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RESEARCH ARTICLE

Vegetative and reproductive responses, oil yield and composition from olive trees (*Olea europaea*) under contrasting water availability during the dry winter-spring period in central Argentina

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Keywords
Flowering; fruiting; oil yield and composition; olive; pre-flowering–flowering period; water deficit.

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Abstract

In Argentina, the climatic pattern of the olive production areas is characterised by a marked water deficit during winter and spring months. A field experiment was carried out to evaluate the effect of water availability during the pre-flowering–flowering period on vegetative, reproductive and yield responses of olive trees grown in central Argentina. From the end of autumn to mid-spring, four irrigation treatments were imposed to olive trees (*Olea europaea*, cv. Arbequina and Manzanilla) at 0, 25, 50 and 75% estimated crop evapotranspiration (ETc). Also, a control treatment was kept at 100% ETc for the entire year. For the first crop year evaluated, water deficit applied at early June, approximately 4 months prior to bloom, reduced the vegetative shoot growth and delayed the flowering time, resulting in shortening of the fruit maturation period and, ultimately, decreased fructification. Trees irrigated with high (75% of ETc) and full (100% of ETc) winter-spring water supply presented significantly higher values of flower density, fruit density and final fruit yield which resulted in water productivity (kg fruits mm^{-1} of irrigation/ha) enhancements of about 500% (cv. Arbequina) and 330% (cv. Manzanilla) with respect to those obtained from the corresponding unirrigated treatments. Differences between treatments in oil content and composition were primarily attributed to variations in fruit maturity. Differences in fatty acid composition were stronger in cv. Arbequina where a gradual increase in oleic acid content was registered in parallel to the increase in irrigation water supply. From a practical stand point, results obtained from most of the analysed parameters were quite similar for both T75 and T100 treatments. Thus, the possible convenience of irrigation at T75% ETc should be considered since it may warrant profitable olive production while saving a considerably quantity of irrigation water in the olive production area in central Argentina.

Introduction

The evergreen olive tree is one of the most characteristic tree crops from the Mediterranean Basin, where it has traditionally been cultivated under conditions of limited water availability. The expansion of olive production has taken olives into non-Mediterranean climates, e.g. subtropics in Australia and South America, where the response of the crop to water availability has not yet been studied in detail. Particularly, in arid and semiarid Argentina, evapotranspiration is high and rainfall is minimal during the winter and spring months, a period which coincides with floral development, bloom and initial fruit set.

One of the most rapid changes that is currently taking place in olive cultivation is the expansion of irrigated orchards. Strategies using regulated deficit irrigation (RDI) have been proposed to optimise water use in olive growing (Grattan *et al.*, 2006; Pérez-López *et al.*, 2007). The aim of RDI is to optimise irrigation by supplying the crop with water levels which are less than actual water requirements but keeping the crop performance as close as possible to its maximum potential. In addition, many studies on RDI have been aimed at better establishing the water requirements for specific phenological periods (Pérez-López *et al.*, 2008; Rousseaux *et al.*, 2008; Rapoport *et al.*, 2012). According to this purpose, most of such RDI strategies involved supplying enough irrigation water during the phenological stages at which the crop is more sensitive to water stress, and reducing or even withholding irrigation for the rest of the crop cycle (Fereres & Soriano, 2007). Most of the published work on RDI has been carried out in countries in the Mediterranean region where irrigation is normally suspended during the winter months because fairly cold and cloudy conditions lead to low values of evapotranspiration, and autumn and/or winter rains provide sufficient water for flowering and subsequent fruit set. Thus, most of the studies on RDI have focused on evaluating irrigation needs under hot dry summer conditions and concern irrigation reductions during the fruit development period (Grattan *et al.*, 2006; Tognetti *et al.*, 2006; Lavee *et al.*, 2007; Pérez-López *et al.*, 2007; Martín-Vertedor *et al.*, 2011).

Few studies on water deficit effects during the developmental phases prior to fruit set have been undertaken. Hartmann & Panetsos (1961) reported that severe reductions in soil moisture content during both inflorescence formation and flower development produced both fewer flowers per inflorescence and reduced the percentage of perfect flowers. A work by Rousseaux *et al.* (2008) examined the physiological and productive responses to the suspension of irrigation during the winter season in an arid region of olive cultivation in La Rioja province (Argentina). When compared to fully irrigated plants, the study found that olive trees which were not irrigated for 6–7 weeks reported similar values of net photosynthetic rate. Coincidently, there was no significant difference in fruit yield at harvest time between the unirrigated and irrigated treatments. An innovative work by Rapoport *et al.* (2012) showed that water deficit during the reduced winter growth had no effect on either flowering or fruiting parameters. However, water deprivation during inflorescence development reduced many different flowering parameters and ovule development. Recently, we observed that full or elevated levels of water availability [100% or 75% of the estimated crop evapotranspiration (ETc)] during winter and spring months protected photosynthetic pigments from oxidative degradation and markedly improved the CO_2 assimilation rate (Pierantozzi *et al.*, 2013). These results suggest that supplemental irrigation during the pre-flowering–flowering period may enhance olive reproductive (flowering and fruiting) performance.

This study concentrates on the effect of different water irrigation levels–applied between the end of autumn and the end of the flowering period (mid-spring)–on vegetative and reproductive responses, oil yield and composition from olive trees of the two major Spanish cultivars grown in central Argentina.

Materials and methods

Plant material and experimental design

A field experiment was conducted in a commercial olive orchard located in Cruz del Eje, in the north-western region of Córdoba province, Argentina. Cruz del Eje is located in the dry Chaco Forest phytogeographical region, at 450 m above sea level. This area has a typical arid Chaco climate, characterised by short and dry winters with a few days of frost. Summer is the rainy season and the total spring-summer rainfall is about 480 mm. The precipitation during the autumn-winter period reaches approximately 90 mm. The average relative humidity is 53%, with the lowest levels during September and October.

The soil of the region in which the olive orchard is located was classified as typical Haplustol (60–65 cm in depth), characterised by volumetric water content of 17.7% at field capacity (soil matric potential of −0.03 MPa) and 9.73% at wilting point (soil matric potential of −1.5 MPa). Soil water content was measured using a soil auger at 1 m from the trunk and at a soil depth of 0–90 cm. Measurements were done on soil samples taken from each experimental plot (three plots for each combination of irrigation level × cultivar). Soil samples were immediately placed in hermetic plastic bags and transported to the laboratory where initial and dried (72 h at 80°C) weights were recorded. At the beginning of the RDI experiment, the average soil water content was around 13.5% for both 2009 and 2010 crop years evaluated. The water used for irrigation had an electrical conductivity equal to 0.20 dS m^{-1} and low sodification risk (Sodium adsorption ratio equal to 1.4).

Two olive (*Olea europaea* L.) cultivars 'Arbequina' and 'Manzanilla' were selected from a 70-year-old orchard with tree spacing of 10 m × 10 m. Arbequina and Manzanilla are the most extensively planted olive cultivars in central Argentina. While Arbequina is a

Table 1 Average monthly temperatures (°C) and rainfall (mm), Penman–Monteith reference evapotranspiration (ETo, mm day^{-1}) and estimated crop evapotranspiration (ETc, mm day^{-1}) in Cruz del Eje during both 2009/2010 and 2010/2011 experimental crop seasons evaluated

	2009/2010 Crop season				2010/2011 Crop season			
Month	Temperature	Rainfall	ETo	ETc	Temperature	Rainfall	ETo	ETc
April	21.8	26.6	3.93	2.67	17.7	69	2.98	2.03
May	16.1	19.1	2.54	1.01	12.9	38	1.94	0.78
June	12.6	0	2.36	0.95	11.7	1.6	1.61	0.64
July	10.2	16	2.16	0.87	10.0	0	2.33	0.93
August	16.6	0	3.55	1.42	12.6	1.6	3.35	1.34
September	14.3	26	4.10	2.79	17.1	9.8	4.82	3.28
October	21.6	5.4	6.09	4.14	19.4	2.6	5.50	3.74
November	25.2	30	7.03	4.78	22.2	42.4	6.04	4.11
December	25.0	117	6.28	4.27	25.4	79	6.97	4.74
January	26.9	35.6	7.10	4.83	25.2	125.1	6.09	4.14
February	25.5	124.1	5.29	3.60	23.4	208.1	4.97	3.38
March	23.8	173.7	4.57	3.11	23.2	26	4.54	3.09

typical oil-producing cultivar, Manzanilla is devoted to both oil production and table olive production. During the 2009 and 2010 crop years, four irrigation treatments were applied to trees of both cultivars. The experimental design included a treatment irrigated at 100% of ETc during the entire year (T100), and three RDI treatments, at 25%, 50% and 75% of ETc (T25, T50 and T75, respectively), applied between the end of autumn (mid-June) and the end of the flowering period (mid-spring). Furthermore, olive trees growing without irrigation between the end of autumn and mid-spring were used as a control (rain-fed) treatment (T0). During the rest of the year, T0, T25, T50 and T75 treatments were irrigated at 100% of ETc. During both 2007 and 2008 crop years, the trees had been irrigated with drip irrigation. These two crop years were taken as an adaptation period and they were not considered for the scheduled analyses.

A randomised block design with three plots for each combination of irrigation level × cultivar was used. Each experimental plot consisted of 12 trees, where the two central trees were selected for all measurements, while the surrounding trees were considered border-guard trees.

Irrigation water was delivered using two pairs of drip lines around each tree, except for plants from T0 treatment which had only one pair of drip lines. Drip lines were placed at 1 m and 1.80 m from the trunk. During the course of the differential irrigation application period, drip lines had drip emitters giving 4 L h^{-1} (T100 and T75 treatments) or 2 L h^{-1} (T50 and T25 treatments). During the rest of the year, drip emitters were changed to 3 L h^{-1} for all RDI treatments. Irrigation events were performed weekly.

Irrigation scheduling was carried out following the methodology proposed by Allen *et al.* (1998), using a simplified water balance method. The crop evapotranspiration was estimated as: $\mathrm{ETc} = \mathrm{ETo} \times K_c \times K_r$, where ETo is the reference evapotranspiration, K_c is the crop coefficient, and K_r is the coefficient of reduction related to the percentage of area shaded by the canopy. The ETo was calculated using a Class A evapotranspiration pan, located next to the experimental area, and the tank coefficient (*K*pan) proposed by Allen *et al.* (1998). During the April–August period, a K_c value equal to 0.4 was assumed, as suggested by Rousseaux *et al.* (2008) for olive trees growing under the conditions prevailing in La Rioja province (Argentina). For the rest of the year, we used a K_c value equal to 0.68 as proposed by Girona *et al.* (2002). The K_r coefficient was calculated using the relation proposed by Fereres *et al.* (1981). For example, a K_r of 1 was used for the 53% crop cover.

Table 1 summarises climatic conditions, ETo and ETc in Cruz del Eje during both 2009/2010 and 2010/11 experimental crop seasons evaluated. Meteorological data were recorded using an automatic weather station (Metos, Pessl Instruments, Weiz, Austria) placed within the experimental orchard.

Stem water potential

Stem water potential (Ψ_{stem}) was measured at midday (between 12:00 h and 13:00 h), every week from the beginning of the experimental treatments, using a Scholander-type pressure chamber according to Shackel *et al.* (2000). The measurements were done on terminal shoots that had been bagged in plastic envelopes covered with aluminium foil at least 2 h before measurements. From each selected tree, two short terminal shoots of the current year with four fully expanded leaves were used. Shoots were selected from mid-canopy on the shaded zones of the trees. After detachment from the canopy, shoots were immediately enclosed in the pressure chamber.

Growth, flowering and fruiting measurements

From each selected tree, six branches chosen from the entire canopy were tagged. For each branch, an average of eighty inflorescences was used to measure inflorescence growth, flowering timing, flower density (number of flowers/inflorescence, Fln/Infl), fruit density (number of fruits/inflorescence, Frn/Infl) and the percentage of fruit set [(Frn/Fln) × 100]. At harvest time, each individual tree was hand-harvested and fruit yield was quantified. From each tree, three independent fruit samples of 1 kg each were taken to determine the maturity index (MI), oil yield, fatty acid composition and the total polyphenol content.

Fruit maturity was determined using the MI proposed by Beltrán *et al.* (2008). From each fruit sample, 100 randomly selected fruits were classified into the following categories: 0–olives with intense green or dark green epidermis; 1–olives with yellow or yellowish green epidermis; 2–olives with yellowish epidermis but with reddish spots or areas over less than half of the fruit; 3–olives with reddish or light violet epidermis over more than half of the fruit; 4–olives with black epidermis and totally white pulp; 5–olives with black epidermis and less than 50% purple pulp; 6–olives with black epidermis and violet (more than 50%) or purple pulp and 7–olives with black epidermis and totally dark pulp. With a to h being the number of fruits in each category, the MI was calculated using the following equation:

$$\begin{aligned}\mathrm{MI} = (&a \times 0 + b \times 1 + c \times 2 + d \times 3 + e \times 4\\ &+ f \times 5 + g \times 6 + h \times 7)/100\end{aligned}$$

Oil yield, fatty acid composition and total phenol content

Olive fruit samples were ground (knife mill) and lyophilised until complete dehydration. From each lyophilised sample, a 20-g aliquot was extracted with n-hexane using a Soxhlet apparatus following the IUPAC Standard Method (IUPAC, 1992). The solvent was removed using a rotary vacuum evaporator at 40°C. The oil content was gravimetrically determined and expressed as weight percent on dry basis ($\mathrm{g\,kg^{-1}}$ fruit, DB).

For fatty acid composition, oil samples of 0.5 g were subjected to alkaline saponification (1 N KOH in methanol). Unsaponificable matter was extracted with n-hexane. The fatty acid methyl esters of total lipids were obtained using 1 N H_2SO_4 in methanol and analysed by gas chromatography. Separations were made on a Supelcowax 10 fused-silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). Peaks were identified by comparing their retention times with those of authentic reference compounds (Torres *et al.*, 2009).

Total phenol content (TPC) in the fruit was analysed using 1-g aliquots of lyophilised fruit samples. The samples were homogenised with 40 mL n-hexane for 10 min. The hexane fraction was removed and the residue was twice re-extracted to allow removal of pigments and most of the lipid fraction. The residue was then shaken for 10 min with 80% (v/v) methanol (3 × 20 mL). After centrifugation (10 min at 3000 g), the hydromethanolic phases were combined and filtered through a 0.45 μm nylon filter. The filtrate was diluted properly and combined with the Folin–Ciocalteu reagent. The absorbance of the solution was measured at 725 nm. TPC was expressed as mg caffeic acid $\mathrm{g^{-1}}$ fruit.

Statistical analyses

Statistical differences between irrigation treatments were estimated from ANOVA test at the 5% level ($P \leq 0.05$) of significance, for all parameters evaluated. Whenever ANOVA indicated a significant difference, a pair-wise comparison of means by least significant difference (LSD) was carried out. The ANOVA took account of the blocks and tested the main effects and interaction between the two factors of cultivar and irrigation (F-test). Correlation analysis was performed employing Pearson's test. All statistical analyses were performed using the InfoStat program (InfoStat version 2008, National University of Córdoba, Córdoba, Argentina).

Results

Stem water potential

During the course of the differential irrigation application period, Ψ_{stem} evolution of both Arbequina and Manzanilla cultivars showed similar tendencies; in general, results from the first year matched well with those obtained for the second year (Fig. 1). There were minor differences among treatments during the first 2.5 months of the RDI application period. These mostly coincide with the colder winter period. The Ψ_{stem} values for T75 were fairly constant. In T0, T25 and T50 treatments the Ψ_{stem} decreased progressively throughout the course of the RDI application period. At the end of the experiment, the Ψ_{stem} decreased markedly in T0 and T25 treatments to values below −3.5 MPa, indicating a moderate water stress. The Ψ_{stem} measured 11 days after rewatering showed no significant differences among

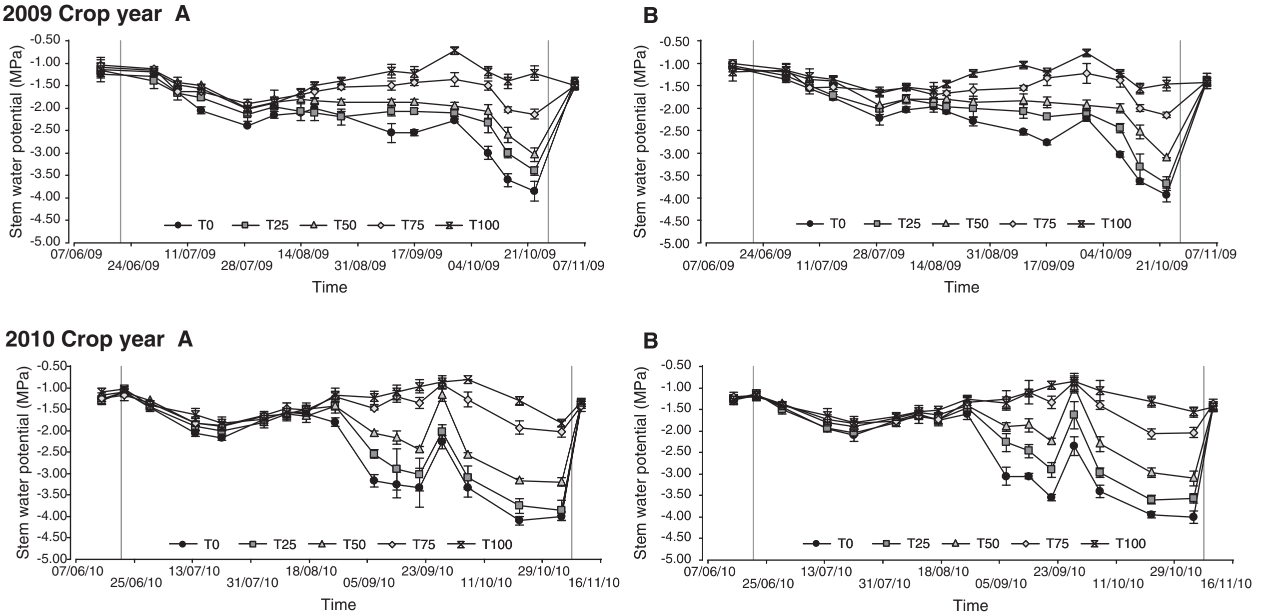

Figure 1 Midday stem water potentials from Arbequina (A) and Manzanilla (B) olive cultivars grown under different irrigation levels. T0, rain-fed treatment; T25, 25% of Etc; T50, 50% of Etc; T75, 75% of Etc; T100, full-irrigated (100% of Etc) treatment. Each point represents the average value (with standard deviation bar). From each selected tree (six trees per irrigation treatment) two independent measurements were done. Vertical bars indicate the period of regulated deficit irrigation.

irrigation treatments, and reached the values obtained before the beginning of the water deprivation period.

Vegetative shoot growth, flowering, fruiting and productive parameters

Irrigation rate significantly affected the current-year vegetative shoot growth in both 2009 and 2010 crop years (Fig. 2). For the first crop year, the final shoot length was reduced under the non-irrigated treatment (T0) by 82% and 66% of the shoot growth on fully irrigated trees (T100) of cvs Arbequina and Manzanilla, respectively. During the second crop year shoot growth reduction was approximately 72% in both of the two cultivars. In T75 and T100 treatments, the shoots developed intensely from the end of the winter season until the beginning of October, when their growth rate decreased.

Results from the first crop year showed that flowering and fruiting parameters were significantly influenced by water deprivation (Table 2). When compared to treatments with higher irrigation rates, the lower irrigation rate treatments (T0, T25 and T50) produced a delay in the beginning of the flowering time in both cultivars (Fig. 3). The flowering period of the more stressed trees was also shorter but continued somewhat longer than that from the non-stressed treatment. No difference was found between T0 and T25 treatments in inflorescence length, flower and fruit density. These two treatments had significantly lower values than the T75 and T100 treatments, which likewise showed similar results. The T50 treatment differed significantly from all other treatments. The fruit set percentage showed no variation between T75 and T100 treatments, but it was increased by 26.4% (Arbequina) and 40.3% (Manzanilla) with respect to T0 and T25 treatments. All the above-mentioned flowering and fruiting parameters presented highly significant positive correlations with Ψ_{stem} values (Table 2). The MI and fruit weight decreased in parallel to increased irrigation rates. Fruits from treatments with reduced water supply began to mature sooner and had significantly higher fruit weights than those from treatments with higher water supply (Table 2).

Fruit yield increased dramatically with increased water supply (Table 2). The olive cultivars evaluated were affected differently by the irrigation levels employed. In ‘Arbequina’, the full-irrigated treatment had an average fruit yield of 90 kg tree^{-1}, almost 10 times higher than the average yield obtained from the control treatment (T0). At the full winter and spring irrigation (T100), the fruit yield was not significantly different from that of the T75 treatment. At these irrigation levels, fruit yield reached a plateau, suggesting that for the agro-ecological conditions of olive growing in central Argentina, ‘Arbequina’ olive trees irrigated until after fruit set at 75% of ETc could

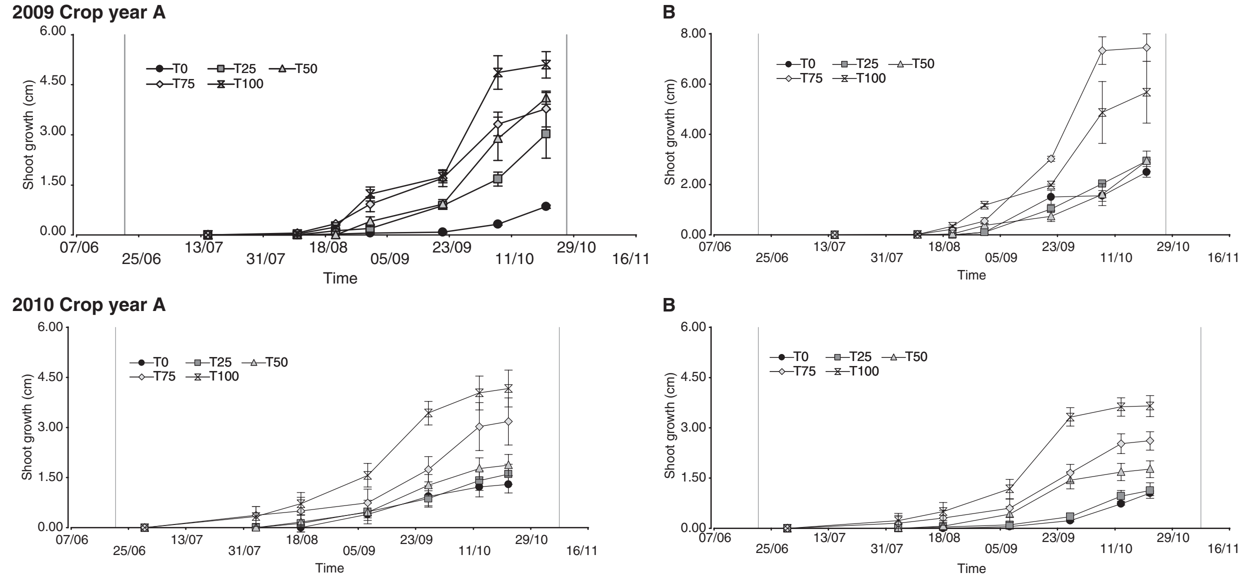

Figure 2 Vegetative shoot growth from Arbequina (A) and Manzanilla (B) olive cultivars grown under different irrigation levels. T0, rain-fed treatment; T25, 25% of Etc; T50, 50% of Etc; T75, 75% of Etc; T100, full-irrigated (100% of Etc) treatment. Each point represents the average value (with standard deviation bar). From each selected tree (six trees per irrigation treatment) six independent measurements were done. Vertical bars indicate the period of regulated deficit irrigation.

Table 2 Flowering and fruiting parameters from Arbequina and Manzanilla olive cultivars grown under different water irrigation levels during the 2009 crop year[a]

Cultivar, treatment	IL	Fln/I	Frn/I	Frn/Fln	MI	Fr W	Fr Y	Frn/T	WP
Cultivar									
Arbequina	18.28	15.98	0.52	2.42	2.69	1.72	47.8	29024	9.94
Manzanilla	20.61	14.32	0.36	3.16	1.85	2.93	71.6	26356	15.09
Treatment									
T0	16.08	11.70	0.25	2.12	2.89	2.57	14.7	5576	4.31
T25	16.60	13.45	0.31	2.24	2.85	2.52	31.6	12244	8.17
T50	18.05	15.05	0.48	3.18	2.02	2.41	48.7	20410	11.01
T75	22.65	17.30	0.56	3.18	1.80	2.09	89.6	44428	18.17
T100	23.85	18.25	0.60	3.22	1.81	2.05	114.0	55792	20.92
LSD$_{\text{Cultivar}}$	1.68	1.47	0.06	0.15	0.20	0.23	11.3	ns	2.26
LSD$_{\text{Treatment}}$	2.68	2.34	0.09	0.23	0.24	0.27	18.0	7462	3.60
LSD$_{\text{Interaction}}$	ns	ns	ns	0.33	ns	ns	ns	ns	ns
r	0.84^{*}	0.72^{*}	0.66^{*}	0.51^{*}	-0.86^{*}	-0.92^{*}	0.90^{*}	0.92^{*}	0.86^{*}

IL, inflorescence length (mm); Fln/I, flower number/inflorescence; Frn/I, fruit number/inflorescence; Frn/Fln, fruit number/flower number (%); MI, maturity index; Fr W, fruit weigth (g); Fr Y, fruit yield (kg tree^{-1}); Frn/T, fruit number/tree; WP, water productivity (kg of fresh fruit/mm of irrigation/ha). T0, rain-fed treatment; T25, 25% of Etc; T50, 50% of Etc; T75, 75% of Etc; T100, full-irrigated (100% of Etc) treatment; ns = non-significant ($P < 0.05$); *r*: Pearson's correlation coefficients between stem water potential and each flowering and fruiting parameters.

* Significant at $P \leq 0.01$.

[a] The least significant difference (LSD) is shown when ANOVA indicates a significant effect ($P < 0.05$).

reach the maximum yield potential. Fruit yield of 'Manzanilla' trees increased linearly in accordance with the increased water supply during the early stage of the season. The rate of increment, however, was lower than that observed for cv. Arbequina. Thus, the average yield was approximately seven times higher at the T100 treatment than that of T0.

Fruit yield was also estimated as a function of the winter and spring irrigation water applied per area. This yield estimation is known as water productivity (WP) and has been reported as a useful parameter to evaluate the efficacy of irrigation management strategies (Moriana *et al.*, 2003; Fereres & Soriano, 2007; Correa-Tedesco *et al.*, 2010). During the first crop year analysed, the

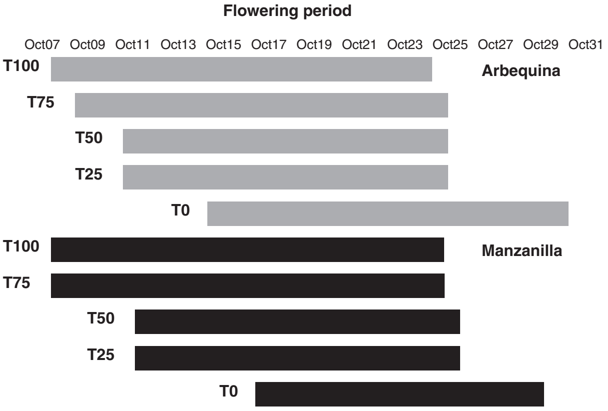

Figure 3 Flowering time from cvs. Arbequina and Manzanilla grown under different water irrigation levels. T0, rain-fed treatment; T25, 25% of Etc; T50, 50% of Etc; T75, 75% of Etc; T100, full-irrigated (100% of Etc) treatment. From each selected tree (six trees per irrigation treatment) six independent measurements were done.

treatments T100 and T75 did not present significant differences in the estimated WP. In these treatments, WP values increased in cv. Arbequina about 5X and in cv. Manzanilla 3.3X compared to those obtained from the respective T0 treatments.

During the second crop year the different levels of winter-spring irrigation had no significant effect on the flowering time (data not shown), fruiting and production parameters (Table 3).

Oil accumulation, fatty acid composition and TPC

Results from the first crop year showed that oil yield (kg tree^{-1}) was significantly affected by irrigation treatments, being observed higher oil yield in the less water-stressed treatments (Table 4). The opposite trend was observed for oil content (g kg^{-1} fruit). During the second crop year, there were no significant differences among treatments in oil yield and oil content (Table 5).

For the first crop year evaluated the oils from cv. Arbequina subjected to the different winter-spring irrigation treatments showed significant variations in the concentrations of the major fatty acids (Table 4), being observed higher oleic acid contents in the most irrigated treatments. The winter-spring irrigation treatments had no significant effect on fatty acid composition of oils from cv. Manzanilla (Tables 4 and 5).

For the first crop year analysed, in both cultivars evaluated, the irrigation treatments with the highest winter-spring water supplies (T100 and T75) gave fruits with significantly higher TPCs (Table 4). For the second crop year, no significant differences in TPC were found among the RDI treatments (Table 5).

Discussion

Stem water potential

Stem water potential (Ψ_{stem}) measurements were used to monitor the response of the water status in olive trees in relation to irrigation. There are no references for optimal Ψ_{stem} values for the agroecological conditions of olive cultivation in central Argentina. Our Ψ_{stem} initial values coincided with those observed by Rousseaux *et al.* (2008), who reported mild reductions in leaf water potentials from olive trees that were not irrigated during the winter in arid northwestern Argentina.

In this study, we applied a low K_c (0.4) for irrigating at 100% ETc during the winter months of June, July, and August and then switched to a higher K_c of 0.68 for September and October. By mid-winter, low water potentials (< -1.5 MPa) were recorded, even in plants under high (75%) or full (100%) ETc irrigation rates. Looking at the Ψ_{stem} records obtained from trees irrigated at 100% Etc, and taking into account the threshold values suggested by Moriana *et al.* (2012) (-1.2 MPa before the beginning of the massive pit hardening and -1.4 MPa during this period and until harvest), it appears that those trees could have undergone a mild level of water stress. However, two main aspects should be considered. First, the mentioned threshold values were obtained during the fruit development period. Second, and perhaps more important, Ψ_{stem} thresholds are function of climate and soil conditions. Therefore, Ψ_{stem} records are not always really comparable between time and regions. In any case, those low water potentials obtained at mid-winter do not seem to have influence on crop performance. According to Pavel & Fereres (1998), they may be attributed to the colder winter temperatures which have been shown to affect the Ψ_{stem} values even under conditions where soil water content is not limiting. To sum up at this point, it could say that the K_c values used for full irrigation (100% ETc) seem to be sufficient to maintain Ψ_{stem} at appropriate levels during the dry winter-spring period in the agroecological conditions of olive cultivation in central Argentina. However, the behaviour observed after the water deficit period indicates a rapid response to rewatering and suggests good hydraulic conductance characteristics, in spite of the large size (4–6 m in height) and advanced age (70 years old) of the olive plants employed. Thus, most of the Ψ_{stem} recovery could be attributed to a large increase in root flow.

Flowering, fruiting and productive parameters

According to Rapoport *et al.* (2012), under limiting water availability, the development process of inflorescences, flowers, ovary and seed primordial could affect the

Table 3 Flowering and fruiting parameters from Arbequina and Manzanilla olive cultivars grown under different water irrigation levels during the 2010 crop year[a]

Cultivar, treatment	IL	Fln/I	Frn/I	Frn/Fln	MI	Fr W	Fr Y	Frn/T	WP
Cultivar									
Arbequina	19.34	17.10	0.30	1.74	3.91	1.42	9.68	6898	4.92
Manzanilla	19.17	13.34	0.18	1.33	2.42	2.62	15.81	6014	8.15
Treatment									
T0	18.70	14.70	0.23	1.53	3.10	1.99	12.08	6049	10.96
T25	18.53	14.77	0.21	1.36	3.09	1.99	14.25	7659	8.47
T50	19.45	15.10	0.27	1.72	3.19	1.94	11.67	5977	5.11
T75	19.80	15.95	0.24	1.43	3.20	1.96	12.63	6369	4.31
T100	19.80	15.58	0.27	1.63	3.27	2.25	13.10	6225	3.83
$\mathrm{LSD}_{\mathrm{Cultivar}}$	ns	1.41	0.06	0.28	0.40	0.15	1.30	ns	0.68
$\mathrm{LSD}_{\mathrm{Treatment}}$	ns	ns	ns	ns	ns	ns	ns	ns	1.09
$\mathrm{LSD}_{\mathrm{Interaction}}$	ns	ns	ns	ns	ns	ns	ns	ns	1.54

IL, inflorescence length (mm); Fln/I, flower number/inflorescence; Frn/I, fruit number/inflorescence; Frn/Fln, fruit number/flower number (%); MI, maturity index; Fr W, fruit weigth (g); Fr Y, fruit yield (kg tree^{-1}); Frn/T, fruit number/tree; WP, water productivity (kg of fresh fruit/mm of irrigation/ha). T0, rain-fed treatment; T25, 25% of Etc; T50, 50% of Etc; T75, 75% of Etc; T100, full-irrigated (100% of Etc) treatment; ns = non-significant ($P < 0.05$).

[a] The least significant difference (LSD) is shown when ANOVA indicates a significant effect ($P < 0.05$).

Table 4 Oil yield and chemical parameters of olive fruits from Arbequina and Manzanilla cultivars grown under different water irrigation levels during the 2009 crop year[a]

				Fatty acids					
Cultivar, treatment	OY	OC	TPC	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic
Cultivar									
Arbequina	9.04	475.4	6740	20.68	2.71	1.57	52.14	21.97	0.87
Manzanilla	10.64	384.8	13996	16.23	1.88	1.46	75.86	3.60	0.98
Treatment									
T0	2.58	463.2	7515	18.98	2.47	1.49	62.53	13.44	0.89
T25	5.47	450.4	9056	18.70	2.46	1.57	62.72	13.60	0.95
T50	8.15	430.0	9846	18.08	2.08	1.44	64.93	12.61	0.87
T75	14.53	405.1	12460	18.37	2.30	1.58	64.53	12.26	0.97
T100	18.47	401.9	12963	18.12	2.16	1.50	65.31	12.01	0.94
$\mathrm{LSD}_{\mathrm{Cultivar}}$	ns	12.3	1489	0.42	0.20	ns	1.03	0.75	0.06
$\mathrm{LSD}_{\mathrm{Treatment}}$	2.84	19.6	2369	ns	ns	ns	1.63	1.19	ns
$\mathrm{LSD}_{\mathrm{Interaction}}$	ns	27.9	3375	ns	ns	ns	2.33	1.69	ns

OY, oil yield (kg tree^{-1}) /; OC, oil content (g kg^{-1} fruit, DB); TPC, total phenol content (mg g^{-1} fruit, DB); fatty acid composition (% of total fatty acids). T0, rain-fed treatment; T25, 25% of Etc; T50, 50% of Etc; T75, 75% of Etc; T100, full-irrigated (100% of Etc) treatment; ns = non-significant ($P < 0.05$).

[a] The least significant difference (LSD) is shown when ANOVA indicates a significant effect ($P < 0.05$).

fruitfulness of the olive tree. In this study, we found that the relationship number of fruits/number of flowers was significantly lower in the treatments with higher water restriction (T0 and T25) indicating that water deficit during the pre-flowering–flowering period, besides limiting floral development, also causes a decrease in fruit set.

The maturation period of the olive fruit is variable and is mainly affected by the varietal characteristics, climatic conditions and fruit load level (Beltrán *et al.*, 2008). Considering that during fruit ripening all olive plants received the same amount of water, the differences in maturation cannot be directly attributed to a reduced water status, but rather to fruit load. The effect of fruit load on fruit size and maturation has previously been established worldwide (Gucci *et al.*, 2007; Beltrán *et al.*, 2008; Martín-Vertedor *et al.*, 2011). In our experiments, we also observed a highly significant negative correlation between fruit load (number of fruit/tree) and the rate of maturation expressed as MI ($r = -0.87$, $P < 0.01$ for cv. Arbequina and $r = -0.85$, $P < 0.01$ for cv. Manzanilla) and fruit weight ($r = -0.98$, $P < 0.01$ for cv. Arbequina and $r = -0.91$, $P < 0.01$ for cv. Manzanilla).

The second crop year may be considered an 'off' year with a limited potential of inflorescence initiation and thus basically also a low crop yield. This initial low production potential in the second year could be, at

Table 5 Oil yield and chemical parameters of olive fruits from Arbequina and Manzanilla cultivars grown under different water irrigation levels during the 2010 crop year[a]

				Fatty acids					
Cultivar, treatment	OY	OC	TPC	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic
Cultivar									
Arbequina	2.00	452.8	6.20	19.23	2.45	1.54	56.65	19.32	0.85
Manzanilla	2.56	399.1	12.58	16.28	1.93	1.44	75.50	3.71	0.93
Treatment									
T0	2.17	434.4	9.27	17.93	2.21	1.51	65.40	11.84	0.88
T25	2.57	424.3	9.29	17.58	2.14	1.49	66.11	11.51	0.88
T50	2.11	428.5	9.64	17.77	2.18	1.44	66.41	11.32	0.93
T75	2.21	429.9	9.36	17.84	2.22	1.51	65.99	11.56	0.90
T100	2.32	412.7	9.40	17.64	2.21	1.48	66.47	11.34	0.90
$LSD_{Cultivar}$	0.27	1.69	0.40	0.23	0.17	ns	0.65	0.42	ns
$LSD_{Treatment}$	ns	2.69	ns	ns	ns	ns	ns	ns	ns
$LSD_{Interaction}$	ns	3.83	ns	ns	ns	ns	ns	ns	ns

OY, oil yield (kg tree^{-1}); OC, oil content (g kg^{-1} fruit, DB); TPC, total phenol content (mg g^{-1} fruit, DB); fatty acid composition (% of total fatty acids). T0, rain-fed treatment; T25, 25% of Etc; T50, 50% of Etc; T75, 75% of Etc; T100, full-irrigated (100% of Etc) treatment; ns = non-significant ($P < 0.05$).

[a] The least significant difference (LSD) is shown when ANOVA indicated a significant effect ($P < 0.05$).

least, a partial reason for the low sensitivity of the trees to winter-spring water limiting irrigation treatments. As the second crop year studied was in the 'off' phase its potential and actual final yield were significantly lower than the yield in the first studied year, which was in the 'on' phase of the production cycle.

In nature, the olive is an alternate fruit-bearing species, with alternate seasons of high ('on') and low ('off') yielding years. This was also the case in the orchard we used for our study. As in most intensive olive growing orchards worldwide, our results showed that even full irrigation conditions (T100) throughout the whole study (two crop years) could not sustain fruit set and production in the second ('off') crop year. In our study, lower yields recorded in less irrigated treatments during the first ('on') year did not correlate with higher yields in the following 'off' season. These results highlight the alternate bearing nature of both olive cultivars we used and evaluated. Thus, the average fruit yields of all treatments in the 'off' year was only 10.6% in 'Arbequina' and 11.4% in 'Manzanilla' of the respective fruit yields obtained at fully irrigated condition during the first ('on') crop year. It could be concluded that reducing the available water during inflorescence development and flowering has a direct impact on olive flower development, and thus yield, regardless of whether the trees are entering an 'on' or 'off' year.

Oil accumulation, fatty acid composition and TPC

Contradictory results about olive oil yield responses to changes in water supply are found in the literature. Motilva *et al.* (2000) observed a trend in which oil yield at harvest was higher under limiting irrigation conditions, probably as a consequence of lower fruit water content. Patumi *et al.* (2002) reported that the irrigation regime did not cause any variation in oil accumulation in the fruit; therefore, oil yield reflected the fruit yield pattern. Gucci *et al.* (2007) hypothesised that the decrease of oil concentration in the fruits of high fruit yielding trees is associated with the reduced availability of photo-assimilates affecting oil synthesis, rather than fruit growth itself. However, Trentacoste *et al.* (2010) found that low source–sink ratios reduced both oil and fresh fruit weight, thus conserving the fruit oil concentration.

Considering the period in which RDI was applied, it is improbable that irrigation had a direct influence on oil accumulation. Oil yield results from a combination of different parameters, such as fruit yield–which is related to both fruit weight and number of fruits per tree–and fruit oil content, which is primarily dependent on the summer thermal environment and the fruit maturation stage. Differences in oil yield between irrigation treatments during the first crop year can be primarily attributed to differences in fruit yield. Data from both oil yield and fruit yield adjusted well to a linear regression model with R^2 values close to 0.95. However, the oil yield response should also be considered in relation to fruit oil content and fruit maturity. So, although there was a significant increase in oil content found at lower water application levels, this phenomenon can be explained by differences in fruit maturity at the time of harvest based on the various irrigation treatments. The highly significant positive correlations between fruit oil content and MI give support to this hypothesis. For the first crop year evaluated, the MI of the 'Arbequina' olives from trees under higher water deficit (T0 and T25) was around 3.5. In this cultivar, the oil content, expressed as % of dry matter, increased during ripening until an MI of 3–4 is reached (Torres *et al.*, 2009). This means that the oil in fruits from the most stressed treatments reached their potential maximum at the time the olives were harvested. These data also indicate that, in order to optimise oil yield, it may be more beneficial to harvest trees with a high fruit load (T100 and T75) later than those with a low fruit load.

During the second crop year, the behaviour observed in oil yield and oil content in the fruits was in line with the lack of variation in fruit yield and fruit maturity. This behaviour reinforces the relationship between all fruiting parameters–where reduced fruiting and productive performance likewise affected oil accumulation.

Regarding fatty acid composition, the most noteworthy finding was a gradual increase in oleic acid content parallel to the increase in irrigation water supply. This fact was only observed in cv. Arbequina and might be explained as a function of the fruit maturity, rather than a direct effect of the irrigation level. Recently, we observed that oleic acid content in oil from cv. Arbequina cultivated in central Argentina falls as soon as an MI of 2–2.5 is reached (unpublished data). In this study, fruits from the most water-stressed treatments reached the highest MI (around 3.5) and their oils had the lowest oleic acid contents. However, results from the second crop year showed that fruits harvested from the various irrigation treatments did not vary in their MI (3.89 in average) (Table 2) and their oils did not present significantly different oleic acid contents (Table 4). Differently, the irrigation treatments did not show significant variations in oleic acid content of oils from cv. Manzanilla. It is possible that at the fruit maturity obtained at harvest (1.85 and 2.10 average for the first and second crop years, respectively) this cultivar reached the highest potential oleic acid content. We have observed that during fruit ripening the cv. Manzanilla early reaches the maximum oleic acid content (approximately 75%) at MI between 1.5 and 2.5, and then it remains constant until the end of the fruit maturation process (unpublished data).

During the first crop year, differences between RDI treatments in TPC may be mainly attributed to differences in fruit maturity. In fruits from cv. Arbequina, the TPC reaches the highest concentration at MI between 2 and 2.5, after which it decreases (Uceda *et al.*, 2008). For the second crop year, no significant differences in TPC were found among irrigation treatments, thereby enhancing our hypothesis that crop load affects the MI, and the latter the TPC.

Conclusions

Data from this study indicate that water deficit imposed to olive trees during winter and spring months (a period with an intensifying water shortage and high evapotranspiration in the olive growing areas in Argentina) has a clear negative impact on reproductive and productive parameters of both Arbequina and Manzanilla cultivars evaluated. The phenological phases coinciding with the pre-flowering–flowering period were affected markedly by water availability indicating strong crop sensitivity to water deficit in that period. Water deficit applied at early June, approximately 4 months prior to bloom, reduced vegetative shoot growth and affected flowering timing, resulting in weakening of flowering, shortening of the fruit maturation period and, ultimately, decreased fructification. For the first crop year evaluated, irrigation treatments with high (75% of ETc) and full (100% of ETc) winter-spring water supply presented significantly higher values of flower density, fruit density and final fruit yield which resulted in WP (kg fruits mm^{-1} of irrigation ha^{-1}) enhancements of about 500% (cv. Arbequina) and 330% (cv. Manzanilla) with respect to those obtained from the corresponding unirrigated treatments. A strong drop in flowering and fruiting parameters was observed during the second crop year. As a result, a marked difference in fruit production was found between the first ('on') and the second ('off) crop years, showing a strong alternate bearing pattern for both cultivars tested. Reducing the available water during inflorescence development and flowering had a deleterious effect on fruit set regardless of whether the trees were entering an 'on' or 'off' year. Likewise, the maintenance of the fully irrigated condition throughout the whole irrigation experiment (two crop years) did not sustain fruit yield performance in the second year. Regarding fruit oil content and composition, differences observed between irrigation treatments may be primarily attributed to variations in fruit maturity at the time of harvest, rather than a direct effect of the irrigation level itself. Significant differences in fatty acid composition were only found for the first crop year evaluated. These were stronger in cv. Arbequina where a gradual increase in oleic acid content was registered in parallel to the increase in irrigation water supply. Finally, from a practical stand point, it is important to note that results obtained from most of the analysed parameters were quite similar for both T75 and T100 treatments. Thus, the possible convenience of irrigation at T75% ETc should be considered since it may warrant profitable olive production while saving consistent amount of irrigation water in the olive production area in central Argentina.

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