 0.05	1.8 ± 0.11

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Tab. 4. 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) obtained from olive fruits sampled at harvest (155 and 140 days after full bloom in 2012 and 2013, respectively) from olive trees (cv. Frantoio) grown under FI, RDI 1 or RDI 2 irrigation (see caption of Table 3 for abbreviations). Values are means ± standard error of four different VOO replicates per irrigation treatment (*n=4*). Different letters indicate significant differences between irrigation treatments after analysis of variance within each year ($p \leq 0.05$)

	2012			2013		
	FI	RDI 1	RDI 2	FI	RDI 1	RDI 2
Free acidity	0.27 ± 0.007 b	0.31 ± 0.007 a	0.25 ± 0.023 b	0.45 ± 0.04 a	0.45 ± 0.02 a	0.33 ± 0.005 b
Peroxide value	5.4 ± 0.29	6.7 ± 0.50	5.5 ± 1.05	3.9 ± 0.74	3.0 ± 0.40	4.2 ± 0.20
K_{232}	1.68 ± 0.03	1.82 ± 0.04	1.67 ± 0.07	1.83 ± 0.07 ab	1.90 ± 0.003 a	1.70 ± 0.002 b
K_{270}	0.12 ± 0.004 ab	0.13 ± 0.007 a	0.10 ± 0.012 b	0.17 ± 0.015 ab	0.18 ± 0.005 a	0.14 ± 0.00 b
ΔK	-0.001 ± 0.0003	-0.002 ± 0.007	-0.002 ± 0.0003	-0.002 ± 0.001	-0.002 ± 0.0001	-0.001 ± 0.0002
Total phenols	458 ± 8.0 a	633 ± 36.6 b	515 ± 14.6 a	789 ± 14.8 a	958 ± 5.8 b	774 ± 29.3 a
Ortho-diphenols	165 ± 3.4 a	220 ± 13.9 b	185 ± 5.0 a	284 ± 6.9 a	367 ± 22.5 b	279 ± 11.5 a

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Tab. 5. Fatty acids composition (%) of virgin olive oils (VOOs) from olive trees (cv. Frantoio) grown under grown under FI, RDI 1 or RDI 2 irrigation in 2012 and 2013 (see caption of Table 3 for abbreviations). Values are means ± standard error of four different VOO replicates (*n=4*). Different letters indicate least significant differences between irrigation treatments after analysis of variance (ANOVA) within each year ($p \leq 0.05$). Data were transformed by arcsine transformation prior to ANOVA.

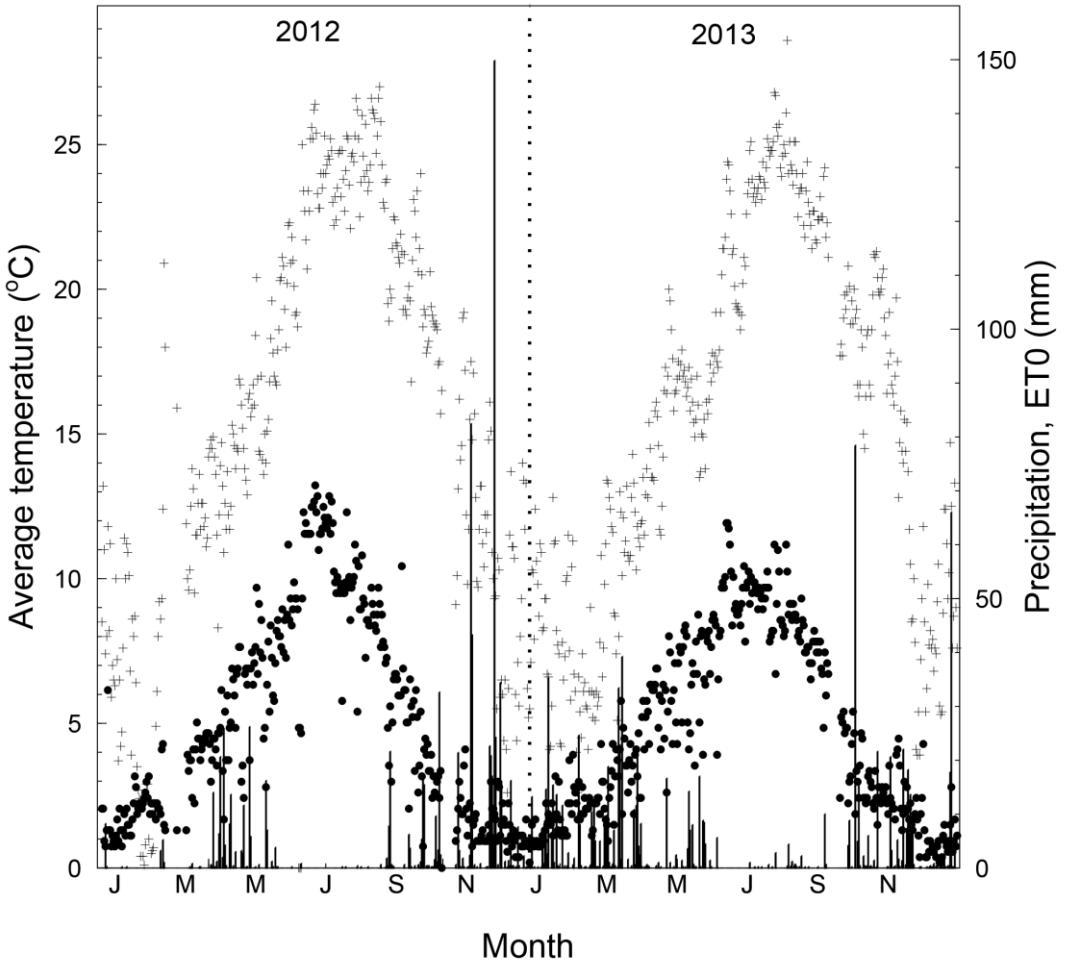
	2012			2013		
	FI	RDI 1	RDI 2	FI	RDI 1	RDI 2
Oleic	73.6 ± 0.18	73.6 ± 0.18	73.5 ± 0.19	74.1 ± 0.34	74.0 ± 0.03	74.1 ± 0.17
Palmitic	15.1 ± 0.25	15.0 ± 0.10	15.1 ± 0.14	13.9 ± 0.17	14.1 ± 0.09	13.9 ± 0.22
Linoleic	6.1 ± 0.13 b	6.6 ± 0.16 a	6.7 ± 0.16 a	7.2 ± 0.22	7.2 ± 0.04	7.3 ± 0.15
Stearic	2.9 ± 0.05 a	2.6 ± 0.14 ab	2.4 ± 0.17 b	2.4 ± 0.17	2.4 ± 0.04	2.3 ± 0.09
Linolenic	0.7 ± 0.01 a	0.7 ± 0.02 a	0.6 ± 0.02 b	0.7 ± 0.01	0.7 ± 0.01	0.7 ± 0.04
Palmitoleic	0.7 ± 0.05	0.6 ± 0.10	0.6 ± 0.02	0.9 ± 0.19	0.9 ± 0.04	0.8 ± 0.04
Arachic	0.41 ± 0.003 ab	0.45 ± 0.02 a	0.33 ± 0.05 b	0.36 ± 0.05	0.32 ± 0.01	0.40 ± 0.06
Margaric	0.044 ± 0.005	0.045 ± 0.002	0.035 ± 0.003	0.02 ± 0.014	0.05 ± 0.01	0.04 ± 0.02
Eptadecenoic	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.0 ± 0.03	0.0 ± 0.02	0.1 ± 0.03

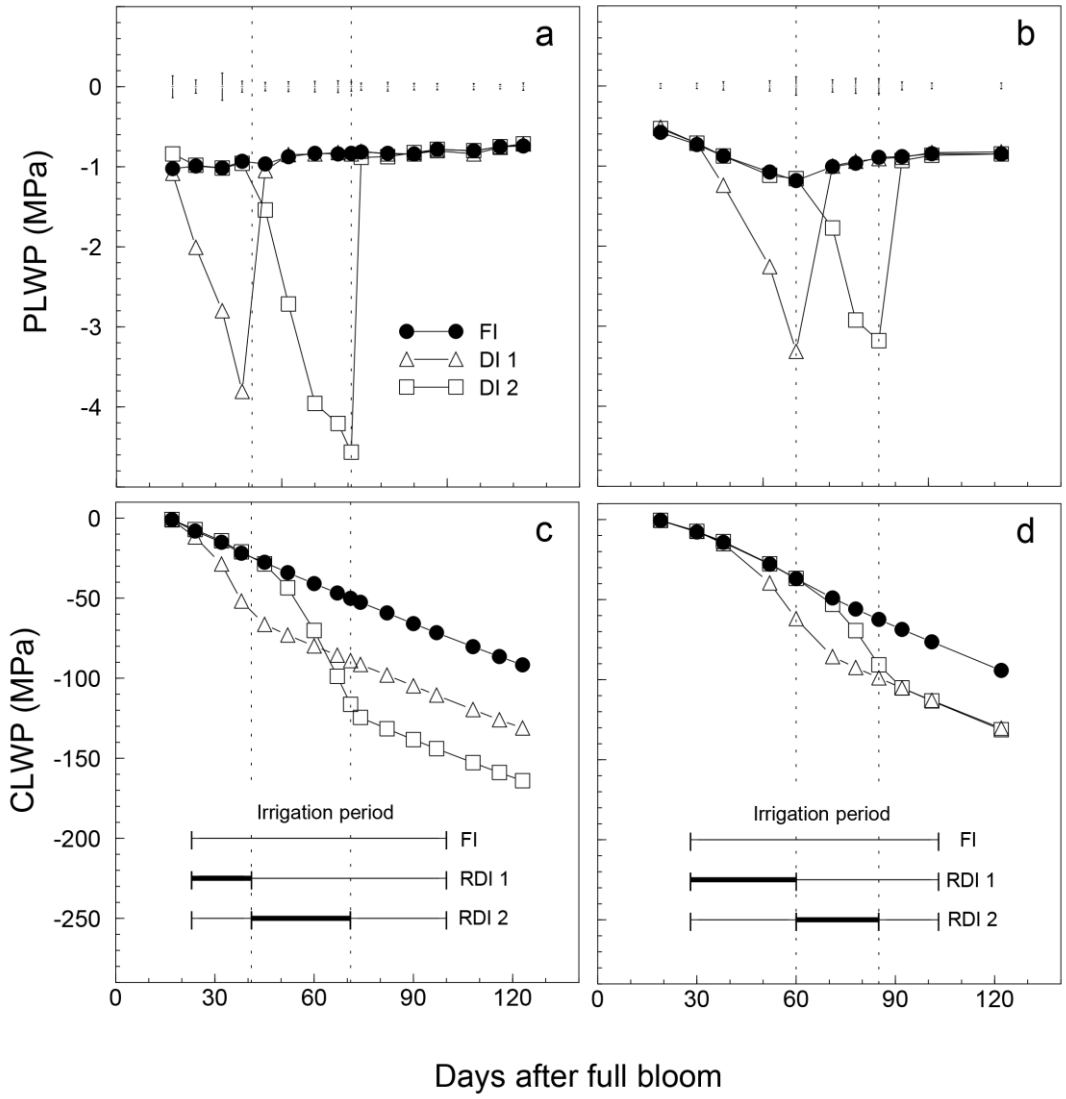
Tab. 6. Phenolic compounds (mg g^{-1} D.W.) in the mesocarp of fruits sampled at harvest from olive trees subjected to FI, RDI 1 or RDI 2 irrigation (see caption of Table 3 for abbreviations). Values are means of three trees for each irrigation treatment (10 fruits for each tree). Different letters indicate significant differences (LSD) between irrigation treatments after analysis of variance ($p \leq 0.05$) within each year.

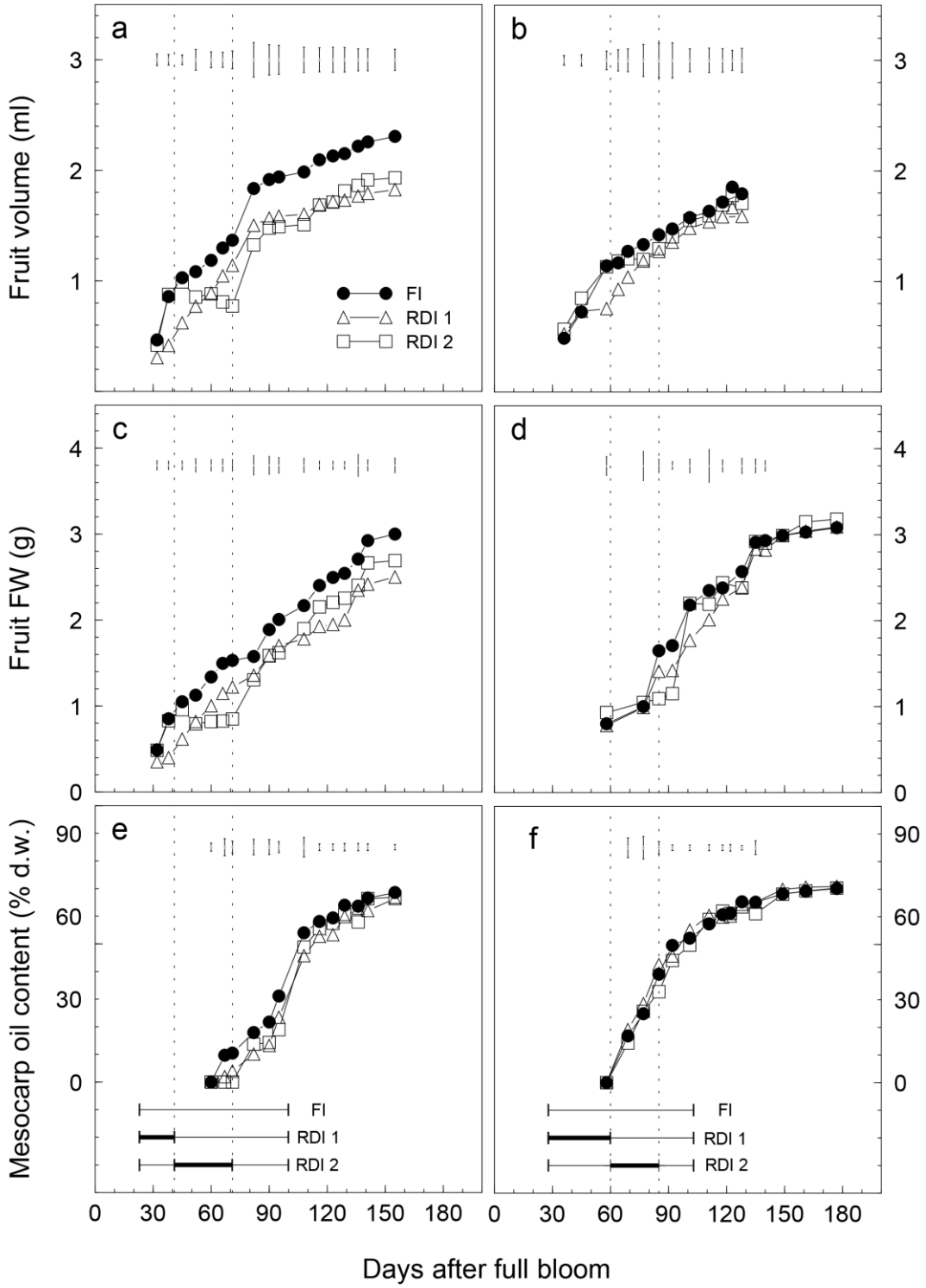
	2012				2013			
	FI	RDI 1	RDI 2	*LSD*	FI	RDI 1	RDI 2	*LSD*
3,4-DHPEA	0.6	0.7	0.5	*1.16*	2.6	2.7	1.5	*1.80*
p-HPEA	0.1	0.1	0.1	*0.07*	1.5 a	0.9 ab	0.4 b	*1.08*
3-4 DHPEA-EDA	39.3 b	49.3 a	41.0 ab	*8.91*	46.1 b	90.8 a	75.0 a	*19.14*
(+)-1-acetoxypinoresinol	0.5 a	0.4 b	0.4 b	*0.04*	0.5 a	0.2 b	0.5 a	*0.23*
(+)-pinoresinol	0.3 b	0.2 c	0.5 a	*0.04*	0.3	0.4	1.4	*1.18*
Verbascoside	9.7 b	15.7 a	8.0 b	*1.86*	9.3 c	20.2 a	14.3 b	*3.23*
Oleuropein	12.9 b	25.9 a	13.7 b	*10.42*	7.7 b	13.4 a	14.1 a	*3.70*
Sum of phenolic fractions	63.5 b	92.3 a	64.2 b	*4.58*	68.0c	128.6 a	107.3 b	*20.9*

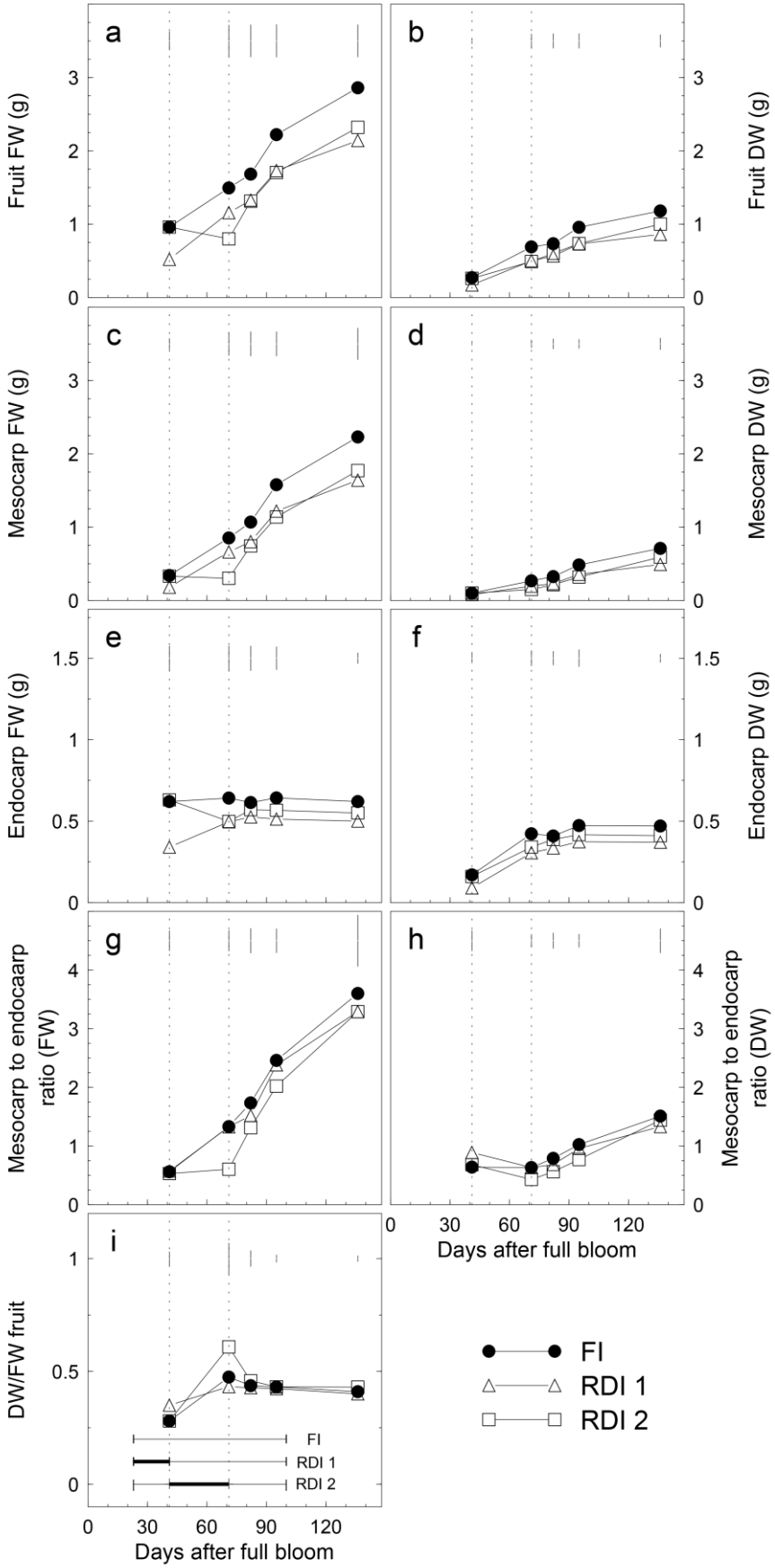
Tab. 7. Phenolic compounds (mg kg^{-1}) of oils obtained from fruits sampled at harvest from olive trees grown under FI, RDI 1 or RDI 2 irrigation (see caption of Table 3 for abbreviations). Values are means of four olive oil samples for each irrigation treatment. Different letters indicate significant differences (LSD) between irrigation treatments after analysis of variance ($p \leq 0.05$) within each year.

	2012				2013			
	FI	RDI 1	RDI 2	*LSD*	FI	RDI 1	RDI 2	*LSD*
3,4-DHPEA	7.3	8.1	7.8	*2.78*	16.8	16.1	11.81	*6.39*
p-HPEA	5.6	5.5	5.6	*3.24*	20.3 a	18.6 a	10.8 b	*5.88*
3-4 DHPEA-EDA	238 b	340 a	274 b	*47.1*	495 b	582 a	489 b	*25.1*
(+)-1-acetoxypinoresinol	18	18	18	*1.09*	16 b	17 b	20 a	*1.09*
(+)-pinoresinol	15	14	14	*0.7*	27	26	25	*2.60*
p-HPEA-EDA	90 a	89 a	71 b	*11.5*	87 c	138 a	109 b	*10.4*
3,4-DHPEA-EA	166 c	266 a	208 b	*35.0*	292 b	505 a	290 b	*42.4*
Sum of phenolic fractions	540 b	740 a	599 b	*86.2*	955 b	1303 a	955 b	*44.2*

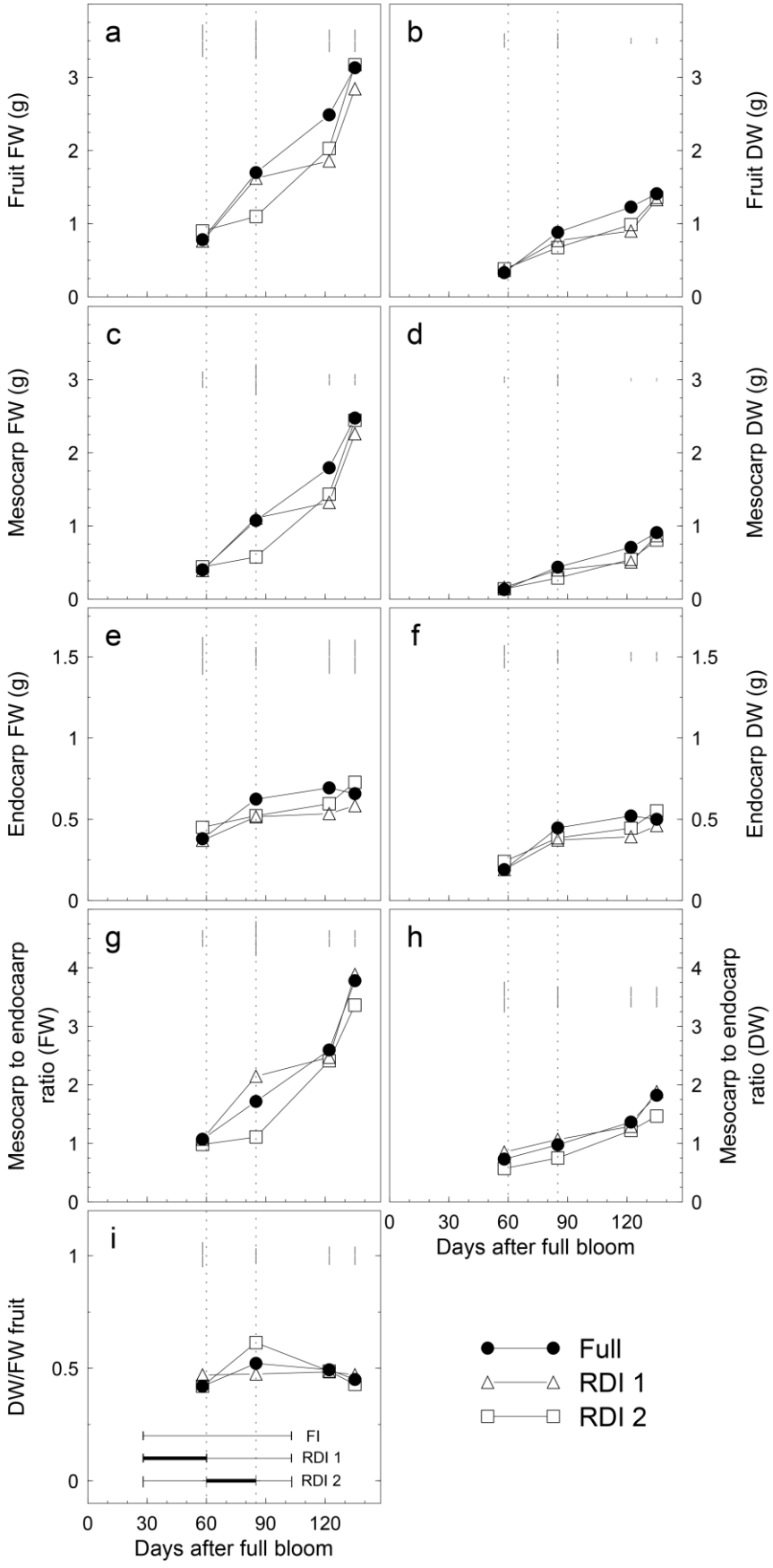

2012
2013
Average temperature (°C)
Precipitation, ET0 (mm)
Month
J
M
M
J
S
N
J
M
M
J
S
N
0
5
10
15
20
25
0
50
100
150


a
b
c
d
PLWP (MPa)
CLWP (MPa)
0
-1
-2
-3
-4
-50
-100
-150
-200
-250
30
60
90
120
FI
DI 1
DI 2
Irrigation period
FI
RDI 1
RDI 2
Days after full bloom


a
b
c
d
e
f
Fruit volume (ml)
Fruit FW (g)
Mesocarp oil content (% d.w.)
Days after full bloom
FI
RDI 1
RDI 2


a
b
c
d
e
f
g
h
i
Fruit FW (g)
Fruit DW (g)
Mesocarp FW (g)
Mesocarp DW (g)
Endocarp FW (g)
Endocarp DW (g)
Mesocarp to endocaarp ratio (FW)
Mesocarp to endocarp ratio (DW)
DW/FW fruit
Days after full bloom
FI
RDI 1
RDI 2

84


a
b
c
d
e
f
g
h
i
Fruit FW (g)
Fruit DW (g)
Mesocarp FW (g)
Mesocarp DW (g)
Endocarp FW (g)
Endocarp DW (g)
Mesocarp to endocaarp ratio (FW)
Mesocarp to endocarp ratio (DW)
DW/FW fruit
Days after full bloom
Full
RDI 1
RDI 2
FI
RDI 1
RDI 2

85