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# Water relations, biochemical – physiological and yield responses of olive trees (*Olea europaea* L. cvs. Arbequina and Manzanilla) under drought stress during the pre-flowering and flowering period

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ABSTRACT

In arid and semiarid regions from Argentina, where the main olive production areas are located, evapotranspiration is high and rainfall is minimal during winter and spring months, as compared with the Mediterranean region where winter rainfall precludes the need of irrigation in such period. The aim of the work was to study water relations, biochemical–physiological and yield responses of olive trees (*Olea europaea* L., Arbequina and Manzanilla cultivars) under different drought stress levels applied during the pre-flowering–flowering period. Increasing levels of water deficit affected plant water relations as measured by pronounced drops of stem water potentials (near −4.0 MPa) in treatments with severe water deprivation at the end of the flowering period. Deficit irrigation was associated with some leaf-level biochemical-physiological responses (accumulation of osmotically active substances, increased concentration of high molecular weight hydrocarbons and cuticle thickening), which can be interpreted as adaptation mechanisms of olive to water deficit. Water stress was also associated with increased lipid peroxidation and decreased levels of photosynthetic pigments, stomatal conductance and photosynthetic rate. During the first crop year analyzed, a significant decrease in fruit set and fruit yield was observed in treatments under water deprivation. Also, all treatments evaluated showed strong drops in fruiting and yield parameters during the second crop year suggesting a marked bearing pattern for both olive cultivars. From a practical standpoint, little irrigation (50% ETc) may be sufficient to maintain adequate plant water potentials for the coldest winter months, but high (75% ETc) or full (100% ETc) irrigation rates could be needed by mid-August (approximately 2 months before flowering) to avoid detrimental effects of water stress on biochemical–physiological and yield parameters of olive trees cultivated in areas with dry winter-spring season.



## 1. Introduction

Olive has been traditionally cultivated in countries from the Mediterranean Basin, under limited water availability conditions. Olive trees are able to tolerate low soil water availability by means of morphological and physiological adaptations acquired in response to drought stress (Bacelar et al., 2004, 2006; Connor, 2005; Sofo et al., 2008). Many studies have reported the capacity of olive to resist arid environments (Connor, 2005; Connor and Fereres, 2005; Tognetti et al., 2005; Sofo et al., 2008; Boughalleb and Hajlaoui, 2011). According to these studies, the biochemical, physiological and yield responses of the olive plant to water deficit are quite variable, depending mainly on the phenological phase, intensity of stress, cultivar and other environmental conditions.

Anatomical responses of olive plants to low water availability are complex and can involve reductions in leaf area and stomatal density, increased sclerophylly and thick cuticle (Bacelar et al., 2004, 2006; Boughalleb and Hajlaoui, 2011). The leaf cuticle provides a protective barrier between the plant and the environment playing a crucial role as a barrier to water loss. Under limited water availability, the plant produces fruits with high cuticular thickness and repellent layers (wax and cutin) (Patumi et al., 2002). Hydrophobicity of the cuticle may be associated to the relative composition of the hydrocarbon fraction of the cuticular wax (Bondada

*Abbreviations:* Car, Carotenoids; Chl-*a*, Chlorophyll a; Chl-*b*, Chlorophyll b; DW, Dry weight; ETc, Estimated crop evapotranspiration; ETo, Reference evapotranspiration; EV, Ending value; GC, Gas chromatography; GC - MS, Gas chromatography–mass spectrometry; gs, Stomatal conductance; IV, Initial value; IWP, Irrigation water productivity; MDA, Malondialdehyde; Phae, Phaeophytin; $P_n$, Photosynthetic rate; PRO, Proline; RDI, Regulated deficit irrigation; ROS, Reactive oxygen substances; TLC, Thin layer chromatography; $\Psi_{stem}$, Stem water potential.

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et al., 1996; Kim et al., 2007). In olive leaves, this fraction is mainly composed of long-chain, higher molecular weight n-alkanes (Bianchi, 1995). Hydrocarbons and other leaf wax components are present in the form of crusts on the leaf surface and assist in the maintenance of turgor by reflecting a high proportion of incident radiation (Baker and Procopiou, 2000). Interestingly, Bondada et al. (1996) and Kim et al. (2007) reported that water deficit increased the long-chain n-alkane content in leaf waxes from cotton and sesame plants. In olive plants, the cuticular wax composition has been described for both leaves and fruits (Bianchi et al., 1992, 1993) but the possible changes in the chemical profile in response to drought stress have not been examined.

Regarding biochemical mechanisms for maintenance of appropriate plant water status during water deficit, the accumulation of osmolytes such as proline (PRO) and sugars is a well-known mechanism against water stress in the olive tree (Sofo et al., 2004; Ben Ahmed et al., 2009). PRO can accumulate to high concentrations without damaging cellular macromolecules, and can prevent membrane damage and protein denaturation during drought stress (Ain-Lhout et al., 2001). An important chemical impairment occurring in plants subjected to water stress is due to the production of reactive oxygen substances (ROS). ROS can promote membrane lipid peroxidation giving hydroperoxy fatty acids that are toxic to cells. Furthermore, when the accumulation of ROS exceeds the removing capacity of the antioxidant system, the effects of oxidative damage can reach cellular proteins and photosynthetic pigments (Bacelar et al., 2006, 2007). So, if the environmental conditions become more stressful, $CO_2$ fixation and net photosynthetic rate might be limited by reductions in chlorophyll content (chlorophyll bleaching).

One of the most significant changes that are currently occurring in olive tree cultivation is the expansion of irrigated orchards. Strategies using regulated deficit irrigation (RDI) have been proposed to optimize water use in olive growing, and several studies have evaluated the effects of RDI on the physiological behavior, yield responses and classical quality parameters of olive oil (Moriana et al., 2003; Bacelar et al., 2007; Lavee et al., 2007; Servili et al., 2007; Sofo et al., 2008). Most of these studies have been conducted in the Mediterranean countries where irrigation is normally suspended during the winter months because of rainfall is high, and cold and cloudy conditions lead to low values of evapotranspiration (Connor and Fereres, 2005). Thus, the effects of water limitation during the developmental phases prior to fruit set have seldom been evaluated (Hartmann and Panetsos, 1961; Rousseaux et al., 2008; Rapoport et al., 2012). Rousseaux et al. (2008) examined the effect of irrigation suppression during the dry winter season at La Rioja province (Argentina). They reported no significant changes in fruit yields from olive trees that were not irrigated for 6–7 weeks (from mid-July to the end of August) as compared with full-irrigated plants. Similarly, Rapoport et al. (2012) showed that water deficit during winter dormancy had no effect on fruiting parameters; however, water deficit applied prior to bloom, or during a period covering flowering and initial fruit set, resulted in lower fruit yields.

Arbequina and Manzanilla are the most extensively planted olive cultivars in central Argentina. While Arbequina is a typical oil-producing cultivar, Manzanilla is devoted to both oil production and table olive production. Despite the importance of these two cultivars in Argentina, there is very little information documenting the olive plant responses to drought during the dry winter-spring season. In this country, the main olive production areas are characterized by a marked water deficit (high evapotranspiration and minimal rainfall) from early winter to mid-spring (Rousseaux et al., 2008), as compared with the Mediterranean region (Connor and Fereres, 2005). Basically, such period covers the winter dormancy, flower differentiation, and floral opening (Rapoport et al., 2012). The objective of the present study was to evaluate water relations, and biochemical–physiological and yield responses in two major Spanish olive cultivars subjected to water deficit during the dry season in central Argentina.

## 2. Materials and methods

### 2.1. Plant material and experimental design

The field experiment was conducted in a commercial olive orchard located near the Cruz del Eje locality, a typical olive growing area in central Argentina. Cruz del Eje (lat. 30°39′S, long. 64°57′W) is located in the dry Chaco Forest phytogeographical area, at 450 m above sea level. The climate in this area represents a typical arid Chaco climate with rains mostly falling in summer and dry winter and spring months. The average value of annual rainfall is 550 mm, with a relative humidity of about 53%. Annual rainfalls are distributed as follow: 330 mm in summer (December 21–March 20), 120 mm in autumn (March 21–June 20), 10 mm in winter (June 21–September 20) and 90 mm in spring (September 21–December 20).

The soil is typical Haplustol (60–65 cm in deep), characterized 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. 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 experiment, the initial soil water content was around 13.5% (W/W) for both 2009 and 2010 crop years evaluated. Soil water content was also near 13.5% at the end of each research period 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 cultivars (Arbequina and Manzanilla) were used. Both cultivars were grown in the same orchard but in different plots. During 2009 and 2010 crop years, four irrigation treatments were applied to 70-year-old trees with planting distances of 10 × 10 m. During both 2007 and 2008 crop years, the trees had been irrigated with drip irrigation. These crop years were taken as an adaptation period and they were not considered for the scheduled biochemical–physiological and yield analyses. Irrigation water was delivered using two drip lines around each tree, with drip emitters (Axios, Palaplast, Thessaloniki, Greece) giving 4 L/h (T75 and T100 treatments) or 2 L/h (T25 and T50 treatments), located at 1 m from the trunk. Irrigation events were performed weekly. The experimental design included a treatment irrigated at 100% of ETc (estimated crop evapotranspiration) during all year (T100), and three RDI treatments, at 25%, 50% and 75% of ETc (T25, T50 and T75 respectively). The differential irrigation treatments (T25, T50 and T75) were imposed to olive trees for approximately 5 months, from the end of autumn to mid-spring. The experimental design also included a rain-fed treatment (T0) consisting of olives trees that were not irrigated between the end of autumn and mid-spring. During the rest of the year, T0, T25, T50 and T75 treatments were irrigated at 100% of ETc. A randomized block design with three sub-plots for each combination of irrigation level × cultivar was used. Each experimental sub-plot consisted of twelve trees, where the two central ones were selected for all measurements, while surrounding trees were considered border-guard trees.

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: $ETc = ETo \times K_c \times K_r$, where ETo is the reference evapotranspiration, $K_c$ is the crop coefficient, and $K_r$ is the coefficient of reduction

**Table 1**
Average monthly temperatures (°C) and rainfall (mm), wind speed (m $s^{-1}$), reference evapotranspiration (ETo, mm $day^{-1}$) and estimated crop evapotranspiration (ETc, mm $day^{-1}$) in Cruz del Eje during the differential irrigation application period for both 2009 and 2010 crop years.

| Month | 2009 crop year | | | | | 2010 crop year | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Temperature | Rainfall | Wind speed | ETo | ETc | Temperature | Rainfall | Wind speed | ETo | ETc |
| June | 12.6 | 0 | 2.47 | 2.36 | 0.95 | 11.7 | 1.6 | 1.06 | 1.61 | 0.64 |
| July | 10.2 | 16 | 2.05 | 2.16 | 0.87 | 10.0 | 0 | 1.86 | 2.33 | 0.93 |
| August | 16.6 | 0 | 2.62 | 3.55 | 1.42 | 12.6 | 1.6 | 2.39 | 3.35 | 1.34 |
| September | 14.3 | 26 | 2.95 | 4.10 | 2.79 | 17.1 | 9.8 | 3.07 | 4.82 | 3.28 |
| October | 21.6 | 5.4 | 3.05 | 6.09 | 4.14 | 19.4 | 2.6 | 2.13 | 5.50 | 3.74 |

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 (Kpan, 0.75) proposed by Allen et al. (1998). During the April–August period, we assumed a $K_c$ value equal to 0.4 as suggested by Rousseaux et al. (2008) for olive trees growing under the conditions prevailing at 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). A Kr of 1 was used for the 53% crop cover.

Table 1 summarizes climatic conditions, ETo and ETc in Cruz del Eje during the differential irrigation application period for both 2009 and 2010 crop years. Meteorological data were recorded by using an automatic weather station (Metos, Pessl Instruments, Weiz, Austria) placed within the experimental orchard. These data were also used to calculate daily ETo values through the Penman-FAO equation (Orgaz and Fereres, 2008). The calculated ETo values were in agreement with those obtained by using the evapotranspiration pan.

### 2.2. Stem water potential and gas exchange measurements

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

Leaf photosynthetic rate ($P_n$) and stomatal conductance (gs) were measured using a portable gas exchange system (LCpro+, ADC Bioscientific Ltd, Hertfordshire, UK) in open-system mode with a flow rate of 0.2 L $min^{-1}$ and leaf temperature within 2 - 3 °C of ambient air temperature (15 - 25 °C). Gas exchange measurements were done at light intensity saturating photosynthesis. Photosynthetic photon flux density was between 1200 - 1500 μmol $m^{-2}$ $s^{-1}$ during all measurements. Measurements were made approximately at biweekly intervals, between 11:00 and 12:00 h. From each selected tree, four mature, fully expanded leaves from North-exposed mid-canopy (current-year shoot) were used.

### 2.3. Biochemical parameters

During both 2009 and 2010 crop years, selected olive trees were sampled at two times: early June (initial value, IV) and the end of October (ending value, EV - 5 days before the irrigation replacement at 100% of ETc). From each tree, 100 g of sun-exposed, fully expanded, young and healthy leaves from the apical extreme of the shoots, selected from the mid-canopy of the entire perimeter of the tree, were collected. A portion of 5 g were immediately lyophilized, frozen and stored at -20 °C. The frozen leaves were finely ground in liquid nitrogen, and the frozen powder was used to determine PRO, malondialdehyde (MDA) and pigment concentrations. On the other hand, 10 fresh leaves from each selected tree were used for cuticle thickness, wax content, and hydrocarbon profile determinations.

#### 2.3.1. Proline, malondialdehyde and pigment contents

Free PRO and MDA contents were determined following the methods employed by Sofo et al. (2004) with some modifications.

PRO. A 5-ml aliquot of 3% (w/v) sulfosalicylic acid was added to 0.5 g of leaf powder and boiled in a water bath at 100 °C during 30 min in glass capped-tubes. The mixture was centrifuged at 2000 × *g* for 5 min at 25 °C. A 200-μL aliquot of the supernatant was mixed with 400 μL distilled water and 2 mL of the reagent mixture (30 mL glacial acetic acid, 20 mL distilled water and 0.5 g of ninhydrin), and boiled at 100 °C for 1 h. After cooling, the reaction mixture was mixed with 6 mL toluene. The toluene fraction containing the chromophore was separated and measured spectrofotometrically at 520 nm (Pelkin-Elmer, Shelton, CT, USA), using toluene as a blank. PRO concentration was calculated by fitting the results to a six-point standard curve made with known concentrations of the standard (L-proline).

MDA. A 0.2-g aliquot of frozen powder was added to 10 mL 0.5% (w/v) thiobarbituric acid in 20% (w/v) trichloroacetic acid. The mixture was heated at 100 °C for 30 min and then quickly cooled at ambient (22 °C) temperature. After centrifugation at 10000 × *g* for 10 min, the supernatant was filtrated through Whatman N° 1 filter paper, and the absorbance at 532 and 600 nm was measured. The value for specific absorption at 600 nm was subtracted to correct the results from the interference of soluble sugars in samples.

Pigment content. The procedures followed for the quantification of chlorophyll-a (Chl-*a*), chlorophyll-b (Chl-*b*), phaeophytins (Phae) and carotenoids (Car) are explained in detail in Carreras and Pignata (2001).

#### 2.3.2. Cuticle thickness, wax content and hydrocarbon profile

The cuticle thickness was assessed in leaf cross sections (10 fresh leaves from each selected tree) prepared for microscopic examination according to the protocol proposed by Maácz and Vagás (1961). Sections were taken from the middle of the leaves to avoid differential thickness along the leaf blade. The samples were fixed in FAE (formalin - acetic acid - ethanol), dehydrated through a series of ethanol/xylene, and embedded in Paramat. Sections were cut at 10 μm thickness, mounted serially and stained with astral blue and basic fuchsine. Upper cuticle thickness was registered with a photomicroscopy (Axiophot, Carl Zeiss, Oberkochen, Germany) and analysed using the PIC 486-LP (Kontron Elektronik, Eching, Germany) software.

For wax extraction, 10 g of whole, lyophilized leaves were immersed for 1 min in 100 mL chloroform at room temperature. The chloroform was evaporated under vacuum at 40 °C and total wax content was determined gravimetrically (Bianchi et al., 1993).

For n-alkane composition determinations, a 0.1-g aliquot of each wax sample was diluted with 1 mL chloroform and the solution was fractionated on preparative thin layer chromatography (TLC, silica gel, 0.5 mm), developed with n-hexane. After developing, the plate was revealed under iodine vapors. A separated zone containing the hydrocarbon fraction was removed from the plate and extracted with chloroform for subsequent gas chromatography (GC) and GC-mass spectrometry (GC-MS) analyses. GC (Perkin-Elmer, Shelton, CT, USA) used a VF–5 ms (Varian, Walnut Creek, CA, USA) capillary column (30 m × 0.25 mm i.d.) coated with a 0.25 μm layer of 5% phenyl, 95% polydimethylsiloxane. The column temperature was programmed from 70 to 300 °C at 4 °C $min^{-1}$, injector and detector temperatures at 320 °C, carrier gas $N_2$ at 1 mL $min^{-1}$. GC-MS (Hewlett-Packard, Palo Alto, CA, USA) used helium (flow rate 1 mL $min^{-1}$) as carrier gas. The column, injector and detector temperatures were as for GC analysis. Hydrocarbons were identified by their retention times compared with those of authentic reference compounds (Sigma-Aldrich, St. Louis, MO, USA) and comparison of their mass spectra data with those of the Wiley mass spectra search library.

### 2.4. Fruiting and yield parameters

From each selected tree, six branches having approximately three years-old, chosen from the mid-canopy, were tagged. For each branch, an average of eighty inflorescences (Farinelli et al., 2012) was used to measure the percent fruit set [(Frn/Fln) × 100], where Frn is the fruit number per inflorescence and Fln is the flower number per inflorescence. At harvest time, each individual tree was hand-harvested and fruit production (kg/tree) was quantified. Irrigation water productivity (IWP) was estimated as kg of fresh fruit per mm of irrigation per ha.

### 2.5. Statistical analyses

Statistical differences among 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. Correlation analyses were performed employing Pearson's test. All statistical analyses were performed using the InfoStat program (National University of Córdoba, Córdoba, Argentina).

## 3. Results and discussion

### 3.1. Plant water relations

Midday stem water potential ($\Psi_{stem}$) has been recommended as a useful tool to monitor the response of the olive tree water status to irrigation (Moriana et al., 2012). There are not references of optimal $\Psi_{stem}$ values for olive cultivation in central Argentina. From measurements in central Spain, midday $\Psi_{stem}$ values between -1.2 and -1.4 MPa are recommended as thresholds for irrigating mature olive orchards (Moriana et al., 2003).

For the first crop year evaluated, during the course of the differential irrigation application period, $\Psi_{stem}$ evolution for both Arbequina and Manzanilla cultivars showed similar patterns (Fig. 1). These matched well with those obtained for the second year (data not showed). In general, there were minor differences among treatments during the first 75 days of the RDI experiment. These mostly coincide with the colder, winter dormancy period. Similarly, Rousseaux et al. (2008) reported mild reductions in leaf water potentials from olive trees that were not irrigated during the winter in arid northwestern Argentina.

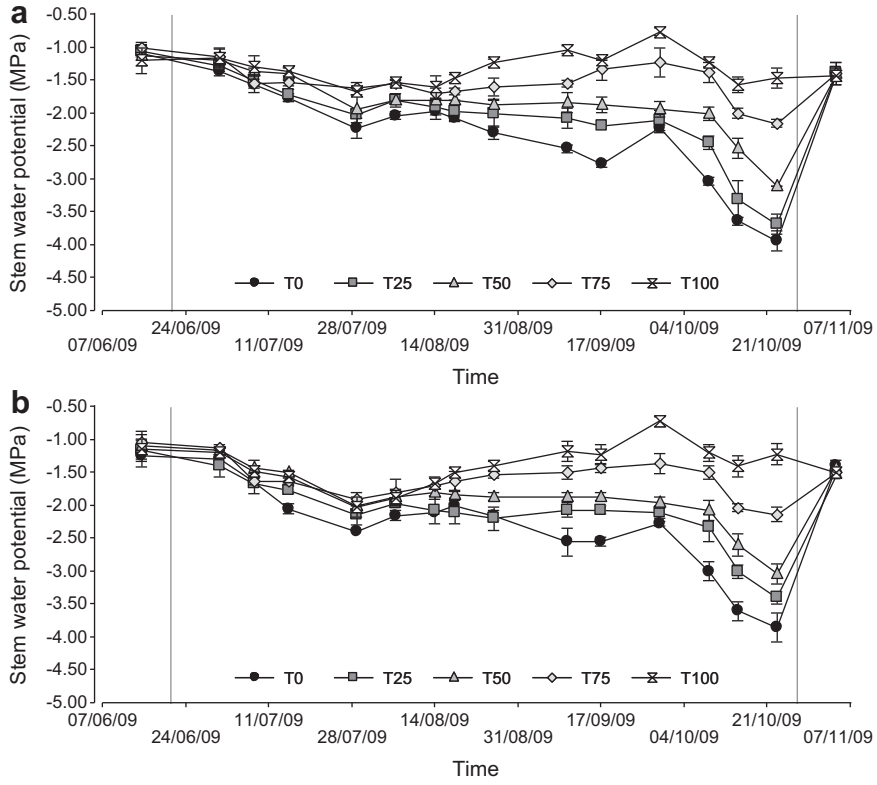


**Figure 1.** Midday stem water potentials obtained from Arbequina (a) and Manzanilla (b) cultivars growing under different water irrigation levels during the 2009 crop year. T0, rain-fed treatment; T25, 25% of Etc; T50, 50% of Etc; T75, 75% of Etc; T100, full-irrigated (100% of Etc) treatment. Each point represents the average value (with standard deviation bar) of 6 measurements. Vertical bars indicate the period of regulated deficit irrigation.

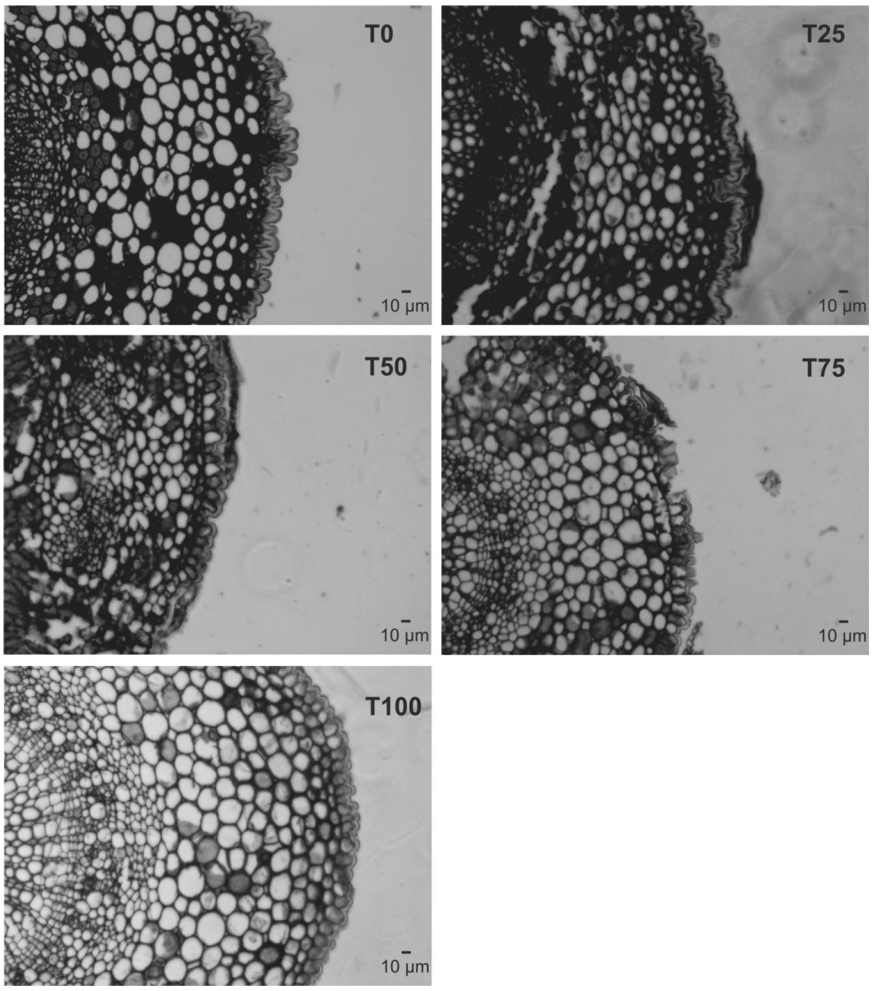


**Figure 2.** Microscopic observations of leaf cross sections (cv. Manzanilla) showing the cuticular layer thickening (μm) in leaves from plants arising from the rain-fed treatment (T0) with respect to RDI treatments (T25, 25% of Etc.; T50, 50% of Etc.; T75, 75% of Etc.) and full-irrigated (T100, 100% of Etc.) plants.

For T75, $\Psi_{stem}$ values were close to those from the full-irrigated treatment but they dropped to -2 MPa at the end of the RDI period. In T0, T25 and T50 treatments the $\Psi_{stem}$ decreased progressively throughout the course of the RDI assay. At the end of the experiment, the $\Psi_{stem}$ decreased markedly (below -3.5 MPa) in T0 and T25 treatments, indicating a moderate water stress.

After the differential irrigation application period, 11 days after rewatering, olive trees recovered the $\Psi_{stem}$ values measured before water deprivation and no significant differences among treatments were found. These observations indicate a rapid response to rewatering and suggest good hydraulic conductance characteristics, in spite of the big size (4 to 6 m in height) and age (70-years-old) of the olive plants employed.

In the present study we assumed a low Kc (0.4) for irrigating at 100% ETc for the winter months of June, July, and August and then switched to a higher Kc of 0.68 for September and October. Only the treatment irrigated at 100% ETc was fit for holding trees under non-water-stress conditions during the whole RDI period evaluated. Looking at the $\Psi_{stem}$ values obtained from this treatment and taking into account the threshold values suggested by Moriana et al. (2003), the Kc values used here could be sufficient to maintain $\Psi_{stem}$ at appropriate levels for the agroecological conditions of olive cultivation in central Argentina. Nevertheless, we registered low water potentials (< -1.5 MPa) by mid-winter even in plants under high (75% ETc) or full (100% ETc) irrigation rates. These records may be attributed to the coldest temperatures which are showed to affect the $\Psi_{stem}$ values even under conditions in which soil water content is not limiting (Pavel and Fereres, 1998).

### 3.2. Biochemical parameters

Microscopic observations of leaf cross sections indicated that irrigation treatments affected markedly the cuticular layer thickness (Fig. 2). In each crop year, at the end of the RDI application period (EV, Tables 2 and 3), significant increases in cuticle thickness were observed in T0, T25 and T50 with respect to T75 and T100 treatments. In Manzanilla cultivar, leaves from unirrigated plants (T0) had cuticles almost threefold thicker than leaves from full-irrigated (T100) plants. For both olive cultivars analyzed, significant negative correlations were found between $\Psi_{stem}$ and cuticle thickness (Table 4). This leaf-level plant response presented a reversible pattern: cuticle thickness increased undergoing water deprivation, but after drought-stress suppression and rewatering (IV from 2010 crop year, Table 3) cuticle thickness showed similar values to those obtained at the beginning of the RDI application period (IV from 2009 crop year, Table 2).

Tables 2 and 3 also show the results for leaf wax content. For each crop year analyzed, wax yields obtained before the RDI application period were in general agreement with those from two common olive cultivars cultivated in Italy (Bianchi et al., 1993). Some studies have reported that drought stress may cause an increase in the amount of wax deposited on leaves of many plants (Cameron

**Table 2**
Cuticle thickness (CT, μm), cuticular wax content (WC, mg/g) and n-alkane composition (%) from leaves of Arbequina and Manzanilla olive cultivars growing under different water irrigation levels during the 2009 crop year.

| | | Irrigation treatment | | | | |
|---|---|---|---|---|---|---|
| | | T0 | T25 | T50 | T75 | T100 |
| Arbequina | | | | | | |
| CT | IV | $8.32^{a,A} \pm 2.34$ | $8.27^{a,A} \pm 2.65$ | $9.41^{a,A} \pm 2.61$ | $9.27^{a,A} \pm 2.83$ | $7.58^{a,A} \pm 3.01$ |
| | EV | $11.8^{b,A} \pm 4.10$ | $12.4^{b,B} \pm 3.21$ | $8.25^{ab,A} \pm 2.36$ | $7.77^{a,A} \pm 2.54$ | $5.82^{a,A} \pm 2.03$ |
| WC | IV | $15.0^{aA} \pm 3.69$ | $15.7^{a,A} \pm 1.86$ | $15.7^{a,A} \pm 1.72$ | $15.9^{a,A} \pm 5.12$ | $15.2^{a,A} \pm 2.77$ |
| | EV | $15.5^{a,A} \pm 5.10$ | $22.0^{a,\ B} \pm 0.88$ | $14.8^{a,A} \pm 3.13$ | $16.6^{a,A} \pm 4.83$ | $20.2^{a,A} \pm 4.09$ |
| $C_{29}$ | IV | $11.9^{a,B} \pm 0.91$ | $11.8^{a,B} \pm 3.11$ | $10.7^{a,B} \pm 1.44$ | $13.9^{a,B} \pm 2.75$ | $11.0^{a,A} \pm 1.04$ |
| | EV | $6.85^{a,A} \pm 0.51$ | $6.84^{a,A} \pm 0.79$ | $6.42^{a,A} \pm 0.45$ | $9.14^{b,A} \pm 0.43$ | $9.63^{b,A} \pm 0.13$ |
| $C_{30}$ | IV | $2.65^{a,B} \pm 0.33$ | $2.67^{a,B} \pm 0.31$ | $2.46^{a,B} \pm 0.11$ | $2.86^{a,B} \pm 0.21$ | $2.41^{a,A} \pm 0.16$ |
| | EV | $1.81^{a,A} \pm 0.20$ | $1.98^{ab,A} \pm 0.06$ | $2.13^{b,A} \pm 0.11$ | $2.46^{c,A} \pm 0.18$ | $2.70^{c,A} \pm 0.22$ |
| $C_{31}$ | IV | $32.2^{a,B} \pm 1.49$ | $32.5^{a,B} \pm 0.99$ | $31.0^{a,A} \pm 1.26$ | $33.2^{a,A} \pm 2.18$ | $30.4^{a,A} \pm 0.36$ |
| | EV | $24.5^{a,A} \pm 1.42$ | $29.5^{b,A} \pm 1.95$ | $31.9^{b,A} \pm 1.28$ | $31.1^{b,A} \pm 1.78$ | $31.8^{b,B} \pm 0.46$ |
| $C_{32}$ | IV | $5.60^{a,A} \pm 0.21$ | $5.85^{a,A} \pm 0.41$ | $5.92^{a,A} \pm 0.20$ | $5.68^{a,A} \pm 0.36$ | $5.40^{a,A} \pm 0.22$ |
| | EV | $5.50^{a,A} \pm 0.31$ | $5.53^{a,A} \pm 0.23$ | $5.86^{a,A} \pm 0.09$ | $5.51^{a,A} \pm 0.31$ | $5.57^{a,A} \pm 0.35$ |
| $C_{33}$ | IV | $32.1^{a,A} \pm 1.59$ | $31.7^{a,A} \pm 3.07$ | $34.0^{a,A} \pm 2.31$ | $29.9^{a,A} \pm 2.53$ | $33.1^{a,A} \pm 1.26$ |
| | EV | $36.9^{c,B} \pm 1.02$ | $35.4^{b,A} \pm 0.55$ | $35.7^{bc,A} \pm 1.24$ | $33.8^{a,B} \pm 1.02$ | $33.1^{a,A} \pm 0.34$ |
| $C_{34}$ | IV | $3.62^{a,A} \pm 0.18$ | $3.65^{a,A} \pm 0.16$ | $3.48^{a,A} \pm 0.17$ | $3.30^{a,A} \pm 0.55$ | $3.82^{a,A} \pm 0.38$ |
| | EV | $5.32^{b,B} \pm 0.30$ | $4.36^{a,B} \pm 0.42$ | $3.99^{a,A} \pm 0.32$ | $4.02^{a,A} \pm 0.29$ | $3.89^{a,A} \pm 0.10$ |
| $C_{35}$ | IV | $11.9^{a,A} \pm 1.38$ | $11.7^{a,A} \pm 1.54$ | $12.4^{a,A} \pm 0.18$ | $11.1^{a,A} \pm 2.45$ | $13.7^{a,A} \pm 0.21$ |
| | EV | $19.1^{c,B} \pm 0.63$ | $16.3^{b,B} \pm 2.03$ | $13.9^{a,B} \pm 2.75$ | $13.9^{a,A} \pm 0.43$ | $13.3^{a,A} \pm 0.33$ |
| Manzanilla | | | | | | |
| CT | IV | $10.6^{a,A} \pm 2.91$ | $11.5^{a,A} \pm 2.78$ | $12.7^{a,A} \pm 3.98$ | $11.1^{a,A} \pm 2.82$ | $11.9^{a,B} \pm 2.66$ |
| | EV | $23.3^{c,B} \pm 3.91$ | $20.1^{c,B} \pm 3.69$ | $11.9^{b,A} \pm 2.03$ | $8.88^{a,A} \pm 2.43$ | $8.12^{a,A} \pm 1.94$ |
| WC | IV | $11.7^{a,A} \pm 0.79$ | $14.5^{a,A} \pm 2.07$ | $14.1^{a,A} \pm 0.71$ | $14.5^{a,A} \pm 2.43$ | $14.0^{a,A} \pm 2.65$ |
| | EV | $20.0^{a,B} \pm 4.57$ | $12.6^{a,A} \pm 2.50$ | $13.5^{a,A} \pm 0.76$ | $17.8^{a,A} \pm 2.07$ | $19.1^{a,A} \pm 4.86$ |
| $C_{29}$ | IV | $10.8^{a,A} \pm 2.20$ | $12.2^{a,B} \pm 0.77$ | $11.7^{a,B} \pm 2.76$ | $12.3^{a,B} \pm 1.47$ | $11.6^{a,A} \pm 2.02$ |
| | EV | $6.77^{ab,A} \pm 0.25$ | $7.55^{b,A} \pm 0.90$ | $6.08^{a,A} \pm 0.68$ | $9.11^{c,A} \pm 0.87$ | $9.60^{c,A} \pm 0.28$ |
| $C_{30}$ | IV | $2.34^{a,A} \pm 0.29$ | $2.64^{a,B} \pm 0.03$ | $2.88^{a,B} \pm 0.33$ | $2.55^{a,A} \pm 0.16$ | $2.67^{a,A} \pm 0.13$ |
| | EV | $1.95^{a,A} \pm 0.09$ | $2.04^{a,A} \pm 0.08$ | $2.02^{a,A} \pm 0.25$ | $2.56^{b,A} \pm 0.20$ | $2.53^{b,A} \pm 0.11$ |
| $C_{31}$ | IV | $30.4^{a,B} \pm 0.87$ | $30.8^{a,A} \pm 0.17$ | $30.6^{a,A} \pm 1.38$ | $30.9^{a,A} \pm 0.81$ | $30.7^{a,A} \pm 0.92$ |
| | EV | $24.7^{a,A} \pm 0.88$ | $29.4^{b,A} \pm 1.58$ | $30.4^{b,A} \pm 0.29$ | $31.1^{bc,A} \pm 2.09$ | $32.7^{c,B} \pm 1.37$ |
| $C_{32}$ | IV | $5.44^{a,A} \pm 0.18$ | $5.70^{a,A} \pm 0.27$ | $5.72^{a,A} \pm 0.46$ | $5.59^{a,A} \pm 0.27$ | $5.76^{a,A} \pm 0.45$ |
| | EV | $5.49^{a,A} \pm 0.38$ | $5.49^{a,A} \pm 0.30$ | $5.80^{a,A} \pm 0.55$ | $5.74^{a,A} \pm 0.51$ | $5.45^{a,A} \pm 0.27$ |
| $C_{33}$ | IV | $33.3^{a,A} \pm 2.72$ | $33.1^{a,A} \pm 0.86$ | $32.8^{a,A} \pm 2.01$ | $33.7^{a,A} \pm 2.46$ | $32.0^{a,A} \pm 1.25$ |
| | EV | $36.8^{d,A} \pm 0.52$ | $35.0^{bc,B} \pm 0.48$ | $36.5^{cd,B} \pm 0.48$ | $33.4^{ab,A} \pm 1.37$ | $32.8^{a,A} \pm 1.01$ |
| $C_{34}$ | IV | $3.94^{a,A} \pm 0.07$ | $3.70^{a,A} \pm 0.13$ | $3.86^{a,A} \pm 0.38$ | $3.52^{a,A} \pm 0.11$ | $3.75^{a,A} \pm 0.32$ |
| | EV | $3.70^{b,A} \pm 0.13$ | $4.17^{a,A} \pm 0.28$ | $4.23^{a,A} \pm 0.19$ | $4.09^{a,B} \pm 0.43$ | $3.93^{a,A} \pm 0.07$ |
| $C_{35}$ | IV | $13.7^{a,A} \pm 0.40$ | $11.7^{a,A} \pm 1.32$ | $12.3^{a,A} \pm 0.84$ | $11.4^{a,A} \pm 0.44$ | $13.4^{a,A} \pm 0.39$ |
| | EV | $19.3^{d,B} \pm 0.16$ | $16.4^{c,B} \pm 1.15$ | $14.9^{b,B} \pm 0.84$ | $13.9^{ab,B} \pm 0.80$ | $12.9^{a,A} \pm 0.63$ |

Mean values (± standar deviation) in each row with different superscript small letters present significant differences ($P \leq 0.05$) among irrigation treatments. Values in each column with different superscript capital letters present significant differences ($P \leq 0.05$) between initial and ending values (IV and EV, respectively) for each biochemical parameter in each irrigation treatment. T0, rain-fed treatment; T25, 25% of Etc; T50, 50% of Etc; T75, 75% of Etc; T100, full-irrigated (100% of Etc) treatment. n-Alkane ($C_{29}$–$C_{35}$) abbreviations: $C_{29}$, nonacosane; $C_{30}$, triacontane; $C_{31}$, hentriacontane; $C_{32}$, dotriacontane; $C_{33}$, tritriacontane; $C_{34}$, tetratriacontane; $C_{35}$, pentatriacontane.

et al., 2006; González and Ayerbe, 2010). In some species, increased amounts of cuticular waxes were also associated with improved drought tolerance (Goodwin and Jenks, 2005; Zhang et al., 2005). Even though the induction of plant waxes seems to be a near-universal plant response to drought, results from the present study did not show a clear tendency toward increasing wax accumulation in leaves from water-stressed plants.

Hydrocarbon constituents of olive leaf cuticular waxes consisted of odd and even-numbered n-alkanes of carbon atoms from $C_{29}$ to $C_{35}$, with odd-numbered compounds predominating largely (Tables 2 and 3). The major alkanes were the $C_{31}$ (hentriacontane) and $C_{33}$ (tritriacontane) homologues, representing more than 60% of the total n-alkane content. In agreement with results from Bianchi et al. (1993), minor quantitative differences were observed between cultivars. Water availability had an effect on the chain length distribution of cuticular wax alkanes. A significant increase in the amounts of the heaviest ($C_{33}$ and $C_{35}$) compounds was observed in T0, T25 and T50 treatments at the end of the RDI application period (EV, Tables 2 and 3) for both 2009 and 2010 crop years. Moreover, the $\Psi_{stem}$ correlated negatively with both $C_{33}$ and $C_{35}$ n-alkanes (Table 4). These findings agree with those from Bondada et al. (1996) who found that water stress increased the levels of long-chain, higher molecular weight alkanes in leaf waxes from cotton plants. It is known that the increase in molecular weight across the n-alkane homolog series results in raised hydrophobicity (Wu and Prausnitz, 2008); so, the cell surface hydrophobicity could be enhanced via accumulation at the cell surface of higher amounts of n-alkane molecules with higher chain length. Although water-stress conditions do not seem to increase cuticular wax content, leaves from water-stressed plants accumulated higher levels of long-chain alkanes in comparison with full-watered plants. It is possible that under the environmental conditions in which olive plants are growing, olive leaves produce enough wax to be able to adequately regulate water loss from the cuticle. Jordan et al. (1984) have observed that certain amount of wax per unit of leaf area is associated with an optimal water permeability coefficient; further wax deposition does not significantly increase resistance to water loss. Alternatively, the ability to alter cuticular wax composition in response to water availability could provide one mechanism whereby olive plants may limit transpiration rate and improve water conservation.

Another biochemical mechanism adopted by the olive tree to face water deficit is osmotic adjustment. It may depend on both active synthesis and accumulation of osmolytes within cells (active osmotic adjustment), and water loss from cells which leads to osmolyte concentration (passive osmotic adjustment). Sofo et al.

**Table 3**
Cuticle thickness (CT, μm), cuticular wax content (WC, mg/g) and n-alkane composition (%) from leaves of Arbequina and Manzanilla olive cultivars growing under different water irrigation levels during the 2010 crop year.

| | | Irrigation treatment | | | | |
|---|---|---|---|---|---|---|
| | | T0 | T25 | T50 | T75 | T100 |
| Arbequina | | | | | | |
| CT | IV | $8.27^{a,A} \pm 2.71$ | $8.21^{a,A} \pm 2.27$ | $8.35^{a,A} \pm 2.38$ | $8.12^{a,A} \pm 2.21$ | $8.63^{a,B} \pm 2.75$ |
| | EV | $13.6^{c,B} \pm 3.54$ | $13.1^{c,B} \pm 3.75$ | $9.23^{b,A} \pm 2.51$ | $6.97^{a,A} \pm 2.91$ | $6.22^{a,A} \pm 2.28$ |
| WC | IV | $13.8^{a,A} \pm 2.02$ | $12.8^{a,A} \pm 2.43$ | $13.2^{a,A} \pm 2.46$ | $12.2^{a,A} \pm 0.54$ | $15.0^{a,A} \pm 2.68$ |
| | EV | $15.5^{a,A} \pm 2.93$ | $17.8^{a,A} \pm 3.63$ | $20.3^{a,B} \pm 2.23$ | $17.4^{a,B} \pm 2.67$ | $17.0^{a,A} \pm 2.43$ |
| $C_{29}$ | IV | $14.4^{a,B} \pm 0.67$ | $12.9^{a,B} \pm 3.51$ | $11.7^{a,B} \pm 0.23$ | $12.3^{a,A} \pm 1.61$ | $14.2^{a,A} \pm 1.92$ |
| | EV | $5.83^{a,A} \pm 1.61$ | $5.36^{a,A} \pm 2.18$ | $7.64^{a,A} \pm 2.48$ | $11.8^{b,A} \pm 0.26$ | $11.8^{b,A} \pm 0.67$ |
| $C_{30}$ | IV | $2.71^{a,B} \pm 0.09$ | $2.74^{a,A} \pm 0.77$ | $2.89^{a,B} \pm 0.21$ | $2.67^{a,A} \pm 0.06$ | $2.62^{a,A} \pm 0.28$ |
| | EV | $1.69^{a,A} \pm 0.38$ | $1.85^{a,A} \pm 0.30$ | $2.09^{ab,A} \pm 0.28$ | $2.45^{b,A} \pm 0.22$ | $2.45^{b,A} \pm 0.16$ |
| $C_{31}$ | IV | $31.8^{a,A} \pm 1.19$ | $32.4^{a,A} \pm 3.16$ | $33.4^{a,A} \pm 2.19$ | $31.9^{a,B} \pm 0.97$ | $31.5^{a,A} \pm 1.14$ |
| | EV | $29.4^{a,A} \pm 5.36$ | $28.7^{a,A} \pm 3.38$ | $31.5^{a,A} \pm 0.93$ | $29.1^{a,A} \pm 1.21$ | $31.0^{a,A} \pm 0.39$ |
| $C_{32}$ | IV | $5.44^{a,A} \pm 0.32$ | $6.12^{a,A} \pm 0.50$ | $6.70^{a,A} \pm 1.08$ | $5.96^{a,B} \pm 0.27$ | $5.43^{a,A} \pm 0.06$ |
| | EV | $6.12^{a,A} \pm 0.80$ | $5.90^{a,A} \pm 0.17$ | $6.23^{a,A} \pm 0.79$ | $5.36^{a,A} \pm 0.40$ | $5.67^{a,A} \pm 0.35$ |
| $C_{33}$ | IV | $30.1^{a,A} \pm 0.33$ | $30.9^{a,A} \pm 3.40$ | $31.8^{a,A} \pm 2.17$ | $31.3^{a,A} \pm 0.96$ | $29.8^{a,A} \pm 0.84$ |
| | EV | $36.4^{c,B} \pm 1.74$ | $35.6^{bc,A} \pm 2.88$ | $34.0^{abc,A} \pm 1.67$ | $32.2^{a,A} \pm 0.73$ | $32.6^{ab,B} \pm 0.76$ |
| $C_{34}$ | IV | $3.42^{a,A} \pm 0.23$ | $3.43^{a,A} \pm 0.83$ | $3.66^{a,A} \pm 0.87$ | $3.52^{a,A} \pm 0.32$ | $4.26^{a,A} \pm 1.72$ |
| | EV | $4.43^{a,A} \pm 1.07$ | $4.75^{a,B} \pm 0.56$ | $4.09^{a,A} \pm 0.13$ | $4.06^{a,B} \pm 0.27$ | $4.46^{a,A} \pm 0.90$ |
| $C_{35}$ | IV | $11.9^{a,A} \pm 1.15$ | $11.4^{a,A} \pm 3.31$ | $9.75^{a,A} \pm 3.01$ | $12.3^{a,A} \pm 1.30$ | $12.1^{a,A} \pm 1.22$ |
| | EV | $16.0^{ab,A} \pm 0.37$ | $17.8^{b,B} \pm 3.11$ | $14.4^{a,A} \pm 0.57$ | $12.5^{a,A} \pm 4.57$ | $11.9^{a,A} \pm 0.62$ |
| Manzanilla | | | | | | |
| CT | IV | $10.4^{a,A} \pm 2.38$ | $10.4^{a,A} \pm 2.68$ | $10.8^{a,A} \pm 2.72$ | $9.70^{a,A} \pm 2.51$ | $9.80^{a,A} \pm 2.66$ |
| | EV | $18.8^{c,B} \pm 3.82$ | $17.5^{c,B} \pm 3.66$ | $12.4^{b,A} \pm 2.99$ | $8.12^{a,A} \pm 2.46$ | $7.22^{a,A} \pm 2.67$ |
| WC | IV | $13.8^{a,A} \pm 1.42$ | $16.5^{a,A} \pm 0.41$ | $17.1^{a,A} \pm 0.18$ | $15.3^{a,A} \pm 1.76$ | $12.6^{a,A} \pm 3.07$ |
| | EV | $19.8^{a,A} \pm 2.60$ | $19.5^{a,A} \pm 3.61$ | $16.3^{a,A} \pm 2.55$ | $17.2^{a,A} \pm 2.0$ | $16.0^{a,A} \pm 4.63$ |
| $C_{29}$ | IV | $13.1^{a,B} \pm 0.35$ | $13.8^{a,B} \pm 4.45$ | $11.0^{a,B} \pm 2.11$ | $10.9^{a,A} \pm 1.24$ | $12.3^{a,A} \pm 3.47$ |
| | EV | $4.38^{a,A} \pm 1.45$ | $5.72^{a,A} \pm 0.68$ | $5.65^{a,A} \pm 1.12$ | $10.1^{b,A} \pm 1.93$ | $13.1^{c,A} \pm 0.90$ |
| $C_{30}$ | IV | $2.72^{a,B} \pm 0.11$ | $2.76^{a,B} \pm 0.12$ | $2.60^{a,B} \pm 0.01$ | $2.68^{a,A} \pm 0.23$ | $2.55^{a,A} \pm 0.15$ |
| | EV | $1.67^{a,A} \pm 0.19$ | $1.86^{a,A} \pm 0.01$ | $1.80^{a,A} \pm 0.16$ | $2.61^{c,A} \pm 0.28$ | $2.30^{b,A} \pm 0.17$ |
| $C_{31}$ | IV | $31.7^{a,A} \pm 0.69$ | $30.5^{a,A} \pm 1.31$ | $31.9^{a,B} \pm 0.56$ | $30.1^{a,A} \pm 0.30$ | $31.5^{a,A} \pm 1.06$ |
| | EV | $25.1^{a,A} \pm 4.92$ | $27.6^{ab,A} \pm 2.26$ | $28.5^{abc,A} \pm 1.64$ | $30.9^{bc,A} \pm 1.30$ | $31.6^{c,A} \pm 1.28$ |
| $C_{32}$ | IV | $5.43^{a,A} \pm 0.62$ | $5.32^{a,A} \pm 0.99$ | $6.10^{a,A} \pm 0.92$ | $5.94^{a,A} \pm 0.08$ | $5.36^{a,A} \pm 0.77$ |
| | EV | $5.96^{a,A} \pm 0.16$ | $5.95^{a,A} \pm 0.20$ | $5.47^{a,A} \pm 0.19$ | $6.20^{a,A} \pm 0.51$ | $5.87^{a,A} \pm 0.78$ |
| $C_{33}$ | IV | $31.4^{a,A} \pm 0.05$ | $30.7^{a,A} \pm 1.93$ | $32.8^{a,A} \pm 1.35$ | $32.1^{a,A} \pm 1.29$ | $32.1^{a,A} \pm 2.48$ |
| | EV | $37.6^{b,B} \pm 0.49$ | $37.9^{b,B} \pm 1.65$ | $36.8^{b,B} \pm 1.12$ | $32.5^{a,A} \pm 2.38$ | $29.6^{a,A} \pm 2.35$ |
| $C_{34}$ | IV | $3.32^{a,A} \pm 0.18$ | $4.09^{a,A} \pm 1.29$ | $3.53^{a,A} \pm 0.27$ | $4.00^{a,A} \pm 0.13$ | $3.49^{a,A} \pm 0.41$ |
| | EV | $5.37^{a,A} \pm 1.31$ | $4.72^{a,A} \pm 0.37$ | $4.59^{a,B} \pm 0.42$ | $3.54^{a,A} \pm 0.41$ | $4.40^{a,A} \pm 1.02$ |
| $C_{35}$ | IV | $12.2^{a,A} \pm 0.13$ | $12.6^{a,A} \pm 2.18$ | $12.0^{a,A} \pm 0.22$ | $14.2^{a,A} \pm 0.45$ | $12.7^{a,A} \pm 1.18$ |
| | EV | $19.8^{b,B} \pm 0.52$ | $16.2^{b,B} \pm 1.92$ | $17.0^{b,B} \pm 1.94$ | $14.0^{a,A} \pm 1.46$ | $12.9^{a,A} \pm 2.57$ |

Mean values (± standar deviation) in each row with different superscript small letters present significant differences ($P \leq 0.05$) among irrigation treatments. Values in each column with different superscript capital letters present significant differences ($P \leq 0.05$) between initial and ending values (IV and EV, respectively) for each biochemical parameter in each irrigation treatment. T0, rain-fed treatment; T25, 25% of Etc; T50, 50% of Etc; T75, 75% of Etc; T100, full-irrigated (100% of Etc) treatment. n-Alkane ($C_{29}$–$C_{35}$) abbreviations: $C_{29}$, nonacosane; $C_{30}$, triacontane; $C_{31}$, hentriacontane; $C_{32}$, dotriacontane; $C_{33}$, tritriacontane; $C_{34}$, tetratriacontane; $C_{35}$, pentatriacontane.

(2008) found that, for values of $\Psi_{stem}$ below -3.2 MPa, the osmotic adjustment due to the accumulation of PRO is completely active and allows the conservation of water in olive tissues.

In each crop year, at the end of the RDI application period, water deficit was related to increments in PRO concentrations (Tables 5 and 6). This leaf-level response to water deprivation was observed for both olive cultivars analyzed and correlated negatively with $\Psi_{stem}$ values (Table 4). The PRO values obtained for T0 and T100 treatments matches well with those from Tunisian cultivars grown in water deficit and full irrigation conditions (Ben Ahmed et al., 2009). Although it has been proved that PRO accumulates in leaves of olive plants undergoing water deprivation (Sofo et al., 2004, 2008; Ben Ahmed et al., 2009), the accumulation pattern of this compound is not clear yet. Data obtained in this

**Table 4**
Correlation coefficients among stem water potential and selected biochemical and physiological parameters in leaves from olive plants growing under different water irrigation levels.

| | 2009 Crop year | | 2010 Crop year | |
|---|---|---|---|---|
| | Arbequina | Manzanilla | Arbequina | Manzanilla |
| CT | −0.97** | −0.86** | −0.96** | −0.92** |
| $C_{33}$ | −0.81** | −0.81** | −0.72** | −0.86** |
| $C_{34}$ | −0.63** | −0.66** | −0.20 | −0.44* |
| $C_{35}$ | −0.74** | −0.86** | −0.57** | −0.76** |
| PRO | −0.91** | −0.88** | −0.91** | −0.88** |
| MDA | −0.73** | −0.75** | −0.84** | −0.87** |
| TChl | 0.68** | 0.78** | 0.65** | 0.65** |
| Car | 0.71** | 0.75** | 0.62** | 0.61** |
| $P_n$ | | | 0.99** | 0.96** |
| *gl* | | | 0.98** | 0.94** |

Abbreviations: CT, cuticle thickness; $C_{33}$, tritriacontane content; $C_{34}$, tetratriacontane content; $C_{35}$, pentatriacontane content; PRO, proline content; MDA, malondialdehyde content; TChl, total chlorophyll content; Car, carotenoid content; $P_n$, leaf photosynthetic rate; *gl*, stomatal conductance. * Significant at $P \leq 0.05$. ** Significant at $P \leq 0.01$.

**Table 5**
Proline (μMol/mg), malondialdehyde (nMol/g) and pigment contents (mg/g) from leaves of Arbequina and Manzanilla olive cultivars growing under different water irrigation levels during the 2009 crop year.

| | | Irrigation treatment | | | | |
|---|---|---|---|---|---|---|
| Arbequina | | T0 | T25 | T50 | T75 | T100 |
| PRO | IV | $0.67^{a,A} \pm 0.06$ | $0.73^{a,A} \pm 0.06$ | $0.75^{a,A} \pm 0.11$ | $0.71^{a,A} \pm 0.06$ | $0.75^{a,A} \pm 0.06$ |
| | EV | $1.89^{e,B} \pm 0.29$ | $1.63^{d,B} \pm 0.20$ | $1.36^{c,B} \pm 0.22$ | $0.67^{b,A} \pm 0.12$ | $0.50^{a,A} \pm 0.09$ |
| MDA | IV | $44.7^{a,A} \pm 1.87$ | $44.4^{a,A} \pm 3.73$ | $47.7^{a,A} \pm 5.24$ | $48.1^{a,A} \pm 6.37$ | $46.0^{a,A} \pm 2.11$ |
| | EV | $117.9^{d,B} \pm 2.35$ | $98.3^{c,B} \pm 2.14$ | $61.9^{b,B} \pm 4.63$ | $62.7^{b,B} \pm 4.77$ | $46.9^{a,A} \pm 5.29$ |
| Chl-*a* | IV | $8.50^{a,A} \pm 0.65$ | $8.63^{a,B} \pm 0.52$ | $8.30^{a,A} \pm 1.03$ | $8.27^{a,A} \pm 0.51$ | $8.30^{a,A} \pm 0.39$ |
| | EV | $7.29^{a,A} \pm 3.38$ | $5.88^{a,A} \pm 1.25$ | $7.48^{a,A} \pm 2.46$ | $9.54^{b,B} \pm 0.32$ | $10.9^{b,B} \pm 1.11$ |
| Chl-*b* | IV | $4.48^{a,B} \pm 0.36$ | $4.20^{a,B} \pm 0.42$ | $4.43^{a,A} \pm 0.43$ | $4.27^{a,A} \pm 0.54$ | $4.30^{a,A} \pm 0.39$ |
| | EV | $3.63^{a,A} \pm 1.25$ | $3.27^{a,A} \pm 0.88$ | $3.80^{a,A} \pm 1.13$ | $5.24^{b,B} \pm 0.74$ | $5.52^{b,B} \pm 0.62$ |
| Chl-*a*/Chl-*b* | IV | $1.90^{a,A} \pm 0.05$ | $2.07^{b,B} \pm 0.25$ | $1.87^{a,A} \pm 0.14$ | $1.95^{a,A} \pm 0.18$ | $1.94^{a,A} \pm 0.14$ |
| | EV | $1.94^{a,A} \pm 0.30$ | $1.84^{a,A} \pm 0.24$ | $1.96^{a,A} \pm 0.18$ | $1.85^{a,A} \pm 0.23$ | $1.97^{a,A} \pm 0.12$ |
| Total Chl | IV | $13.0^{a,A} \pm 0.99$ | $12.8^{a,B} \pm 0.72$ | $12.7^{a,A} \pm 1.40$ | $12.5^{a,A} \pm 1.00$ | $12.6^{a,A} \pm 0.72$ |
| | EV | $10.9^{a,A} \pm 4.61$ | $9.15^{a,A} \pm 2.05$ | $11.3^{a,A} \pm 3.57$ | $14.8^{b,B} \pm 0.88$ | $16.4^{b,B} \pm 1.67$ |
| Car | IV | $5.62^{a,A} \pm 1.10$ | $5.82^{a,B} \pm 0.96$ | $5.69^{a,B} \pm 0.74$ | $5.63^{a,A} \pm 0.83$ | $5.70^{a,A} \pm 0.42$ |
| | EV | $4.46^{a,A} \pm 1.71$ | $3.73^{a,A} \pm 0.83$ | $4.59^{a,A} \pm 1.34$ | $6.19^{b,A} \pm 0.57$ | $6.74^{b,B} \pm 0.83$ |
| Total Phae | IV | $10.2^{a,A} \pm 1.07$ | $10.3^{a,B} \pm 0.87$ | $9.76^{a,A} \pm 0.94$ | $10.0^{a,A} \pm 0.98$ | $10.2^{a,A} \pm 0.84$ |
| | EV | $8.59^{a,A} \pm 3.97$ | $7.40^{a,A} \pm 1.34$ | $8.99^{a,A} \pm 3.07$ | $11.5^{b,B} \pm 0.75$ | $12.9^{b,B} \pm 1.23$ |
| Phae/Chl | IV | $0.79^{b,A} \pm 0.03$ | $0.80^{b,A} \pm 0.04$ | $0.77^{a,A} \pm 0.03$ | $0.80^{b,A} \pm 0.03$ | $0.81^{b,A} \pm 0.04$ |
| | EV | $0.77^{a,A} \pm 0.05$ | $0.82^{a,A} \pm 0.09$ | $0.79^{a,A} \pm 0.03$ | $0.78^{a,A} \pm 0.02$ | $0.79^{a,A} \pm 0.02$ |
| Manzanilla | | | | | | |
| PRO | IV | $0.75^{a,A} \pm 0.05$ | $0.85^{b,A} \pm 0.07$ | $0.85^{b,A} \pm 0.03$ | $0.75^{a,B} \pm 0.08$ | $0.81^{b,B} \pm 0.06$ |
| | EV | $1.92^{e,B} \pm 0.10$ | $1.66^{d,B} \pm 0.16$ | $1.05^{c,B} \pm 0.19$ | $0.55^{b,A} \pm 0.07$ | $0.39^{a,A} \pm 0.06$ |
| MDA | IV | $41.9^{a,A} \pm 0.94$ | $49.4^{b,A} \pm 4.80$ | $45.9^{a,A} \pm 2.99$ | $49.6^{b,A} \pm 7.77$ | $43.7^{a,A} \pm 2.92$ |
| | EV | $114.3^{d,B} \pm 3.83$ | $90.9^{c,B} \pm 7.96$ | $72.4^{b,B} \pm 7.81$ | $58.9^{a,B} \pm 9.60$ | $50.8^{a,B} \pm 6.47$ |
| Chl-*a* | IV | $10.2^{a,B} \pm 0.90$ | $9.91^{a,B} \pm 0.56$ | $10.1^{a,B} \pm 0.57$ | $9.91^{a,A} \pm 0.69$ | $10.1^{a,A} \pm 0.63$ |
| | EV | $7.04^{a,A} \pm 0.46$ | $7.73^{a,A} \pm 0.88$ | $8.20^{a,A} \pm 1.50$ | $10.1^{b,A} \pm 1.96$ | $13.2^{c,B} \pm 2.68$ |
| Chl-*b* | IV | $5.18^{a,B} \pm 0.44$ | $5.08^{a,B} \pm 0.40$ | $5.41^{a,B} \pm 0.71$ | $5.02^{a,A} \pm 0.58$ | $5.20^{a,A} \pm 0.51$ |
| | EV | $3.96^{a,A} \pm 0.98$ | $4.31^{a,A} \pm 1.05$ | $4.14^{a,A} \pm 0.94$ | $5.24^{b,A} \pm 0.26$ | $5.52^{b,A} \pm 0.41$ |
| Chl-*a*/Chl-*b* | IV | $1.97^{a,A} \pm 0.16$ | $1.96^{a,A} \pm 0.18$ | $1.89^{a,A} \pm 0.17$ | $1.99^{a,A} \pm 0.14$ | $1.96^{a,A} \pm 0.19$ |
| | EV | $1.86^{a,A} \pm 0.36$ | $1.85^{a,A} \pm 0.28$ | $2.01^{b,B} \pm 0.20$ | $2.01^{b,A} \pm 0.15$ | $2.37^{c,B} \pm 0.20$ |
| Total Chl | IV | $15.4^{a,B} \pm 1.19$ | $15.0^{a,B} \pm 0.74$ | $15.5^{a,B} \pm 1.22$ | $14.9^{a,A} \pm 1.21$ | $15.3^{a,A} \pm 0.96$ |
| | EV | $11.0^{a,A} \pm 1.36$ | $12.0^{a,A} \pm 1.84$ | $12.3^{a,A} \pm 2.39$ | $15.1^{b,A} \pm 2.78$ | $18.3^{c,B} \pm 3.94$ |
| Car | IV | $6.94^{a,B} \pm 0.64$ | $7.13^{a,B} \pm 0.58$ | $6.82^{a,B} \pm 0.75$ | $6.95^{a,B} \pm 0.55$ | $6.99^{a,A} \pm 0.55$ |
| | EV | $4.72^{a,A} \pm 0.78$ | $5.17^{a,A} \pm 0.86$ | $5.28^{a,A} \pm 1.06$ | $6.17^{b,A} \pm 1.05$ | $9.25^{c,B} \pm 2.11$ |
| Total Phae | IV | $12.9^{a,B} \pm 0.86$ | $12.9^{a,B} \pm 0.58$ | $12.8^{a,B} \pm 1.30$ | $12.4^{a,A} \pm 1.01$ | $13.1^{a,A} \pm 0.79$ |
| | EV | $8.33^{a,A} \pm 0.53$ | $8.93^{a,A} \pm 0.89$ | $9.51^{a,A} \pm 1.78$ | $12.1^{b,A} \pm 2.10$ | $17.1^{c,B} \pm 3.66$ |
| Phae/Chl | IV | $0.84^{a,B} \pm 0.02$ | $0.86^{a,B} \pm 0.03$ | $0.83^{a,B} \pm 0.04$ | $0.83^{a,A} \pm 0.04$ | $0.85^{a,B} \pm 0.03$ |
| | EV | $0.77^{a,A} \pm 0.08$ | $0.75^{a,A} \pm 0.07$ | $0.78^{a,A} \pm 0.06$ | $0.80^{a,A} \pm 0.08$ | $0.77^{a,A} \pm 0.05$ |

Mean values (± standar deviation) in each row with different superscript small letters present significant differences ($P \leq 0.05$) among irrigation treatments. Values in each column with different superscript capital letters present significant differences ($P \leq 0.05$) between initial and ending values (IV and EV, respectively) for each biochemical parameter in each irrigation treatment. T0, rain-fed treatment; T25, 25% of Etc; T50, 50% of Etc; T75, 75% of Etc; T100, full-irrigated (100% of Etc) treatment. Abbreviations: PRO, proline; MDA, malondialdehyde; Chl, chlorophyll; Car, carotenoids; Phae, phaeophytins.

study indicate that significant increments in PRO levels occurred in T0, T25 and T50 treatments, which reached $\Psi_{stem}$ values between −2.5 and −4 MPa at the end of the water-stress period, suggesting that PRO accumulation in olive leaves may be induced at relatively mild water stress. Comparing the 2009 EV with 2010 IV, a pronounced drop in PRO concentration was observed for T0, T25 and T50 treatments. The 2010 IV resulted, in turn, similar to 2009 IV indicating that olive leaves recover their natural PRO concentration after drought-stress suppression. These observations suggest that olives leaves can accumulate PRO rapidly, in response to water deprivation, according to an opportunistic and reversible pattern.

In olive plants, drought stress is often associated with increased cellular levels of malondialdehyde (MDA). This compound arises from ROS-mediated PUFA (mainly linolenic acid) oxidation. Considering the results obtained by Guerfel et al. (2008), i.e., the increase in linolenic acid content–arising mainly from membrane lipids–in water-stressed olive plants, the results obtained in the present study reinforce the importance of oxidative stress in olive plants under water deprivation conditions. Water deprivation increased significantly the MDA levels in all RDI treatments (IV *vs* EV, Tables 5 and 6) indicating that oxidative cell membrane damage took place even under mild water stress conditions (T50 and T75 treatments). The MDA increase was dependent from the water deficit level and correlated negatively with $\Psi_{stem}$ (Table 5). Similarly to the effect observed from PRO accumulation, after drought-stress suppression MDA contents decreased at levels similar to those observed at the beginning of the RDI application period (2010 IV *vs* 2009 IV). Considering the MDA concentration as a biochemical marker for the ROS-mediated membrane injury, data obtained in this study and other related (Sofo et al., 2004; Bacelar et al., 2006) support the hypothesis that drought-stress suppression and rewatering can reduce membrane lipid peroxidation because of repairing mechanisms start to keep pace with oxidative damage.

Water stress conditions may provoke destruction of photosynthetic pigments (Bacelar et al., 2006, 2007; Guerfel et al., 2008; Boughalleb and Hajlaoui, 2011). According to Smirnoff (1993) the decrease in chlorophyll content is a typical symptom of oxidative stress and may be the result of pigment degradation. In general, for both crop years analyzed, at the end of the RDI application period, leaves from trees under water stress conditions (T0, T25 and T50) showed significant reductions in total chlorophyll and carotenoid concentrations (Tables 5 and 6). For both olive cultivars tested, significant positive correlations were found among $\Psi_{stem}$ values and both chlorophyll and carotenoid contents (Table 4). In general, at the end of the RDI application period, the ratio Chl-*a*/Chl-*b* showed lower values in RDI treatments with higher water deprivation. This fact may be explained in terms of differential stability of these pigments – under abiotic stress conditions – which could affect the stability of light-harvesting complexes involved in light absorption (Hoober et al., 2010) and, consequently, the photosynthetic rate.

**Table 6**
Proline (μMol/mg), malondialdehyde (nMol/g) and pigment contents (mg/g) from leaves of Arbequina and Manzanilla olive cultivars growing under different water irrigation levels during the 2010 crop year.

| | | Irrigation treatment | | | | |
|---|---|---|---|---|---|---|
| Arbequina | | T0 | T25 | T50 | T75 | T100 |
| PRO | IV | $0.67^{a,A} \pm 0.04$ | $0.67^{a,A} \pm 0.12$ | $0.60^{a,A} \pm 0.11$ | $0.69^{a,A} \pm 0.07$ | $0.72^{a,A} \pm 0.14$ |
| | EV | $2.07^{d,B} \pm 0.10$ | $2.18^{d,B} \pm 0.24$ | $1.25^{c,B} \pm 0.13$ | $0.94^{b,B} \pm 0.15$ | $0.65^{a,A} \pm 0.05$ |
| MDA | IV | $37.2^{b,A} \pm 3.12$ | $32.8^{a,A} \pm 2.80$ | $33.7^{a,A} \pm 2.40$ | $36.8^{b,A} \pm 3.85$ | $32.6^{a,A} \pm 2.47$ |
| | EV | $151.0^{e,B} \pm 5.13$ | $102.3^{d,B} \pm 4.68$ | $79.2^{c,B} \pm 3.20$ | $70.8^{b,B} \pm 5.47$ | $52.8^{a,B} \pm 1.66$ |
| Chl-*a* | IV | $8.24^{a,B} \pm 0.34$ | $8.45^{a,A} \pm 0.52$ | $8.30^{a,A} \pm 0.52$ | $8.33^{a,A} \pm 0.50$ | $8.52^{a,A} \pm 0.58$ |
| | EV | $4.77^{a,A} \pm 0.68$ | $6.65^{a,B} \pm 2.05$ | $10.5^{c,B} \pm 0.49$ | $9.25^{c,B} \pm 0.88$ | $9.27^{c,A} \pm 2.58$ |
| Chl-*b* | IV | $3.81^{a,B} \pm 0.25$ | $3.91^{a,A} \pm 0.43$ | $4.03^{a,A} \pm 0.59$ | $3.83^{a,A} \pm 0.11$ | $4.12^{a,A} \pm 0.37$ |
| | EV | $2.81^{a,A} \pm 0.34$ | $3.68^{b,A} \pm 0.94$ | $4.75^{b,B} \pm 0.87$ | $4.60^{c,B} \pm 0.39$ | $4.43^{c,A} \pm 1.14$ |
| Chl-*a*/Chl-*b* | IV | $2.18^{a,B} \pm 0.21$ | $2.18^{a,B} \pm 0.21$ | $2.09^{a,A} \pm 0.21$ | $2.18^{a,B} \pm 0.16$ | $2.07^{a,A} \pm 0.14$ |
| | EV | $1.73^{a,A} \pm 0.38$ | $1.80^{a,A} \pm 0.26$ | $2.20^{b,B} \pm 0.19$ | $2.02^{b,A} \pm 0.20$ | $2.08^{b,A} \pm 0.14$ |
| Total Chl | IV | $12.0^{a,B} \pm 0.31$ | $12.4^{a,B} \pm 0.83$ | $12.4^{a,A} \pm 0.83$ | $12.2^{a,A} \pm 0.48$ | $12.6^{a,A,} \pm 0.87$ |
| | EV | $7.58^{a,A} \pm 0.63$ | $10.3^{b,B} \pm 2.94$ | $15.2^{c,B} \pm 1.34$ | $13.9^{c,B} \pm 1.08$ | $13.5^{c,A} \pm 3.67$ |
| Car | IV | $4.93^{a,B} \pm 0.81$ | $4.43^{a,A} \pm 0.84$ | $4.86^{a,A} \pm 1.02$ | $4.38^{a,A} \pm 0.41$ | $4.73^{a,A} \pm 0.79$ |
| | EV | $3.50^{a,A} \pm 0.28$ | $4.59^{b,A} \pm 1.33$ | $6.94^{c,B} \pm 0.62$ | $5.92^{c,B} \pm 0.31$ | $5.91^{c,B} \pm 1.39$ |
| Total Phae | IV | $10.4^{a,B} \pm 0.72$ | $10.2^{a,B} \pm 0.77$ | $10.3^{a,A} \pm 0.77$ | $10.1^{a,A} \pm 0.75$ | $10.3^{a,A} \pm 0.90$ |
| | EV | $5.89^{a,A} \pm 1.04$ | $7.74^{b,A} \pm 2.39$ | $12.2^{c,B} \pm 0.33$ | $10.8^{c,A} \pm 1.01$ | $10.8^{c,A} \pm 2.70$ |
| Phae/Chl | IV | $0.86^{a,B} \pm 0.05$ | $0.83^{a,B} \pm 0.07$ | $0.83^{a,B} \pm 0.03$ | $0.83^{a,A} \pm 0.04$ | $0.81^{a,A} \pm 0.04$ |
| | EV | $0.77^{a,A} \pm 0.09$ | $0.74^{a,A} \pm 0.04$ | $0.75^{a,A} \pm 0.06$ | $0.79^{a,A} \pm 0.10$ | $0.81^{a,A} \pm 0.06$ |
| Manzanilla | | | | | | |
| PRO | IV | $0.68^{a,A} \pm 0.07$ | $0.73^{a,A} \pm 0.13$ | $0.69^{a,A} \pm 0.13$ | $0.68^{a,A} \pm 0.08$ | $0.70^{a,B} \pm 0.24$ |
| | EV | $2.21^{e,B} \pm 0.22$ | $1.78^{d,B} \pm 0.18$ | $1.18^{c,B} \pm 0.29$ | $0.85^{b,B} \pm 0.22$ | $0.50^{a,A} \pm 0.10$ |
| MDA | IV | $34.6^{a,A} \pm 4.27$ | $32.0^{a,A} \pm 1.28$ | $35.3^{a,A} \pm 5.21$ | $33.4^{a,A} \pm 3.88$ | $34.2^{a,A} \pm 2.78$ |
| | EV | $132.7^{e,B} \pm 19.4$ | $92.1^{d,B} \pm 5.66$ | $80.2^{c,B} \pm 3.89$ | $58.3^{b,B} \pm 11.8$ | $47.0^{a,B} \pm 10.66$ |
| Chl-*a* | IV | $11.5^{a,B} \pm 0.66$ | $11.3^{a,B} \pm 0.64$ | $11.8^{a,B} \pm 0.40$ | $11.5^{a,B} \pm 0.56$ | $11.6^{a,A} \pm 0.73$ |
| | EV | $7.08^{a,A} \pm 1.04$ | $9.32^{b,A} \pm 2.97$ | $9.32^{b,A} \pm 2.73$ | $10.3^{b,A} \pm 1.77$ | $12.6^{c,A} \pm 3.93$ |
| Chl-*b* | IV | $5.52^{a,B} \pm 0.33$ | $5.66^{a,B} \pm 0.46$ | $5.80^{a,B} \pm 0.31$ | $5.65^{a,B} \pm 0.26$ | $5.68^{a,A} \pm 0.30$ |
| | EV | $3.73^{a,A} \pm 0.95$ | $4.79^{b,A} \pm 1.82$ | $5.00^{bc,A} \pm 1.03$ | $4.87^{b,A} \pm 0.57$ | $5.88^{c,A} \pm 1.91$ |
| Chl-*a*/Chl-*b* | IV | $2.09^{a,B} \pm 0.18$ | $2.01^{a,A} \pm 0.23$ | $2.04^{a,B} \pm 0.11$ | $2.04^{a,A} \pm 0.07$ | $2.05^{a,A} \pm 0.15$ |
| | EV | $1.91^{a,A} \pm 0.06$ | $1.96^{a,A} \pm 0.05$ | $1.86^{a,A} \pm 0.14$ | $2.11^{b,A} \pm 0.16$ | $2.15^{b,B} \pm 0.10$ |
| Total Chl | IV | $17.1^{a,B} \pm 0.74$ | $17.1^{a,B} \pm 0.74$ | $17.6^{a,B} \pm 0.56$ | $17.2^{a,B} \pm 0.77$ | $17.3^{a,A} \pm 0.85$ |
| | EV | $10.8^{a,A} \pm 1.95$ | $14.1^{b,A} \pm 4.75$ | $14.3^{b,A} \pm 3.70$ | $15.1^{b,A} \pm 2.21$ | $18.5^{c,A} \pm 5.84$ |
| Car | IV | $7.18^{a,B} \pm 0.74$ | $7.23^{a,B} \pm 0.44$ | $7.56^{a,B} \pm 0.47$ | $7.45^{a,B} \pm 0.34$ | $7.44^{a,A} \pm 0.37$ |
| | EV | $5.04^{a,A} \pm 0.47$ | $6.29^{b,A} \pm 1.81$ | $6.17^{c,A} \pm 1.48$ | $6.46^{c,A} \pm 0.73$ | $7.77^{c,A} \pm 2.36$ |
| Total Phae | IV | $13.4^{a,B} \pm 0.58$ | $13.1^{a,B} \pm 0.70$ | $13.7^{a,B} \pm 0.59$ | $13.6^{a,B} \pm 0.84$ | $13.5^{a,A} \pm 0.87$ |
| | EV | $7.61^{a,A} \pm 1.93$ | $10.7^{b,A} \pm 3.56$ | $10.9^{b,A} \pm 2.93$ | $11.9^{b,A} \pm 2.23$ | $14.6^{c,A} \pm 4.21$ |
| Phae/Chl | IV | $0.79^{a,B} \pm 0.03$ | $0.77^{a,A} \pm 0.04$ | $0.78^{a,A} \pm 0.03$ | $0.79^{a,A} \pm 0.05$ | $0.78^{a,A} \pm 0.03$ |
| | EV | $0.73^{a,A} \pm 0.11$ | $0.76^{ab,A} \pm 0.05$ | $0.76^{ab,A} \pm 0.04$ | $0.78^{b,A} \pm 0.06$ | $0.80^{b,A} \pm 0.06$ |

Mean values (± standar deviation) in each row with different superscript small letters present significant differences ($P \leq 0.05$) among irrigation treatments. Values in each column with different superscript capital letters present significant differences ($P \leq 0.05$) between initial and ending values (IV and EV, respectively) for each biochemical parameter in each irrigation treatment. T0, rain-fed treatment; T25, 25% of Etc; T50, 50% of Etc; T75, 75% of Etc; T100, full-irrigated (100% of Etc) treatment. Abbreviations: PRO, proline; MDA, malondialdehyde; Chl, chlorophyll; Car, carotenoids; Phae, phaeophytins.

The phaeophytin/chlorophyll ratio (Phae/Chl) – also known as phaeophytinization index–is frequently used as a parameter to evaluate chlorophyll degradation (Carreras and Pignata, 2001). With the exception of results from the Manzanilla cultivar analyzed at 2010 crop year (Table 7), no variations in Phae/Chl were found among the RDI treatments after the period of water deprivation. These results indicate that chlorophyll content decline at higher water deficit could not be only explained by degradation of the photosynthetic pigment. Reductions in chlorophyll levels have been also associated with pigment synthesis deficiency together with changes in thylakoid membrane structure (Brito et al., 2003). These changes, in turn, may be related to ROS-induced lipid peroxidation.

### 3.3. Gas exchange parameters

Several studies have shown reductions in photosynthetic rate ($P_n$) of olive plants growing under water-stress conditions (Tognetti et al., 2005; Bacelar et al., 2006; Ben Ahmed et al., 2009). During the water stress period both cultivars analyzed had similar photosynthetic performance (Table 7). Two months after the initiation of the RDI application period, the different treatments did not present statistical significant variations in $P_n$ values. Later on, $P_n$ increased significantly in T100 and T75 treatments indicating that full or elevate irrigation levels improve markedly $CO_2$ assimilation rate. Treatments T0 and T25 registered minor changes along the water stress period. For T0, the average values obtained throughout the RDI experiment (3.57 $\mu mol\ m^{-2}\ s^{-1}$ for Arbequina, 3.63 $\mu mol\ m^{-2}\ s^{-1}$ for Manzanilla) were 62 and 61.1% lower than the average values from the respective T100 treatments. These results indicate a strong impact of water deprivation on $P_n$. For both olive cultivars, highly significantly positive correlations were found between $\Psi_{stem}$ and $P_n$ values (Table 4).

Díaz-Espejo et al. (2007) have reported that diffusional limitation of photosynthesis is the main factor determining the distribution of photosynthetic capacity in olive leaves under drought conditions. Their data were obtained during the summer months, in a Mediterranean environment characterized by high irradiance and evaporative demand. In the present study, gas exchange measurements were taken from a period in which climatic conditions (mean temperatures and irradiance) are less severe as compared with those from the summer in the Mediterranean environment. Nevertheless, results show a strong effect of drought conditions on stomatal conductance (gs). Considering the whole RDI period, the average values of gs in T0 were 69.7% (Arbequina) and 67.2% (Manzanilla) lower than the average values from the respective T100 treatments. In both cultivars, gs was affected by water deficit in a similar way (Table 7); a close correlation was obtained between gs and $\Psi_{stem}$ values (Table 4) suggesting a control of gs through a hydraulic feed back mechanism (Jones, 1998).

**Table 7**
Leaf photosynthetic rate ($P_n$) and stomatal conductance (gs) from Arbequina and Manzanilla olive cultivars growing under different water irrigation levels.

| Date | Irrigation treatment | $P_n$ ($\mu$mol m$^{-2}$ s$^{-1}$) | | gs (mmol m$^{-2}$ s$^{-1}$) | |
|---|---|---|---|---|---|
| | | Arbequina | Manzanilla | Arbequina | Manzanilla |
| July 22 | T100 | $3.70^{a,A} \pm 0.26$ | $3.74^{a,A} \pm 0.19$ | $91.7^{b,A} \pm 1.53$ | $88.2^{a,A} \pm 1.48$ |
| | T75 | $3.63^{a,A} \pm 0.13$ | $3.70^{a,A} \pm 0.17$ | $88.0^{a,A} \pm 1.63$ | $93.3^{b,A} \pm 5.51$ |
| | T50 | $3.63^{a,A} \pm 0.32$ | $3.67^{a,A} \pm 0.12$ | $90.0^{ab,D} \pm 2.00$ | $88.0^{a,D} \pm 1.00$ |
| | T25 | $3.60^{a,AB} \pm 0.08$ | $3.57^{a,A} \pm 0.06$ | $88.3^{a,D} \pm 0.50$ | $86.3^{a,E} \pm 0.58$ |
| | T0 | $3.50^{a,BC} \pm 0.10$ | $3.65^{a,A} \pm 0.07$ | $87.7^{a,F} \pm 0.58$ | $89.5^{ab,D} \pm 0.71$ |
| Aug 11 | T100 | $4.47^{b,A} \pm 0.16$ | $4.46^{a,A} \pm 0.21$ | $148.7^{c,B} \pm 1.15$ | $145.4^{c,B} \pm 2.89$ |
| | T75 | $4.45^{b,A} \pm 0.17$ | $4.40^{a,AB} \pm 0.27$ | $145.0^{b,C} \pm 0.82$ | $137.7^{c,B} \pm 2.31$ |
| | T50 | $4.33^{ab,A} \pm 0.15$ | $4.27^{a,A} \pm 0.21$ | $112.0^{a,E} \pm 2.00$ | $120.3^{b,F} \pm 1.53$ |
| | T25 | $4.20^{ab,BCD} \pm 0.28$ | $4.23^{a,AB} \pm 0.16$ | $110.5^{a,E} \pm 1.00$ | $114.7^{a,G} \pm 2.89$ |
| | T0 | $4.13^{ab,CD} \pm 0.16$ | $4.20^{a,B} \pm 0.10$ | $110.3^{a,G} \pm 0.58$ | $110.0^{a,E} \pm 0.10$ |
| Aug 26 | T100 | $8.43^{c,B} \pm 1.68$ | $6.82^{e,B} \pm 0.40$ | $199.0^{c,D} \pm 13.0$ | $190.8^{e,E} \pm 6.46$ |
| | T75 | $5.79^{b,B} \pm 0.57$ | $6.12^{d,BC} \pm 0.11$ | $182.5^{c,G} \pm 7.14$ | $176.7^{d,E} \pm 8.08$ |
| | T50 | $4.50^{b,AB} \pm 0.33$ | $5.07^{c,B} \pm 0.14$ | $79.3^{b,C} \pm 14.8$ | $109.0^{c,E} \pm 21.3$ |
| | T25 | $3.06^{a,A} \pm 0.47$ | $3.71^{b,A,} \pm 0.33$ | $46.0^{ab,C} \pm 14.3$ | $66.0^{b,D} \pm 15.7$ |
| | T0 | $2.67^{a,A} \pm 0.56$ | $2.87^{a,A} \pm 0.11$ | $39.7^{a,E} \pm 15.5$ | $44.5^{a,C} \pm 10.6$ |
| Set 07 | T100 | $9.31^{d,B} \pm 1.23$ | $8.91^{c,C} \pm 0.80$ | $179.3^{c,C} \pm 1.15$ | $177.0^{c,C} \pm 3.32$ |
| | T75 | $7.57^{c,C} \pm 0.62$ | $7.85^{c,CDE} \pm 0.53$ | $175.0^{c,F} \pm 3.56$ | $171.7^{c,DE} \pm 5.77$ |
| | T50 | $5.87^{b,CD} \pm 0.97$ | $6.08^{b,C} \pm 0.77$ | $51.3^{b,B} \pm 2.31$ | $105.0^{b,E} \pm 3.51$ |
| | T25 | $4.91^{b,CDE} \pm 0.95$ | $5.94^{b,CD} \pm 0.05$ | $49.0^{b,C} \pm 1.15$ | $104.7^{b,E} \pm 0.58$ |
| | T0 | $2.96^{a,AB} \pm 0.48$ | $3.34^{a,A} \pm 1.38$ | $35.0^{a,E} \pm 5.00$ | $40.0^{a,F} \pm 0.10$ |
| Set 21 | T100 | $10.9^{c,BC} \pm 2.73$ | $12.78^{c,D} \pm 0.68$ | $180.0^{e,C} \pm 2.00$ | $170.6^{d,C} \pm 4.67$ |
| | T75 | $7.16^{b,C} \pm 0.54$ | $11.35^{c,EF} \pm 0.45$ | $165.5^{d,E} \pm 1.91$ | $169.0^{d,D} \pm 1.73$ |
| | T50 | $5.94^{ab,CD} \pm 1.23$ | $5.48^{b,BC} \pm 0.46$ | $73.7^{c,C} \pm 3.21$ | $55.0^{c,C} \pm 21.8$ |
| | T25 | $4.02^{a,ABC} \pm 0.56$ | $4.33^{a,AB} \pm 0.39$ | $24.0^{b,A} \pm 1.15$ | $33.0^{b,BC} \pm 1.73$ |
| | T0 | $3.73^{a,CD} \pm 0.47$ | $3.24^{a,A} \pm 0.81$ | $18.3^{a,A} \pm 2.52$ | $22.5^{a,AB} \pm 3.54$ |
| Oct 06 | T100 | $13.6^{d,CD} \pm 0.29$ | $13.53^{d,D} \pm 1.99$ | $184.7^{d,C} \pm 5.03$ | $180.0^{d,D} \pm 3.54$ |
| | T75 | $9.29^{c,D} \pm 0.54$ | $9.73^{c,E} \pm 0.88$ | $133.0^{c,B} \pm 4.20$ | $136.7^{c,B} \pm 1.15$ |
| | T50 | $7.00^{bc,DE} \pm 0.14$ | $7.34^{bc,D} \pm 0.55$ | $50.0^{b,B} \pm 5.00$ | $47.0^{b,B} \pm 1.73$ |
| | T25 | $4.54^{ab,BCDE} \pm 0.66$ | $4.66^{ab,AB} \pm 1.02$ | $26.3^{a,A} \pm 16.0$ | $28.3^{a,A} \pm 18.9$ |
| | T0 | $3.83^{a,CD} \pm 0.61$ | $3.87^{a,A} \pm 0.90$ | $28.3^{a,CD} \pm 2.89$ | $28.0^{a,B} \pm 0.10$ |
| Oct 22 | T100 | $15.4^{e,D} \pm 0.14$ | $15.2^{d,E} \pm 0.47$ | $181.7^{e,C} \pm 2.89$ | $183.4^{d,D} \pm 4.22$ |
| | T75 | $14.7^{d,E} \pm 0.18$ | $14.7^{c,F} \pm 0.85$ | $130.5^{b,B} \pm 3.56$ | $138.3^{c,B} \pm 2.89$ |
| | T50 | $7.61^{c,E} \pm 0.22$ | $7.58^{b,D} \pm 0.23$ | $40.0^{a,A} \pm 2.00$ | $42.3^{b,A} \pm 2.52$ |
| | T25 | $5.22^{b,DE} \pm 0.01$ | $5.15^{a,BCD} \pm 0.29$ | $35.3^{a,B} \pm 7.09$ | $35.0^{a,C} \pm 8.66$ |
| | T0 | $4.20^{a,D} \pm 0.08$ | $4.25^{a,B} \pm 0.05$ | $33.3^{a,DE} \pm 5.00$ | $38.0^{ab,C} \pm 17.0$ |

For each date, mean values (± standar deviation) in each column with different superscript small letters present significant differences ($P \leq 0.05$) among irrigation treatments. For each irrigation treatment, values in each column with different superscript capital letters present significant differences ($P \leq 0.05$) among dates. From each selected tree (six trees per treatment) a total of four mature, fully expanded leaves were used. T0, rain-fed treatment; T25, 25% of Etc; T50, 50% of Etc; T75, 75% of Etc; T100, full-irrigated (100% of Etc) treatment.

The decrease in gs is an important mechanism adopted by olive trees under drought (Moriana et al., 2002; Connor and Fereres, 2005; Boughalleb and Hajlaoui, 2011). This adaptative response contributes to maintain internal plant water status, but it can limit the $CO_2$ photosynthetic assimilation (Flexas and Medrano, 2002; Centritto et al., 2003). The close correlation ($r = 0.98$) between $P_n$ and gs suggest that, under water deficit conditions, photosynthesis may be limited severely by stomatal diffusion. However, photosynthetic performance is not only explained by stomatal control. It has been reported that non-stomatal limitations to photosynthesis may also occur in olive plants, particularly at severe drought stress conditions (Bacelar et al., 2007; Boughalleb and Hajlaoui,

**Table 8**
Fruiting and yield parameters from Arbequina and Manzanilla olive cultivars growing under different water irrigation levels during 2009 and 2010 crop years.

| | Irrigation treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| Arbequina | Crop year | T0 | T25 | T50 | T75 | T100 | r |
| Fruit set (%) | 2009 | $2.5^{a} \pm 0.6$ | $2.8^{a} \pm 0.2$ | $3.3^{b} \pm 0.2$ | $3.6^{b} \pm 0.1$ | $3.6^{b} \pm 0.1$ | 0.45* |
| | 2010 | $1.9^{a} \pm 0.3$ | $1.4^{a} \pm 0.5$ | $2.0^{a} \pm 0.4$ | $1.7^{a} \pm 0.5$ | $1.9^{a} \pm 0.5$ | |
| Fr Y (kg/tree) | 2009 | $9.3^{a} \pm 1.15$ | $20.0^{a} \pm 4.08$ | $37.3^{b} \pm 7.51$ | $82.5^{c} \pm 9.57$ | $90.0^{c} \pm 10.0$ | 0.94* |
| | 2010 | $8.5^{a} \pm 0.85$ | $12.4^{a} \pm 3.12$ | $7.8^{a} \pm 1.21$ | $8.1^{a} \pm 2.07$ | $10.9^{a} \pm 2.21$ | |
| IWP (kg/mm/ha) | 2009 | $2.74^{a} \pm 0.34$ | $5.26^{b} \pm 0.82$ | $8.45^{c} \pm 1.70$ | $16.7^{d} \pm 1.94$ | $16.5^{d} \pm 1.84$ | 0.91* |
| | 2010 | $7.73^{b} \pm 0.77$ | $7.38^{b} \pm 1.87$ | $3.45^{a} \pm 0.53$ | $2.86^{a} \pm 0.73$ | $3.18^{a} \pm 0.65$ | |
| Manzanilla | | | | | | | |
| Fruit set (%) | 2009 | $1.7^{a} \pm 0.1$ | $1.7^{a} \pm 0.2$ | $3.0^{c} \pm 0.1$ | $2.8^{b} \pm 0.2$ | $2.9^{bc} \pm 0.1$ | 0.67* |
| | 2010 | $1.2^{a} \pm 0.1$ | $1.4^{a} \pm 0.5$ | $1.5^{a} \pm 0.3$ | $1.2^{a} \pm 0.4$ | $1.4^{a} \pm 0.2$ | |
| Fr Y (kg/tree) | 2009 | $20.0^{a} \pm 7.07$ | $43.3^{a} \pm 17.5$ | $60.0^{ab} \pm 10.0$ | $96.7^{b} \pm 20.8$ | $138.0^{c} \pm 28.6$ | 0.91* |
| | 2010 | $15.6^{a} \pm 0.85$ | $16.1^{a} \pm 2.11$ | $15.3^{a} \pm 1.22$ | $16.3^{a} \pm 1.74$ | $15.3^{a} \pm 1.52$ | |
| IWP (kg/mm/ha) | 2009 | $5.88^{a} \pm 2.08$ | $11.1^{a} \pm 4.50$ | $13.6^{ab} \pm 2.27$ | $19.6^{bc} \pm 4.22$ | $25.3^{c} \pm 5.25$ | 0.86* |
| | 2010 | $14.2^{d} \pm 0.77$ | $9.56^{c} \pm 1.26$ | $6.77^{b} \pm 0.54$ | $5.76^{b} \pm 0.61$ | $4.49^{a} \pm 0.45$ | |

Mean values (± standar deviation) in each row with different superscript letters present significant differences ($P \leq 0.05$) among irrigation treatments. T0, rain-fed treatment; T25, 25% of Etc; T50, 50% of Etc; T75, 75% of Etc; T100, full-irrigated (100% of Etc) treatment. **r**: Pearson's correlation coefficients among stem water potential and each fruiting and yield parameter. *Significant at $P \leq 0.01$. Fruit set was determined on eighty inflorescences per branch. From each selected tree (six trees per treatment) a total of six branches were used. Abbreviations: Fruit set = [(Frn/Fln) × 100], where Frn is the fruit number per inflorescence and Fln is the flower number per inflorescence; Fr Y, fruit yield; IWP, irrigation water productivity (kg of fresh fruit per mm of irrigation per ha).

2011). In the present study, we observed increased ROS-induced lipid peroxidation, together with photosynthetic pigment losses, in drought-stressed olive trees. These drought-induced biochemical changes have been associated to photosynthetic apparatus impairment (Bacelar et al., 2007; Sofo et al., 2008) and may also contribute to reduce photosynthetic activity and functionality.

### 3.4. Fruiting and yield parameters

During the first crop year analyzed, irrigation rate had a strong effect on both fruit set and fruit yield (Table 8). Regardless of the cultivar, treatments T0 and T25 did not differ significantly in the percent fruit set, but they presented significantly lower values than those from the other treatments. On average, treatments irrigated at 50, 75 and 100% of ETc showed fruit set values approximately 24% (Arbequina) and 41% (Manzanilla) higher than the average values obtained from the respective T0 treatments. Regarding fruit production, Arbequina plants receiving 100% of ETc showed to increase almost 10 times the average yield in relation to unirrigated plants (T0). At the fully irrigated condition the fruit yield had not significant difference with that of the T75 treatment. Fruit yield from Manzanilla trees increased linearly with increased water supply, but the rate of increment was lower than that observed for Arbequina cultivar. In T100, the average yield was approximately 7 times higher than that of T0. Similarly, in California, Goldhamer et al. (1994) found that the yield of mature trees (cv. "Manzanillo") responded linearly to the amount of water applied. When fruit yield was estimated as a function of irrigation water applied per area, very important increments in irrigation water productivity (IWP) were observed at the highest irrigation levels. On average, considering values from T75 and T100 together, IWP increased about 500% (Arbequina) and 330% (Manzanilla) with respect to the values from the respective T0 treatments.

During the second crop year, fruiting and yield responses to irrigation showed a strong contrast with respect to the response patterns obtained from the first year. There were not significant variations among irrigation treatments in fruit set and fruit yield. The average (all treatments) fruit yields represented only 10.6% (Arbequina) and 11.4% (Manzanilla) of the respective fruit yields obtained at the fully irrigated condition during the first crop year. As a result, for the second year, the more elevated irrigation levels lower WP.

Differences in fruit production between crop years were very strong, resulting in an "on" year (2009) and an "off" year (2010). The maintenance of the fully irrigated condition during all year (T100) throughout the whole irrigation experiment (two crop years) could not sustain fruit set and production in the second crop year. Likewise, lower yields recorded in less irrigated treatments during the first year did not correlate with higher yields the next season. Why did fruit production fall in tress under full-irrigated levels? One hypothesis suggests that the different plant organs are connected to sources/sinks relationships which determine the partitioning of nutritional resources, thus affecting the reproductive performance from one year to other (Connor and Fereres, 2005). The heaviest crop loads obtained at T75 and T100 treatments indicate that fruit production was the main sink during the "on" (2009) crop year. This fact could result in competition for photo-assimilates between fruits and induction of buds, resulting in lesser rates of floral induction, floral initiation, fruit set, fruit filling and fruit yield. From data obtained in this work, it is clear that the maintenance of the fully irrigated condition is a useful strategy tending to conserve tree water status and avoid detrimental effects on biochemical and physiological parameters, but it is insufficient to compensate that competition and to maintain top fruit yields.

The results discussed previously highlight the alternate bearing behaviour of both olive cultivars evaluated. The olive tree is in nature an alternate fruit-bearing species and has been reported to alternate seasons of high yields ("on" years) with those of low yields ("off" year) (Lavee et al., 2007; Rallo and Cuevas, 2008). This alternate bearing adds variability and difficulty to interpretation of fruiting records. To assess the pattern of yield response to variations in ETc we also used biennal production data. So, when cumulative fruit production for the two years evaluated were considered, Arbequina and Manzanilla cultivars showed to increase fruit yield by 82.4 and 76.8% in fully irrigated plants with regard to the respective rain-fed treatments.

### 3.5. Variability sources for biochemical and yield responses

In order to assess the comparative responses of the two olive cultivars tested and their possible interactions with both the irrigation level and the crop year, the whole data set was analyzed by three-way ANOVA (Table 9). In general, differences between cultivars were less important than differences between irrigation levels and crop years. The irrigation treatment was the main variability source for CT and all biochemical parameters analyzed. Variations in yield parameters were mainly attributed to crop year effect. $\Psi_{stem}$, $P_n$ and gs showed minor, non-significant variations between cultivars along the RDI application period. Cultivar-related differences were significant for CT, TChl, Car, fruit set, fruit yield and IWP. The results also revealed significant cultivar × irrigation treatment interactions for CT, TChl and Car contents.

In view of the marked differences between genotypes in fruit characteristics, it is difficult to assess the statistical significance of cultivar-specific responses to water irrigation levels. Nevertheless, it seems that in the experimental conditions of this study, the studied olive cultivars responded differently to the irrigation levels employed. At the highest irrigation levels (T75 and T100), fruit yield

**Table 9**
Three-way analysis of variance (ANOVA) for some selected biochemical and yield parameters.

| Parameter | Cultivar (C) | Treatment (T) | Crop year (CY) | C × T | C × CY | T × CY | C × T × CY |
|---|---|---|---|---|---|---|---|
| CT | 14.71* | 56.90* | 0.23 | 6.14* | 1.11 | 0.41 | 1.03 |
| $C_{33}$ | 0.43 | 53.8* | 0.69 | 5.42 | 0.64 | 5.81 | 3.84 |
| $C_{34}$ | 0.20 | 29.1* | 1.37 | 2.97 | 0.70 | 6.84 | 5.55 |
| $C_{35}$ | 1.91 | 46.3* | 0.04 | 2.81 | 1.15 | 2.37 | 1.83 |
| PRO | 0.68 | 83.8* | 2.47 | 0.48 | 0.01 | 0.86 | 0.81 |
| MDA | 0.54 | 73.3* | 2.10 | 0.91 | 0.52 | 1.92 | 0.09 |
| TChl | 5.40* | 34.6* | 0.02 | 4.91* | 0.01 | 6.85* | 1.08 |
| Car | 7.09* | 32.6* | 0.38 | 4.88* | 0.00 | 6.89* | 1.63 |
| Fruit set | 11.1* | 11.1* | 53.2* | 0.51 | 0.94 | 6.38* | 1.45 |
| Fruit yield | 3.19* | 19.9* | 31.5* | 0.55 | 1.11* | 19.8* | 0.81 |
| IWP | 8.88* | 7.62* | 18.1* | 0.32 | 0.47 | 37.7* | 1.60 |

Variability expressed as percentage of total sum of squares. * Significant at $P \leq 0.05$. Abreviations: CT, cuticle thickness; $C_{33}$, tritriacontane content; $C_{34}$, tetratriacontane content; $C_{35}$, pentatriacontane content; PRO, proline content; MDA, malondialdehyde content; TChl, total chlorophyll content; Car, carotenoid content; IWP, irrigation water productivity.

from cv. Arbequina reached a plateau, thus suggesting that, for the agro-ecological conditions of olive growing in central Argentina, Arbequina trees irrigated at 75% ETc during winter-spring period could reach the maximum yield potential. On the contrary, cv. Manzanilla responded linearly to the amount of irrigation water applied. Thus, the fruit yield from the most irrigated treatments does not appear to reach their maximum potential at the time the trees were harvested. IWP revealed significant differences in water-use efficiency between Arbequina and Manzanilla. Thus, differences between cultivars in IWP showed cv. Arbequina with higher fruit production than cv. Manzanilla for the same seasonal irrigation water volume. However, this yield response to irrigation water level was strongly affected by the alternate bearing behaviour of both olive cultivars evaluated, as indicated by the tight coupling between both irrigation level and crop year effects.

## 4. Conclusions

Olive has been successfully cultivated over millennia mainly in the Mediterranean Basin, indicating that it is a crop well adapted to the climatic conditions prevailing in that region. The expansion of olive production has taken olives into non-Mediterranean climates, e.g. subtropics in Australia and Argentina, where the response of the crop to water availability has not been yet studied in detail. Particularly, in arid and semiarid Argentina evapotranspiration is high and rainfall is minimal during the winter and spring months, as compared with the Mediterranean region where winter rainfall precludes the need of irrigation in such period; so, most of the irrigation schedules have been developed to cover water requirements for different fruit developmental phases.

In this study, water deficit was imposed to olive trees (Arbequina and Manzanilla cultivars) for a 5-months period (from mid-June to the end of October). Basically, this covers the winter dormancy period, flower differentiation, and flower opening. The two olive cultivars studied responded to water deprivation in a similar way, by developing rapid and reversible leaf-level adaptative responses: accumulation of osmotically active substances, increased concentration of high molecular weight hydrocarbons and cuticle thickening. Although these traits may be considered indicators of tolerance against water stress, they were not able to prevent detrimental effects of water deprivation. These included membrane lipid peroxidation, and decreased levels of photosynthetic pigments. All these responses followed a reversible pattern: the values from the mentioned parameters returned to baseline values after water deprivation suppression and rewatering. Water deficit influenced both photosynthesis and stomatal conductance in a similar way. $CO_2$ stomatal diffusion could be the most important factor affecting the photosynthetic rate. In addition, non-stomatal limitations associated to photosynthetic apparatus impairment could occur at severe water deficit conditions.

During the first crop year analyzed, a significant decrease in fruit set and fruit yield was observed in treatments under moderate (50% ETc) and severe (25 and 0% ETc) water deprivation. In Arbequina cultivar fruit production peaked in correspondence of 100% Etc, though fully irrigated plants showed statistically comparable values to 75% ETc treatment. On the other hand, all treatments evaluated showed strong drops in fruiting and yield parameters during the second crop year suggesting a marked bearing pattern for both olive cultivars analyzed. From a practical standpoint, little irrigation (50% ETc) may be sufficient to maintain adequate plant water potentials for the coldest winter months, but high (75% ETc) or full (100% ETc) irrigation rates could be needed by mid-August (approximately 2 months before flowering) to avoid detrimental effects of water stress on biochemical–physiological and yield parameters of olive trees cultivated in areas with dry winter-spring season.

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