

# Effects of Water Deficit on the Leaf Water Relations of Pot-grown Olive Cultivars

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## ABSTRACT

An experiment was conducted to investigate the water relations of potted young olive trees (*Olea europaea* L.) cultivar 'Biancolilla', 'Giarraffa' and 'Nocellara del Belice' during water stress and after relief from stress. The aim was to highlight the different responses of the three cultivars and obtain a decision-making aid for planning an efficient watering schedule for potted plants grown in nurseries. Leaf water potential ( $\Psi$ ), relative water content (RWC) and conductance to water vapour ( $g_L$ ) were measured in three-year-old plants. All three olive cultivars were able to survive the severe stress imposed by withholding water for three weeks, but gas exchange was strongly impaired and recovery after rewatering was slow, resulting in a potential reduction of growth. The cultivars showed different responses to drought: 'Giarraffa' was the most sensitive, showing the earliest reduction in  $\Psi$  and the lowest recovery in  $g_L$ . 'Biancolilla' showed higher  $g_L$ , leaf hydration and  $\Psi$  under water deficit and appeared to be best adapted to drought. The behaviour of 'Nocellara del Belice' was similar to that of 'Biancolilla', and a particularly good recovery of  $g_L$  after rewatering occurred.

**Keywords:** drought stress, irrigation scheduling, *Olea europaea*, re-watering, stomatal conductance

**Abbreviations:** DW, dry weight; FW, fresh weight;  $g_L$ , leaf stomatal conductance to water vapour; PAR, photosynthetically active radiation; RH, relative humidity; RW, re-watered conditions; RWC, relative water content; S1, S2, S3, one, two and three weeks of water stress; TLP, turgor loss point; TW, turgid weight; W, watered conditions; WSIS, water stress impact on species;  $\Psi$ , leaf water potential;  $\Psi_{MIN}$ , minimum leaf water potential;  $\Psi_{PD}$ , pre-dawn leaf water potential;  $\Psi_{TLP}$ , leaf water potential at turgor loss point

## INTRODUCTION

Olive is widely cultivated in the Mediterranean basin for oil and table fruit consumption. It has also been used as an ornamental tree in gardens and landscaping (Cantini *et al.* 1999). Being a drought tolerant species, it has been traditionally grown under rain-fed conditions, although the demand for its products has led to irrigation practices in many areas. While plants that grow in the field may explore a wide area of soil with their roots in search of water, plants that are grown in pots need to be watered frequently because they are more easily subject to the negative effects of water stress. Drought affects water relation parameters in higher plants, and stomatal closure due to changes in leaf water potential is commonly observed under water stress conditions (e.g. Chartzoulakis *et al.* 1999; Lo Gullo *et al.* 2003). The reduction of gas exchange results in limited CO<sub>2</sub> fixation, and thus reduces productivity (Fischer and Turner 1978) and under severe drought non-stomatal factors may further affect assimilation in olive trees (Angelopoulos *et al.* 1996). Therefore, drought and water use issues are of special concern in the horticulture industry (Hodges and Haydu 2002). In nurseries and greenhouses the latest technology and trends in water management are starting to be used to grow trees in an environmentally-friendly way that promotes water conservation, lowers operating costs and at the same time improves plant health and survival. Some differences among olive cultivars have been observed concerning their adaptation under drought conditions (Chartzoulakis *et al.* 1999; Giorio *et al.* 1999; Moriana *et al.* 2002; Bacelar *et al.* 2007), but there is very limited information available about the ecophysiological behaviour of three olive cultivars commonly grown in Sicily: 'Biancolilla', used for oil production, 'Nocellara del Belice' and 'Giarraffa' mainly used for the production of pickling olives. Grisafi *et al.*

(2004) studied the effects of heat and drought stress on leaf anatomy, growth and photosynthesis in potted plants of 'Biancolilla' and 'Nocellara del Belice', showing that the former cultivar was the most resistant to stress, while other studies mainly regarded the effects of irrigation on yield and oil quality in 'Nocellara del Belice' (Baratta *et al.* 1985; Patumi *et al.* 1999; D'Andria *et al.* 2004).

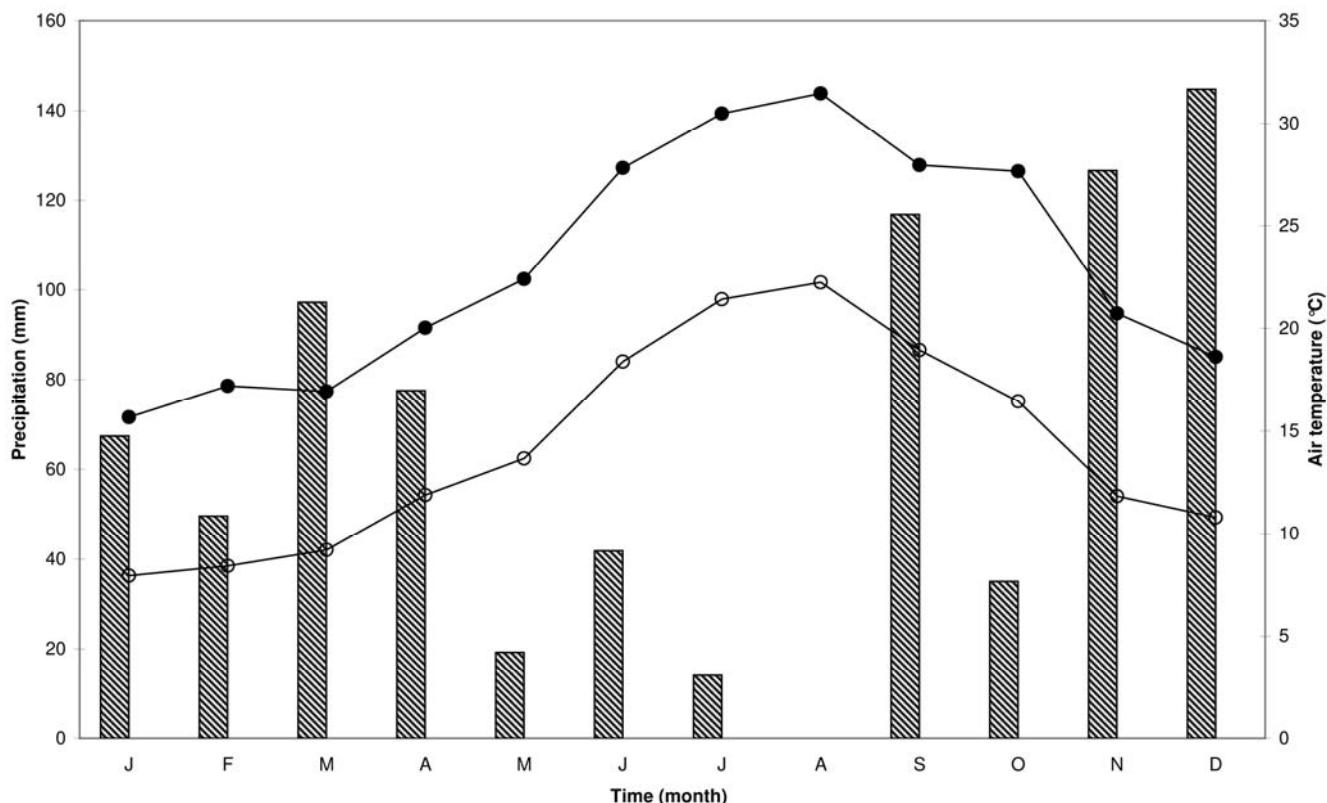
Minimum leaf water potential is often used to quantify stress levels, but it may not be the true expression of the amount of water stress suffered by a plant, as water potential may rise more or less rapidly in different species after reaching minimum values. Therefore, the effect of stress depends also on the length of time the plant is subject to low water potentials, so a more reliable method to assess the impact of water stress on the water relations of a plant is to use an index resulting from the integrals of the curves describing the diurnal pattern of leaf water potential (Mishio and Yokoi 1991; Vertovec *et al.* 2001). A similar index was calculated for gas exchange, from the integrals of the curves describing the diurnal pattern of stomatal conductance.

The aim of this work was to follow the water relations of potted young olive trees of the three cultivars, during a three week period of water stress and a week after relief from stress, and obtain a decision-making aid for planning an efficient watering schedule for potted plants grown in nurseries.

## MATERIALS AND METHODS

### Plant material and growing conditions

Three-year-old olive plants (*Olea europaea* L.) of the cultivars 'Giarraffa', 'Biancolilla' and 'Nocellara del Belice' were grown at the Botanical Garden of Palermo in 50 L pots containing a mixture



**Fig. 1** Mean monthly maximum (solid circles) and minimum (open circles) air temperatures and precipitation (vertical bars) during the year 2004 in Palermo, Italy.

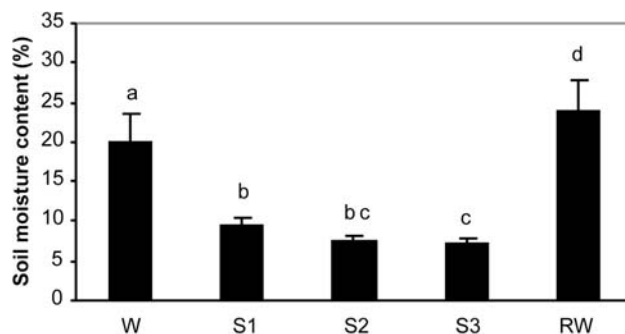
of turf, sand and soil (1:1:1). For each cultivar, twelve plants were divided into a set of six control plants, irrigated twice a week to field capacity (8 L of tap water), and six drought-stressed plants, to which water was withheld for three weeks. After three weeks without water, relief from drought stress was provided by watering the stressed plants like the controls for one week. Of the six plants per treatment, three were used to measure leaf water status parameters and three to determine soil water content. Experiments were carried out in 2004, between the end of June and July, on average the warmest and driest period of the year. The mean monthly air temperatures and precipitation for the year 2004 are reported in **Fig. 1** (data from the Regione Siciliana - SIAS - Servizio Informativo Agrometeorologico Siciliano).

Measurements were taken once a week, at the beginning of the experiment (W, watered conditions), during drought stress (S1, S2, S3, one, two and three weeks after withholding water) and one week after relief from stress (RW, re-watered conditions).

On the days when measurements were taken (June 23 and 30, July 7, 14 and 21 of 2004), photosynthetic active radiation (PAR) was measured with a quantum sensor (LiCor mod. LI-250, LiCor Inc., Lincoln, USA), relative humidity (RH) and air temperature with a thermo-hygrometer (model RMR132HG, Oregon Scientific, Inc. Portland, USA), and soil temperature at a depth of 0.2 m with a probe thermometer (model Checktemp 1, Hanna Instruments). At the beginning of the experiment, minimum and maximum air temperatures were 19 and 24°C respectively, air RH varied between 63 and 80%, and soil temperature varied from 20 to 28°C. At S3, minimum and maximum air temperatures were 20 and 28°C, air RH varied between 36 and 60%, and soil temperature varied from 20 to 32°C, rising 4°C above air temperature at midday. After re-watering, minimum and maximum air temperatures were 23 and 31°C, air RH varied between 55 and 85%, and soil temperature varied from 23 to 32°C. On average, at midday, PAR was around  $1100 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

### Soil water relations

Field capacity of the soil mixture was determined from soil water retention curves obtained by desorption with pressures from 0.03 to 1.5 MPa. To determine changes in soil water content during the experiment, triplicate soil samples were taken from three pots per



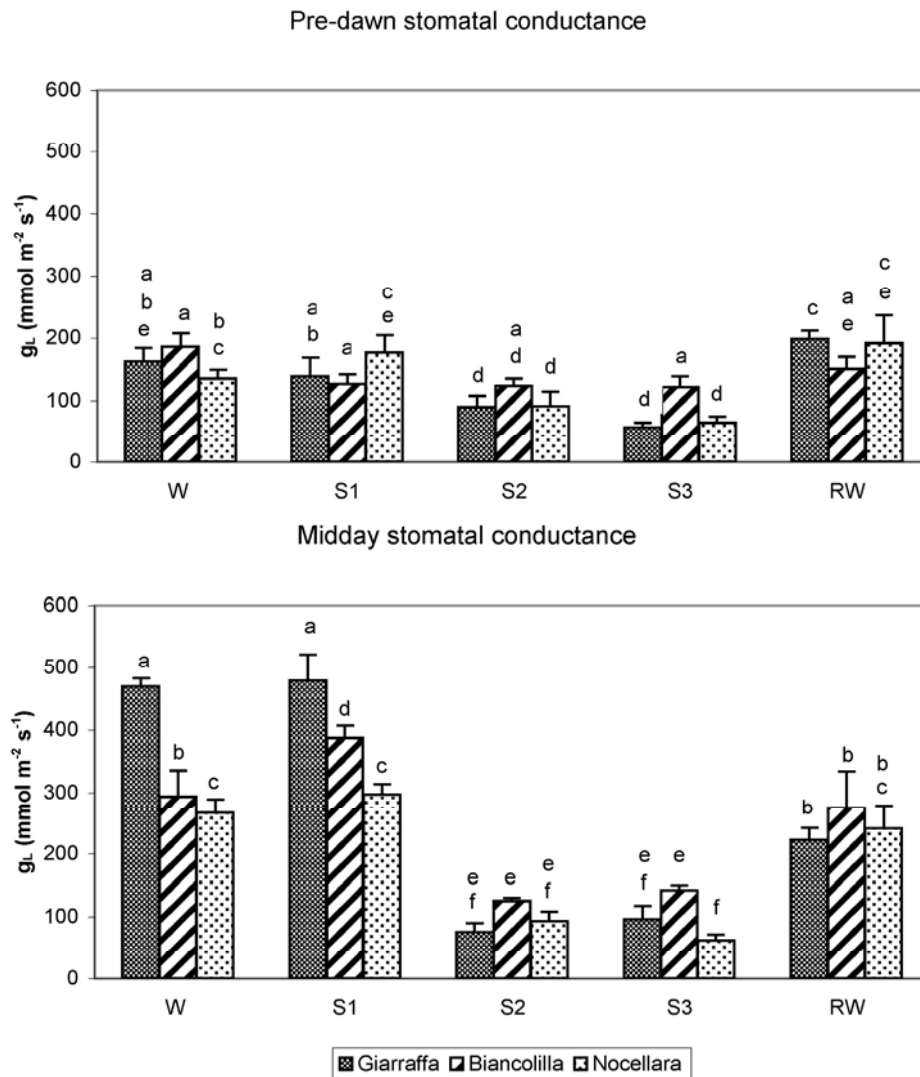
**Fig. 2** Soil moisture content (%) during the course of the experiment. Mean values with SD of soil samples taken at a depth of 0.25 m ( $n = 27$ ). Different letters above the bars indicate significant differences among the means at  $P < 0.05$  for Tukey's test.

cultivar of the drought-stressed trees, at a depth of 0.25 m. The percentage soil water content was determined gravimetrically after drying at 105°C for 3 d (Gardner *et al.* 2001). Soil water content decreased steeply after a week without watering from 20% to 9.5%. At S2 there was still a slight though not significant decrease, down to 7.5%, and at S3 the soil water content did not vary. After re-watering, soil water content returned to the values measured at the beginning of the experiment. Within each treatment there were no significant differences in soil water content among the three cultivars, so data were pooled and mean values during the experiment are shown in **Fig. 2**.

### Leaf water status parameters

All parameters were measured on three plants per treatment and cultivar, on mature, fully expanded leaves. The average leaf size for 'Giarraffa', 'Biancolilla' and 'Nocellara del Belice' was 13.9, 10.7 and 15.6 cm<sup>2</sup>, respectively. Diurnal time course measurements of physiological parameters were taken at regular intervals between 5:30 and 20:00 h.

Leaf conductance to water vapour ( $g_L$ ) was measured on five leaves per plant and time of measurement, using a portable cycling



**Fig. 3** Pre-dawn and midday values of leaf conductance to water vapour ( $g_L$ ) during the course of the experiment in 'Giarraffa', 'Biancolilla' and 'Nocellara del Belice'. Vertical bars are SD of the mean ( $n = 15$ ). Different letters above the bars indicate significant differences among the means at  $P < 0.05$  for Tukey's test.

porometer (model AP4, Delta-T Devices, Ltd., UK).

Leaf water potentials ( $\Psi$ ) were measured according to Lo Gullo *et al.* (2003) using a pressure chamber (model SKPM 1400, Skye Instruments, UK), on twigs of the same length and leaf number. Young tissues influence the average leaf water potential measured on twigs, so new leaf growth had been removed three weeks before the beginning of the experiment. Moist filter paper inside the chamber minimised water loss during measurements. Samples were enclosed in plastic bags, sealed after cutting and immediately transferred to the laboratory where measurements were taken. Before  $\Psi$  measurement, three leaves per plant were collected from the same twigs to determine relative water content (RWC). After recording fresh weight (FW), the leaves were transferred into screw-cap plastic vials, with their cut ends immersed in distilled water. Leaf turgid weight (TW) was measured after 24 h in the dark. Leaves were then dried at  $70^\circ\text{C}$  for 3 d and dry weights (DW) were recorded. Leaf RWC was calculated as  $\text{RWC} = 100 \cdot (\text{FW} - \text{DW}) / (\text{TW} - \text{DW})$ .

Pressure-volume (P-V) isotherms were determined on three twigs per cultivar collected at W and at S3. The P-V isotherms were obtained by the pressure chamber technique (Tyree and Hammel 1972; Salleo 1983). Leaf osmotic potential at full turgor ( $\Psi_{\pi 0}$ ) and leaf water potential at turgor loss point ( $\Psi_{\text{TLP}}$ ) were estimated from the P-V isotherms. The turgor loss point served as a reference to estimate the residual turgor of leaves at minimum diurnal  $\Psi$  values.

The curves describing the pattern of diurnal leaf water potential were used to calculate the water stress impact on species (WSIS), according to the equation:

$$\text{WSIS}_\Psi = \frac{1}{2} \sum_{i=1}^N (\Psi_{(i)} + \Psi_{(i-1)}) \cdot (t_i - t_{i-1})$$

where  $\Psi$  is the leaf water potential in MPa and  $t$  is the time of measurement in h.

The integrated diurnal leaf conductance for each cultivar was calculated using the equation:

$$\text{WSIS}_{g_L} = \frac{1}{2} \sum_{i=1}^N (g_{L(i)} + g_{L(i-1)}) \cdot (t_i - t_{i-1})$$

where  $g_L$  is the leaf stomatal conductance in  $\text{mmol m}^{-2} \text{s}^{-1}$  and  $t$  is the time of measurement in h.

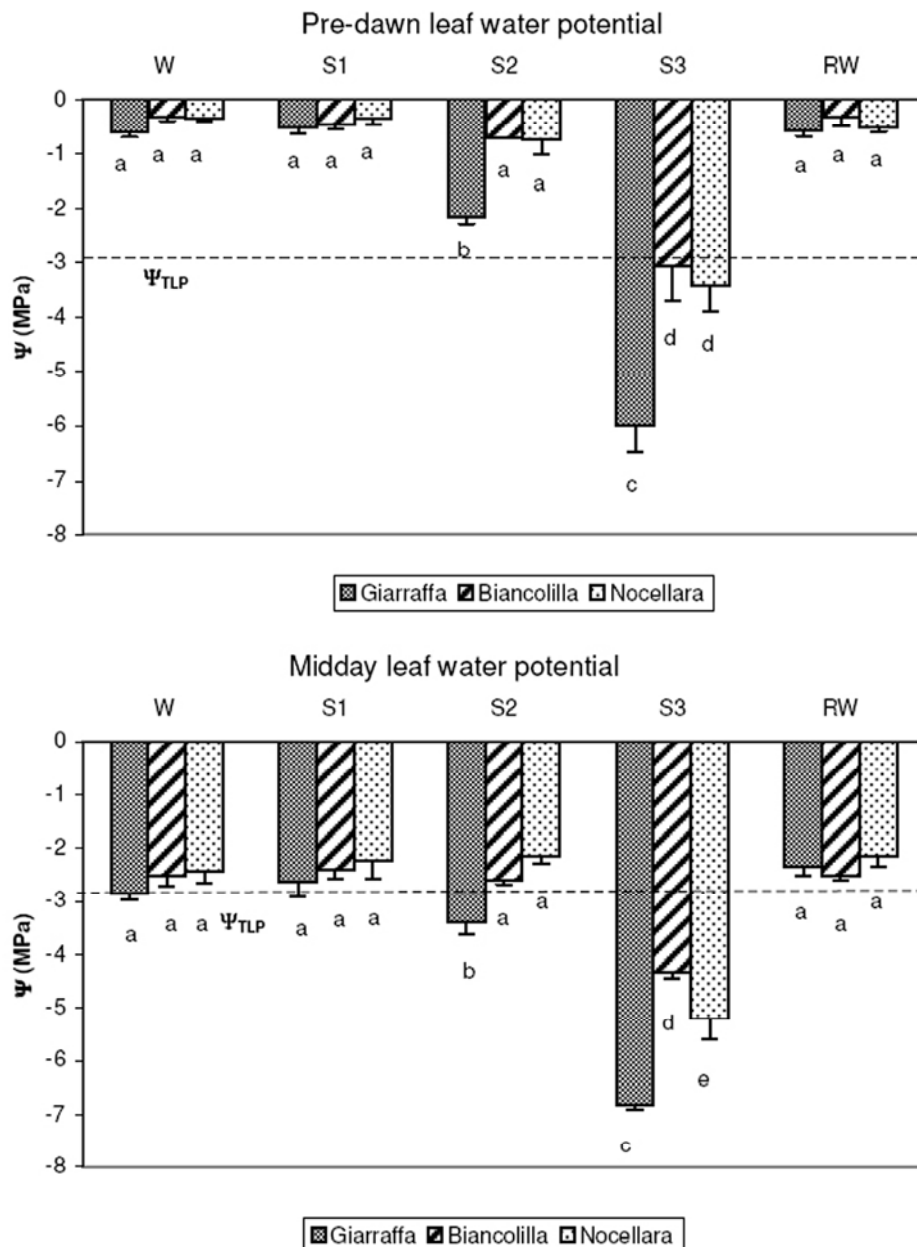
### Statistics

Mean values and standard deviations were calculated and the significance ( $P < 0.05$ ) of differences between sets of data was tested using one-way ANOVA, followed by Tukey's test in cases involving significant  $F$ -values.

## RESULTS

### Leaf water status

Pre-dawn and midday measurements of  $g_L$  during the course of the experiment are shown in **Fig. 3**. Before the onset of stress, 'Giarraffa' showed the highest midday  $g_L$  values,  $470 \text{ mmol m}^{-2} \text{s}^{-1}$ , while 'Biancolilla' and 'Nocellara del Belice' showed lower values,  $290$  and  $270 \text{ mmol m}^{-2} \text{s}^{-1}$ , respectively. Midday  $g_L$  values dropped in all three cultivars two weeks after withholding water, and reached the same values as those measured at pre-dawn, indicating stomatal closure. At S3,  $g_L$  did not change significantly in any of the three cultivars. At RW  $g_L$  increased significantly, reaching



**Fig. 4** Pre-dawn and midday values of leaf water potential ( $\Psi$ ) during the course of the experiment in 'Giarraffa', 'Biancolilla' and 'Nocellara del Belice'. Vertical bars are SD of the mean ( $n = 3$ ). Different letters below the bars indicate significant differences among the means at  $P < 0.05$  for Tukey's test. The horizontal lines represent mean leaf water potential at turgor loss point ( $\Psi_{TLP}$ ).

**Table 1** Leaf osmotic potential at full turgor ( $\Psi_{\pi 0}$ ) and leaf water potential at turgor loss point ( $\Psi_{TLP}$ ) for the three cultivars at the beginning of the experiment (W) and three weeks after withholding water (S3). Mean values are reported  $\pm$  SD ( $n = 3$ ). Values followed by different letters denote significant differences within a column ( $P < 0.05$ ).

Cultivar	W		S3	
	$\Psi_{\pi 0}$ (-MPa)	$\Psi_{TLP}$ (-MPa)	$\Psi_{\pi 0}$ (-MPa)	$\Psi_{TLP}$ (-MPa)
'Giarraffa'	2.32 $\pm$ 0.11 ab	2.89 $\pm$ 0.09 a	2.23 $\pm$ 0.19 ab	2.73 $\pm$ 0.07 a
'Biancolilla'	2.35 $\pm$ 0.03 a	2.71 $\pm$ 0.14 a	2.33 $\pm$ 0.04 a	2.75 $\pm$ 0.07 a
'Nocellara'	2.12 $\pm$ 0.09 b	2.73 $\pm$ 0.05 a	2.42 $\pm$ 0.03 b	2.90 $\pm$ 0.02 b

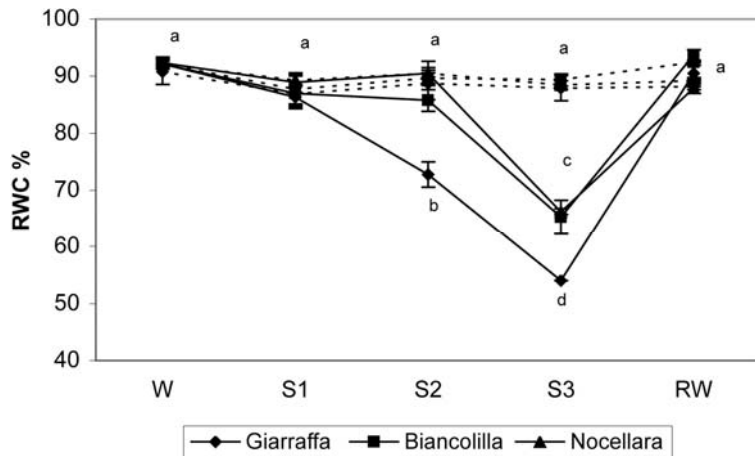
50% of the controls in 'Giarraffa', 95% in 'Biancolilla' and 90% in 'Nocellara del Belice'.

Pre-dawn ( $\Psi_{PD}$ ) and midday ( $\Psi_{MIN}$ ) values of leaf water potential during the course of the experiment are reported in **Fig. 4**. At S1, values of  $\Psi_{MIN}$  did not vary significantly in any of the three cultivars. At S2, 'Giarraffa' showed significant signs of water stress, with a decrease of  $\Psi_{PD}$  from -0.5 to -2.0 MPa, and  $\Psi_{MIN}$  values about 20% lower than the water potential at turgor loss point ( $\Psi_{TLP}$ ) (**Table 1**). In 'Biancolilla' and 'Nocellara del Belice'  $\Psi$  values did not change significantly. At S3, all cultivars showed symptoms of severe water stress, with both  $\Psi_{PD}$  and  $\Psi_{MIN}$  values below turgor loss point. The lowest  $\Psi$  values were measured in 'Giarraffa' (-6.8 MPa), followed by 'Nocellara del Belice' (-5.2 MPa) and 'Biancolilla' (-4.3 MPa). At RW, all three cultivars had recovered, with  $\Psi_{PD}$  values not significantly different from those of the controls and  $\Psi_{MIN}$  values within

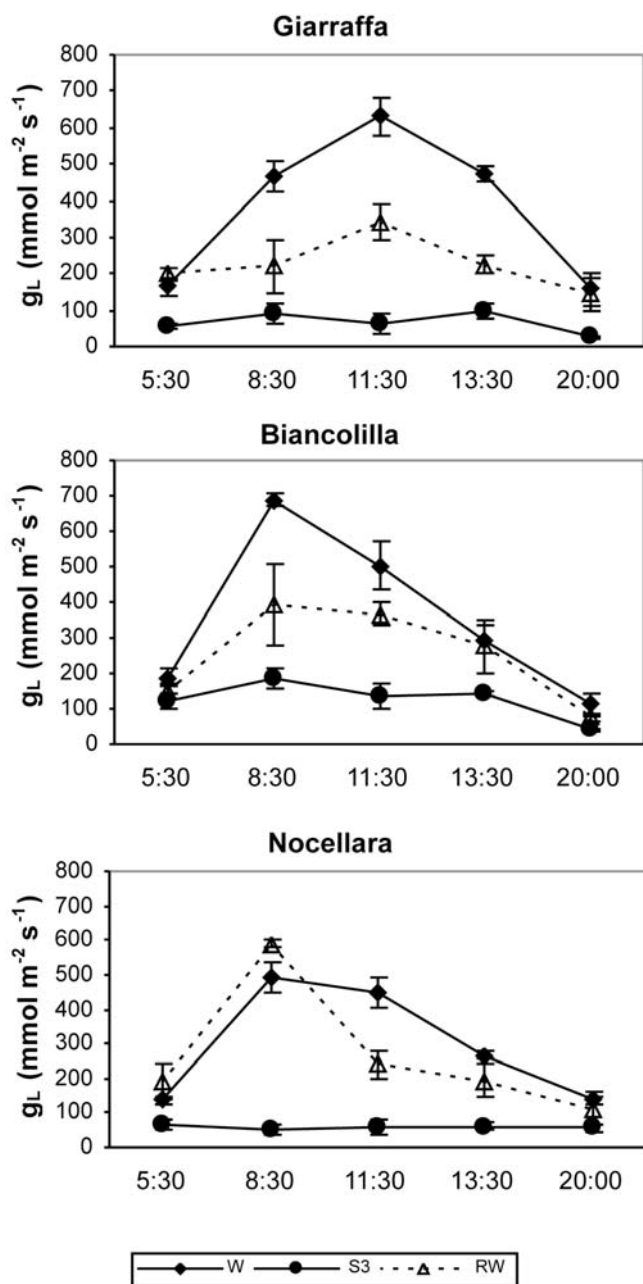
the turgor region.

Leaf RWC followed the same pattern found for  $\Psi$  (**Fig. 5**). At the beginning of the experiment, all cultivars showed RWC values around 9%. At S1, there were no significant changes in RWC of any of the cultivars. At S2, in 'Giarraffa' RWC was 87% at predawn and decreased significantly to 73% at midday. 'Biancolilla' and 'Nocellara del Belice' had predawn RWC values of 92% and minimum RWC of 86 and 90%, respectively, and were not significantly different from the controls. At S3, 'Giarraffa' showed a further significant decrease in RWC, which remained very low throughout the day, around 55%. 'Biancolilla' and 'Nocellara del Belice' showed the same significant decrease in RWC, that varied from 73% at predawn to 65% at midday. At RW, RWC values returned to those of the controls in all cultivars.

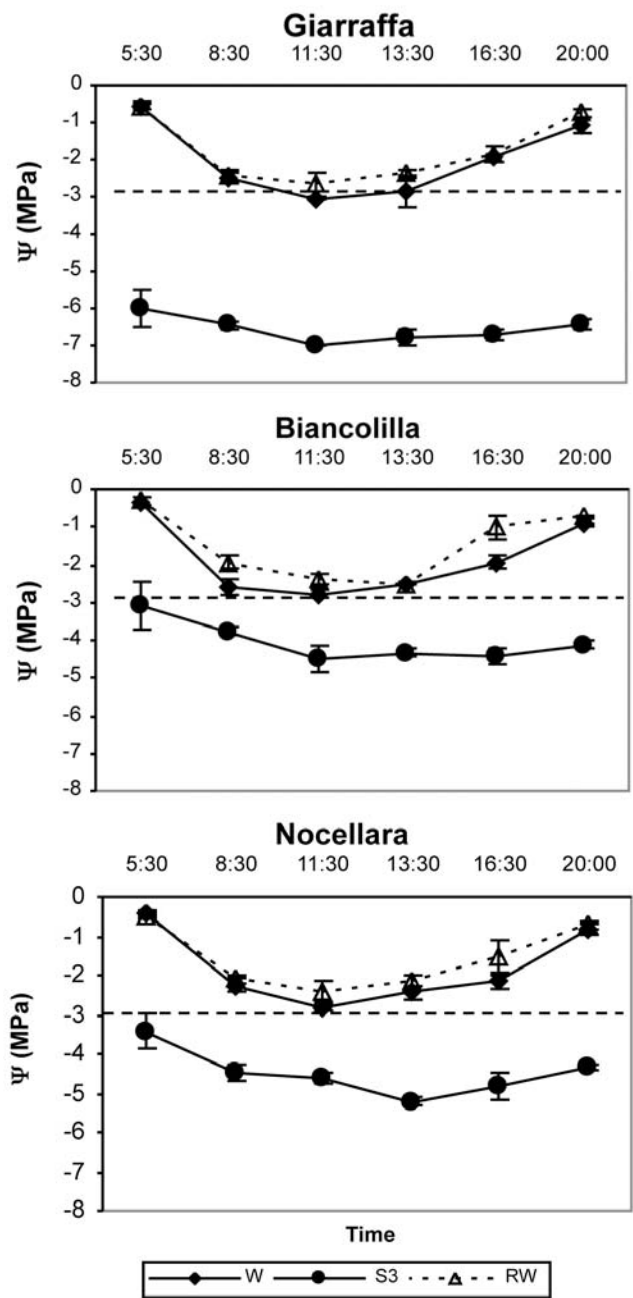
Analysis of leaf water potential isotherms at the begin-



**Fig. 5** Leaf relative water content during the course of the experiment in control (broken lines) and stressed (solid lines) plants of 'Giarraffa', 'Biancolilla' and 'Nocellara del Belice'. Vertical bars are SD of the mean ( $n = 9$ ). Different letters near the symbols indicate significant differences among the means at  $P < 0.05$  for Tukey's test.



**Fig. 6** Diurnal time courses of leaf conductance to water vapour ( $g_L$ ) in 'Giarraffa', 'Biancolilla' and 'Nocellara del Belice' at the beginning of the experiment (W), three weeks after withholding water (S3) and one week after relief from water stress (RW). Vertical bars are SD of the mean ( $n = 15$ ).



**Fig. 7** Diurnal time courses of leaf water potential ( $\Psi$ ) in 'Giarraffa', 'Biancolilla' and 'Nocellara del Belice' at the beginning of the experiment (W), three weeks after withholding water (S3) and one week after relief from water stress (RW). Vertical bars are SD of the mean ( $n = 3$ ). The horizontal lines represent leaf water potential at turgor loss point ( $\Psi_{TLP}$ ).

**Table 2** Water Stress Impact on Species (WSIS<sub>ψ</sub>) calculated as integrated diurnal values for leaf water potential (Ψ) at the beginning of the experiment (W), three weeks after withholding water (S3) and one week after relief from water stress (RW). Values are reported ± percent SD (n = 3). Values followed by different letters denote significant differences within a column (P<0.05).

	WSIS <sub>ψ</sub> (MPa h)	WSIS <sub>ψ</sub> (MPa h)	WSIS <sub>ψ</sub> (MPa h)
	W	S3	RW
'Giarraffa'	32.79 ± 7.9% a	102.50 ± 2.3% a	29.06 ± 7.9% a
'Biancolilla'	30.87 ± 6.3% a	63.83 ± 5.4% b	23.91 ± 10.1% a
'Nocellara'	30.41 ± 7.1% a	71.06 ± 4.7% b	25.65 ± 12.6% a

ning of the experiment and at S3 (Table 1) showed no significant differences in Ψ<sub>π0</sub> and Ψ<sub>TLP</sub> between the treatments for 'Giarraffa' and 'Biancolilla', while in 'Nocellara del Belice' there was a significant decrease both in Ψ<sub>π0</sub> and Ψ<sub>TLP</sub>. At W, Ψ<sub>TLP</sub> was not significantly different in the three cultivars, while at S3 Ψ<sub>TLP</sub> of 'Nocellara del Belice' was significantly lower than in 'Giarraffa' and 'Biancolilla'.

The diurnal time course of g<sub>L</sub> and Ψ can give more detailed information than the minimum midday values on the amount of water stress suffered by the plants. Fig. 6 shows the daily pattern of g<sub>L</sub> in controls, plants at S3 and plants at RW. In control plants of all cultivars, maximum g<sub>L</sub> values were reached between 8:30 and 11:30 h. At S3, stomata remained practically closed throughout the day in 'Giarraffa' and 'Nocellara del Belice'. In 'Biancolilla' g<sub>L</sub> remained slightly higher than in the other two cultivars, constant throughout the day and around 100 mmol m<sup>-2</sup> s<sup>-1</sup>. At RW, in 'Giarraffa' and 'Biancolilla' stomata began opening again, but g<sub>L</sub> remained quite lower than the controls; in 'Nocellara del Belice' the g<sub>L</sub> values reached those of the controls during the first hours of the morning.

The diurnal time course of Ψ (Fig. 7) shows that, for stressed plants, in 'Giarraffa' there was no recovery of Ψ<sub>L</sub> during the night, as predawn values were not significantly different from those measured throughout the day. In 'Biancolilla' and 'Nocellara del Belice' a small drop in Ψ between pre-dawn and the warmer hours of the day was still evident. The diurnal time course of Ψ at RW showed complete recovery of Ψ values in all three cultivars.

The integrated diurnal Ψ values calculated for the three cultivars at W, S3 and RW are reported in Table 2. In well watered conditions, W and RW, the WSIS values of the three cultivars ranged between 24 and 33 MPa h and there were no significant differences among the cultivars. Under severe drought stress, at S3, WSIS increased by 210% in 'Giarraffa', 107% in 'Biancolilla' and 134% in 'Nocellara del Belice', indicating that water stress strongly affected the plants throughout the day and confirming that 'Giarraffa' suffered the greatest degree of water stress.

The integrated diurnal g<sub>L</sub> values (WSIS<sub>g<sub>L</sub></sub>) are reported in Table 3. At W, 'Giarraffa' had the highest WSIS<sub>g<sub>L</sub></sub> value (5724 mmol m<sup>-2</sup> s<sup>-1</sup> h), followed by 'Biancolilla' and 'Nocellara del Belice'. At S3 the greatest reduction in gas exchange, around 80%, occurred for 'Giarraffa' and 'Nocellara del Belice', while 'Biancolilla' showed a 65% reduction with respect to controls. At RW, the WSIS<sub>g<sub>L</sub></sub> values reached 57% and 72% of the controls in 'Giarraffa' and 'Biancolilla', respectively, while 'Nocellara del Belice' showed the highest recovery, reaching 87% of the controls.

## DISCUSSION

All three olive cultivars were able to survive the severe stress imposed by withholding water for three weeks. However, the cultivars showed different responses to drought. 'Giarraffa' appeared to be the most sensitive cultivar: at S2 leaf Ψ fell below Ψ<sub>TLP</sub> values and Ψ<sub>PD</sub> decreased steeply. In 'Biancolilla' and 'Nocellara del Belice' stress suffered at S2 was less intense, and Ψ fell below Ψ<sub>TLP</sub> only at S3 (Fig. 4). Several components determine Ψ<sub>PD</sub> values and these are not always related to the water status of the soil in the root zone, so the use of Ψ<sub>PD</sub> as an indicator for irrigation cannot always be recommended (Améglio *et al.* 1999). Our data also confirm that, for potted plants in our experimental conditions, Ψ<sub>PD</sub> does not necessarily reflect soil Ψ, as different Ψ<sub>PD</sub> values were measured in plants growing in soil with the same water content.

While 'Giarraffa' showed the earliest reduction in leaf Ψ, the effect of withholding water on g<sub>L</sub> was the same in all cultivars, with stomatal conductance reduced to pre-dawn values at S2 (Fig. 3). The reduction in g<sub>L</sub> could therefore be related more to soil drying than to leaf water status. Indeed, when soil water status declines slowly, over several days or more, g<sub>L</sub> often declines without any change in Ψ, due to a drought-sensing mechanism located in the roots that induces the production of ABA (Zhang and Davies 1990; Gollan *et al.* 1992). However, it must be kept in mind that our measurements were taken at 7 day intervals, and that there may have been more subtle differences among the cultivars on a shorter time scale that could have gone undetected. In 'Biancolilla' and 'Nocellara del Belice' stomatal closure at S2 allowed the maintenance of high RWC (Fig. 2), high Ψ<sub>PD</sub>, and Ψ<sub>MIN</sub> above Ψ<sub>TLP</sub> (Fig. 4). In 'Giarraffa', instead, stomatal closure was not sufficient to prevent the drop in RWC, Ψ<sub>PD</sub> and Ψ<sub>MIN</sub>. As drought stress increased, Ψ reached very negative values especially in 'Giarraffa', down to -6.8 MPa, reaching a Ψ<sub>MIN</sub>/Ψ<sub>TLP</sub> ratio of 2.4. At S3, leaf dehydration proceeded in all cultivars notwithstanding stomatal closure, as observed in wild olive trees growing in arid conditions, where leaves underwent consistent dehydration even at quite low g<sub>L</sub> levels (Vertovec *et al.* 2001). This observation was explained by the fact that in *O. oleaster* the root system was extremely vulnerable to drought due to a large reduction in root hydraulic conductance (Lo Gullo *et al.* 1998; Nardini *et al.* 1998). Changes in water relations parameters like leaf water status or stomatal response can be explained by changes in hydraulic properties (Tyree and Ewers 1991; Tardieu and Davies 1993; Salleo *et al.* 2000; Lo Gullo *et al.* 2003; Bacelar *et al.* 2007) and several studies have outlined the importance of xylem cavitation as the factor contributing most to reduction of productivity of plants subjected to environmental stresses (Sperry and Tyree 1988; Hubbard *et al.* 2001). The capability of a given species to maintain high root hydraulic conductance might represent one of the most important factors in determining drought resistance strategy (Terradas and Savé 1992; Nardini *et al.* 1999).

The effect of water stress did not result simply in an episodic lowering of Ψ<sub>MIN</sub> values, but all three cultivars suffered throughout the day a prolonged state of stress, as shown by the WSIS values for Ψ and g<sub>L</sub> (Tables 2 and 3). The degree of stress experienced by 'Biancolilla' and 'Nocellara del Belice' was comparable to that of plants of *Olea oleaster* (WSIS<sub>ψ</sub> values of about 70 MPa h) growing in the

**Table 3** Water Stress Impact on Species (WSIS<sub>g<sub>L</sub></sub>) calculated as integrated diurnal values for leaf conductance to water vapour (g<sub>L</sub>) at the beginning of the experiment (W), three weeks after withholding water (S3) and one week after relief from water stress (RW). Values are reported ± percent SD (n = 3). Values followed by different letters denote significant differences within a column (P<0.05).

	WSIS <sub>g<sub>L</sub></sub> (mmol m <sup>-2</sup> s <sup>-1</sup> h)	WSIS <sub>g<sub>L</sub></sub> (mmol m <sup>-2</sup> s <sup>-1</sup> h)	WSIS <sub>g<sub>L</sub></sub> (mmol m <sup>-2</sup> s <sup>-1</sup> h)
	W	S3	RW
'Giarraffa'	5723.74 ± 9.2% a	1014.77 ± 25.8% a	3229.07 ± 18.8% a
'Biancolilla'	5213.26 ± 10.1% ab	1818.19 ± 13.9% b	3735.08 ± 21.5% a
'Nocellara'	4390.21 ± 8.6% b	836.06 ± 24.7% a	3827.40 ± 14.4% a

field under water stress conditions (Vertovec *et al.* 2001). The overall impact of water stress on 'Giarraffa' was higher than in the other two cultivars. Under well watered conditions, 'Giarraffa' reached higher levels of  $g_L$ , both as maximum and integrated values, so it is possible that this cultivar lost water faster than the other two, reaching turgor loss point earlier. On reaching  $\Psi_{TLP}$  a reduction in fine root hydraulic conductance may occur, leading to cavitation, as reported for potted wild olive seedlings (Lo Gullo *et al.* 1998). With the same soil water availability, 'Biancolilla' and 'Nocellara del Belice', that reach  $\Psi_{TLP}$  only at S3, could rely on a better functioning hydraulic system.

One week after rewatering,  $\Psi$  returned to control values, while  $g_L$  was still impaired. Therefore, it is important to note that  $\Psi$  alone does not give sufficient information on the effects of stress, as shown by the behaviour of all cultivars after rewatering, when  $\Psi$  was restored to the level of controls (Fig. 7), but gas exchange was still affected by the previous drought period (Fig. 6). This lag in the recovery of  $g_L$  is in agreement with reports that in olive rewatering after severe water stress caused a prompt recovery in  $\Psi$  not accompanied by a re-increase in  $g_L$  (Natali *et al.* 1991; Tognetti *et al.* 2005). These data appear in agreement with the hypothesis that  $\Psi$  does not control  $g_L$  (Giorio *et al.* 1999), and on the other hand the observation of a negative correlation between  $g_L$  and  $\Psi$  under well watered conditions, according to Jones (1998) implies a role in the control of plant water status by leaf stomatal conductance, and not *vice versa*. However, although the mechanism by which stomata regulate water loss is still an argument of debate, a great deal of empirical evidence suggests that stomatal guard cells respond by negative feedback to a local measure of  $\Psi$  and a response of  $g_L$  need not require a variation in  $\Psi$  beyond the range of measurement uncertainty (Buckley 2005). Chemical signals such as ABA and sap pH may also control stomatal behaviour, even before changes in  $\Psi$  are evident (Morillon and Chrispeels 2001; Wilkinson and Davies 2002; Buckley 2005).

Among the three cultivars, 'Biancolilla' was the one that under water deficit showed higher  $g_L$ , leaf hydration and  $\Psi$  and appeared to be the cultivar best adapted to drought, in agreement with results on photosynthetic activity (Grisafi *et al.* 2004). The behaviour of 'Nocellara del Belice' was similar to that of 'Biancolilla', and a particularly good recovery of  $g_L$  after rewatering was observed. 'Giarraffa' was the most sensitive to drought, showing the earliest reduction in  $\Psi$ , the highest  $WSIS_{\Psi}$  value under stress and the lowest recovery in  $g_L$  after re-watering. We can therefore conclude that 'Biancolilla' is a very resistant cultivar among those that can be used for oil production, while, 'Nocellara del Belice' is a very resistant cultivar for the production both of oil and pickling olives.

Although it is impossible to give a unique and perfect watering schedule, due to the coexistence of many variables such as soil type, rainfall quantity and regularity, evaporation and transpiration rates, etc., the results obtained show that all three cultivars resist up to three weeks without water, but their gas exchange is strongly impaired and the recovery after rewatering is slow, resulting in a potential reduction of growth. To optimise potted growth conditions, all the cultivars should be watered to field capacity at 8-10 day intervals, in order to avoid reduction of stomatal conductance. However, 'Biancolilla' and 'Nocellara del Belice' can better tolerate up to two weeks without water.

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## REFERENCES

- Améglío T, Archer P, Cohen M, Valancogne C, Daudet F, Dayau S, Cruziat P (1999) Significance and limits in the use of predawn leaf water potential for tree irrigation. *Plant and Soil* **207**, 155-167
- Angelopoulos K, Dichio B, Xiloyannis C (1996) Inhibition of photosynthesis in olive trees (*Olea europaea* L.) during water stress and rewatering. *Journal of Experimental Botany* **301**, 1093-1100
- Bacelar EA, Moutinho-Pereira JM, Gonçalves BC, Ferreira HF, Correia CM (2007) Changes in growth, gas exchange, xylem hydraulic properties and water use efficiency of three olive cultivars under contrasting water availability regimes. *Environmental and Experimental Botany* **60**, 183-192
- Baratta B, Caruso T, Di Marco L, Inglese P (1985) Effetti dell'irrigazione sulle caratteristiche delle olive nella cv. "Nocellara del Belice". *Rivista di Frutticoltura* **3/4**, 61-66
- Buckley TN (2005) The control of stomata by water balance. *New Phytologist* **168**, 275-292
- Cantini C, Cimato A, Sani S (1999) Morphological evaluation of olive germplasm present in Tuscany region. *Euphytica* **109**, 173-181
- Chartzoulakis K, Patakas A, Bosabalidis AM (1999) Changes in water relations, photosynthesis and leaf anatomy induced by intermittent drought in two olive cultivars. *Environmental and Experimental Botany* **42**, 113-120
- d'Andria R, Lavini A, Morelli G, Patumi M, Terenziani S, Calandrelli D, Fragnito F (2004) Effects of water regimes on five pickling and double aptitude olive cultivars (*Olea europaea* L.). *The Journal of Horticultural Science and Biotechnology* **79**, 18-25
- Fischer RA, Turner NC (1978) Plant productivity in the arid and semiarid zones. *Annual Review of Plant Physiology and Plant Molecular Biology* **29**, 277-317
- Gardner CMK, Robinson D, Blyth K, Cooper JD (2001) Soil water content. In: Smith KA, Mullins CE (Eds) *Soil and Environmental Analysis: Physical Methods*, CRC Press, Boca Raton, pp 1-64
- Giorio P, Sorrentino G, d'Andria R (1999) Stomatal behaviour, leaf water status and photosynthetic response in field-grown olive trees under water deficit. *Environmental and Experimental Botany* **42**, 95-104
- Gollan T, Schurr U, Schulze ED (1992) Stomatal response to drying soil in relation to changes in xylem sap composition of *Helianthus annuus*. I. The concentration of cations, anions, amino acids in, and pH of, the xylem sap. *Plant Cell and Environment* **15**, 551-559
- Grisafi F, Bonafede E, Dalla Vecchia F, Rascio N (2004) Some morphological, anatomical, physiological responses of different olive cultivars to high temperatures and drought stress. *Acta Botanica Gallica* **151**, 241-253
- Hodges AW, Haydu JJ (2002) *Economic impacts of the Florida Environmental Horticulture Industry, 2000*. Economic Information Report EI 02-3 Food and Resource Economics Department, Institute of Food and Agricultural Sciences, University of Florida, 87 pp
- Hubbard RM, Ryan MG, Stiller V, Sperry JS (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant Cell and Environment* **24**, 113-121
- Jones HG (1998) Stomatal control of photosynthesis and transpiration. *Journal of Experimental Botany* **49** (Special Issue), 387-398
- Lo Gullo MA, Nardini A, Salleo S, Tyree MT (1998) Changes in root hydraulic conductance ( $K_R$ ) of *Olea oleaster* seedlings following drought stress and irrigation. *New Phytologist* **140**, 25-31
- Lo Gullo MA, Salleo S, Rosso R, Trifilò P (2003) Drought resistance of 2-year-old saplings of Mediterranean forest trees in the field: relations between water relations, hydraulics and productivity. *Plant Soil* **250**, 259-272
- Mishio M, Yokoi Y (1991) A model for estimation of water flow resistance in soil-leaf pathway under dynamic conditions. *Journal of Experimental Botany* **42**, 541-546
- Moriana A, Villalobos FJ, Fereres E (2002) Stomatal and photosynthetic responses of olive (*Olea europaea* L.) leaves to water deficits. *Plant Cell and Environment* **25**, 395-405
- Morillon R, Chrispeels MJ (2001) The role of ABA and transpiration stream in the regulation of the osmotic water permeability of leaf cells. *Proceedings of the National Academy of Sciences USA* **98**, 14138-14143
- Nardini A, Lo Gullo MA, Salleo S (1999) Competitive strategies for water availability in two Mediterranean *Quercus* species. *Plant Cell and Environment* **22**, 109-116
- Nardini A, Salleo S, Lo Gullo MA (1998) Root hydraulic conductance of six forest trees: possible adaptive significance of seasonal changes. *Plant Biosystems* **132**, 97-104
- Natali S, Bignami C, Fusari A (1991) Water consumption, photosynthesis, transpiration and leaf water potential in *Olea europaea* L., cv "Frantoio", at different levels of available water. *Agricoltura Mediterranea* **121**, 205-212
- Patumi M, d'Andria R, Fontanazza G, Morelli G, Giorio P, Sorrentino G (1999) Yield and oil quality of intensively trained trees of three cultivars of olive (*Olea europaea* L.) under different irrigation regimes. *Journal of Horticultural Science and Biotechnology* **74**, 729-737
- Salleo S (1983) Water relation parameters of two Sicilian species of *Senecio* (Groundsel) measured by the pressure bomb. *New Phytologist* **95**, 851-859
- Salleo S, Nardini A, Pitt F, Lo Gullo MA (2000) Xylem cavitation and hydraulic control of stomatal conductance in laurel (*Laurus nobilis* L.). *Plant*

- Cell and Environment* **23**, 71-79
- Sperry JS, Tyree MT** (1988) Mechanism of water stress-induced xylem embolism. *Plant Physiology* **88**, 581-587
- Tardieu F, Davies WJ** (1993) Integration of hydraulic and chemical signalling in the control of stomatal conductance and water status of droughted plants. *Plant Cell and Environment* **16**, 341-349
- Terradas J, Savé R** (1992) The influence of summer and winter stress and water relationships on the distribution of *Quercus ilex* L. *Vegetatio* **99/100**, 137-145
- Tognetti R, d'Andria R, Morelli G, Alvino A** (2005) The effect of deficit irrigation on seasonal variations of plant water use in *Olea europaea* L. *Plant Soil* **273**, 139-155
- Tyree MT, Ewers FW** (1991) The hydraulic architecture of trees and other woody plants. *New Phytologist* **119**, 345-360
- Tyree MT, Hammel HT** (1972) The measurement of the turgor pressure and water relations of plants by the pressure bomb technique. *Journal of Experimental Botany* **23**, 267-282
- Vertovec M, Sakçali S, Ozturk M, Salleo S, Giacomich P, Feoli E, Nardini A** (2001) Diagnosing plant water status as a tool for quantifying water stress on a regional basis in Mediterranean drylands. *Annals of Forest Science* **58**, 113-125
- Wilkinson S, Davies WJ** (2002) ABA-based chemical signalling: the co-ordination of responses to stress in plants. *Plant Cell and Environment* **25**, 195-210
- Zhang MW, Davies WJ** (1990) Changes in the concentration of ABA in xylem sap as a function of changing soil water status can account for changes in leaf conductance and growth. *Plant Cell and Environment* **13**, 277-285