

# Osmotic regulation in leaves and roots of olive trees during a water deficit and rewatering

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**Summary** We evaluated the osmotic adjustment capacity of leaves and roots of young olive (*Olea europaea* L.) trees during a period of water deficit and subsequent rewatering. The trials were carried out in Basilicata (40°24' N, 16°48' E) on 2-year-old self-rooted olive plants (cv. 'Coratina'). Plants were subjected to one of four drought treatments. After 13 days of drought, plants reached mean predawn leaf water potentials of  $-0.45 \pm 0.015$  MPa (control),  $-1.65 \pm 0.021$  (low stress),  $-3.25 \pm 0.035$  (medium stress) and  $-5.35 \pm 0.027$  MPa (high stress). Total osmotic adjustment increased with increasing severity of drought stress. Trees in the high stress treatment showed total osmotic adjustments ranging between 2.4 MPa at 0500 h and 3.8 MPa at 1800 h on the last day of the drought period. Osmotic adjustment allowed the leaves to reach leaf water potentials of about  $-7.0$  MPa. Active osmotic adjustment at predawn decreased during the rewatering period in both leaves and roots. Stomatal conductance and net photosynthetic rate declined with increasing drought stress. Osmotic adjustment in olive trees was associated with active and passive osmotic regulation of drought tolerance, providing an important mechanism for avoiding water loss.

**Keywords:** drought tolerance, gas exchange, *Olea europaea*, osmotic adjustment, turgor potential, water potential.

## Introduction

Osmotic adjustment allows plants to tolerate temporary or prolonged periods of water shortage and is one of the crucial processes involved in plant adaptation to drought (Chaves et al. 2003). Plants subjected to water deficits may synthesize and accumulate amino acids (e.g., proline and aspartic acid), proteins, sugars (e.g., sucrose, glucose and mannitol), methylated quaternary ammonium compounds (e.g., glycine betaine and alanine betaine) and organic acids (Ingram and Bartels 1996). High concentrations of these compatible solutes contribute to the lowering of osmotic potential ( $\Psi_{\pi}$ ) and allow water to move into the cells, thereby maintaining the turgor potential

( $\Psi_p$ ) and increasing tissue tolerance to low soil water potentials (Tyree and Jarvis 1982, Bray 1993). These solutes also sequester water molecules, protect cell membranes and protein complexes and allow the metabolic machinery to continue functioning (Chaves et al. 2003). The lowering of  $\Psi_{\pi}$  as a result of the net accumulation of compatible solutes is defined as "active osmotic adjustment" ( $A\Delta\Psi_{\pi}$ ) and can be determined by measuring osmotic potential at full turgor ( $\Psi_{\pi 100}$ ) (Girma and Krieg 1992). The mechanism by which a net loss of symplastic water in plant tissues causes a reduction in cell volume and an increase in solute concentration is defined as "passive osmotic adjustment" ( $P\Delta\Psi_{\pi}$ ) (Lakso 1985). A correlation between osmotic adjustment and drought stress has been found in several tree species, including *Ziziphus rotundifolia* Lamk. (Arndt et al. 2001), *Vitis vinifera* L. (Patakas et al. 2002), *Eucalyptus* (Ngugi et al. 2003), *Populus tremula* L. and *Tilia cordata* Mill. (Aasamaa et al. 2004).

Olives (*Olea europaea* L.) are a common crop of the Mediterranean basin, where long periods of drought occur during the dry season when temperature and vapor pressure deficit are high. This species exhibits a high degree of drought tolerance (Lo Gullo and Salleo 1988), withstanding water deficits by reducing gas exchange (Xiloyannis et al. 1988, Moriana et al. 2002), controlling transpiration and water uptake (Moreno et al. 1996, Fernández et al. 1997), establishing a high water potential gradient between leaves and roots (Xiloyannis et al. 1999) and increasing the activity of some antioxidant enzymes (Sofa et al. 2004a) that likely protect photosystem II (PSII) (Angelopoulos et al. 1996). Drought stress in olive trees also causes leaf anatomical alterations, which contribute to drought tolerance (Bosabalidis and Kofidis 2002). Moreover, a decrease in the size and a parallel increase in the density of leaf cells, increases in the abundance of leaf hairs and stomata during water shortage (Bosabalidis and Kofidis 2002) and the formation of scleromorphic leaves that are well adapted to the high solar irradiances and temperatures prevailing in semi-arid regions (Jarvis and McNaughton 1986) contribute to drought tolerance of olive trees. Finally, olive trees subjected to a period of rewatering following a drought show rapid recovery of

photosynthetic function (Angelopoulos et al. 1996) that also depends on irradiance (Sofo et al. 2004a).

Many of the responses of olive trees to drought are well documented (Lo Gullo and Salleo 1988, Chartzoulakis et al. 1999), but few studies have focused on the mechanisms of osmotic adjustment, so little is known about the osmoregulatory capacity of this species during periods of water shortage and rewatering. To obtain a more complete picture of the responses of olive trees to drought, we determined total osmotic adjustment ( $\Delta\Psi_{\pi} = A\Delta_{\pi} + P\Delta\Psi_{\pi}$ ) of plants during exposure to different severities of drought stress and during subsequent recovery from drought. We also defined the relative contributions of  $A\Delta_{\pi}$  and  $P\Delta_{\pi}$  to  $\Delta\Psi_{\pi}$ .

## Materials and methods

### Plant material and experimental design

The trials were conducted at the Pantanello Agricultural Experimental Farm in Metaponto (southern Italy, Basilicata Region, 40°24' N, 16°48' E) on 2-year-old self-rooted *Olea europaea* cv. 'Coratina' plants that were about 1.5 m high. Forty olive plants were grown outdoors in 0.016-m<sup>3</sup> pots filled with a 3:1 (v/v) mixture of field soil (73.2% sand, 13.3% silt and 13.5% clay) and peat. The pots were covered with plastic film and aluminum foil to restrict evaporation from the soil surface and to minimize radiant heating of the containers. Plants were weighed every evening and the amount of water transpired daily was determined. Soil water content from transplanting to the start of the drought treatment was held constant at around 85% of soil water holding capacity. Plants were fertilized at 25–30-day intervals throughout the period of vegetative growth with 3 to 4 g of slow-release nitrogen complex fertilizer (Nitrophoska Gold 15, 9, 16, 2, 7 N, P, K, Ca, Mg, Compo Agricoltura, Cesano Maderno, MI, Italy).

The plants were separated into four groups of 10. In the control group, the water added daily was equal to the amount transpired. In the other three groups drought stress was imposed for 13 days by restoring only 75 (low stress), 50 (medium stress) and 25% (high stress) of the total water transpired.

From each treatment, three plants with similar predawn leaf water potentials ( $\Psi_w$ ) were selected for  $\Psi_{\pi}$  measurements. After 13 days of drought, mean predawn  $\Psi_w$  values were  $-0.45 \pm 0.015$  (SE) MPa in control plants,  $-1.65 \pm 0.021$  MPa in low-stress plants,  $-3.25 \pm 0.035$  MPa in medium-stress plants and  $-5.35 \pm 0.027$  MPa in high-stress plants. After the 13-day drought period, plants were watered daily for 30 days by adding the amount of water lost by transpiration.

### Environmental parameters

Throughout the drying and rewatering periods, vapor pressure deficit (VPD) and photosynthetic photon flux (PPF) were monitored at a weather station located within 20 m of the experimental plot. Because conditions were similar throughout the experimental period, data are reported for the last day of the drought period and the first day of the rewatering phase.

### Soil water status

Soil water content at field capacity was determined gravimetrically. After percolation of gravitational water, four 200 g soil samples were taken from four pots without trees and covered with a plastic film to prevent evaporative water loss. Soil water content at the permanent wilting point was measured gravimetrically on four soil samples taken from four randomly selected pots with trees. Soil available water was obtained by calculating the difference between soil water content at field capacity and soil water content at permanent wilting point.

### Gas exchange

At 1, 4, 7, 11 and 13 days from the beginning of the drought treatments, three plants per treatment with similar predawn  $\Psi_w$  values were chosen to measure net photosynthetic rate and stomatal conductance. Measurements were made on three fully expanded leaves from each plant taken from the mid-section of current-year shoots and marked at the beginning of the experiment. The measurements were made with a leaf chamber analyzer LCA-4 (ADC, Hoddesdon, Hertfordshire, U.K.) operated at a flow rate of 200  $\mu\text{mol s}^{-1}$  under the prevailing environmental conditions.

### Plant water status

Throughout the drought treatment, plant water status was determined by simultaneous measurements of  $\Psi_w$  and leaf water content (LWC) on 4–5 fully expanded leaves from each plant taken from the mid-section of current-year shoots. Each excised leaf was immediately put inside a polyethylene bag for  $\Psi_w$  measurement. All predawn and midday  $\Psi_w$  measurements were made with a pressure chamber (Model 600, PMS Instruments, Corvallis, OR), according to Turner (1981).

Values of LWC were determined as:

$$\text{LWC} = 100 (\text{FM} - \text{DM}) / \text{FM} \quad (1)$$

where FM is leaf fresh mass and DM is leaf dry mass. Dry mass was determined after drying the leaf samples at 80 °C for 24 h. Values of LWC were expressed as relative water content (RWC) by determining FM, DM and saturated mass (SM) as:

$$\text{RWC} = (\text{FM} - \text{DM}) / (\text{SM} - \text{DM}) \quad (2)$$

For SM determination, leaves were rehydrated by immersing the petiole in distilled water in a beaker sealed with parafilm. Full rehydration was achieved in 24–48 h in complete darkness at 2–4 °C.

During the last day of the 13-day drought treatment, leaves from plants in each treatment were collected at 0500, 0700, 1200, 1500 and 1800 h, placed in polyethylene bags and immediately frozen at –80 °C. At 0, 6 and 29 days from the beginning of the rewatering period, leaves and roots from plants in each treatment were sampled at 0500 h, placed in polyethyl-

ene bags and stored at  $-80^{\circ}\text{C}$ . Three samples were collected from each plant, with each sample containing 4–5 fully expanded leaves from the mid-section of current-year shoots and 10–15 g FM of roots (diameter = 4 to 5 mm).

Frozen tissues were equilibrated at  $20^{\circ}\text{C}$  for 15 min before determination of  $\Psi_{\pi}$ ,  $\Psi_p$  and  $\Psi_{\pi 100}$ . Cell contents were extracted by squeezing tissues in sterilized plastic syringes. A 100- $\mu\text{l}$  aliquot of the extract was used to determine osmolarity with an osmometer (Wescor model 2000, Logan, UT, USA) calibrated against a salt solution.

Osmotic potential values of expressed sap were calculated from the Van't Hoff relationship as given by Nobel (1983):

$$\Psi_{\pi} = 0.02437 \text{ (osmolarity)} \quad (3)$$

Values of  $\Psi_p$  were calculated as:

$$\Psi_p = \Psi_w - \Psi_{\pi} \quad (4)$$

This determination of  $\Psi_p$  gives a mean value for all cells in the analyzed tissue, providing a valid indication of the general physiological status of the leaf. Negative values of  $\Psi_p$  were considered to indicate zero turgor, according to Ackerson and Krieg (1977). Values of  $\Psi_{\pi 100}$  values were calculated after Morgan (1984) as:

$$\Psi_{\pi 100} \text{LWC}_{100} = \Psi_{\pi} \text{LWC}_{\text{id}} \quad (5)$$

where  $\text{LWC}_{100}$  is leaf water content at full hydration (after nighttime water recovery) and  $\text{LWC}_{\text{id}}$  is the daytime leaf water content of the treated plants. Because artificial rehydration causes a slight oversaturation that significantly alters the pressure–volume relationship and, hence, estimated  $\Psi_{\pi}$  (Parker and Pallardy 1987),  $\text{LWC}$  was used as a more accurate estimation of  $\Psi_{\pi 100}$ .

#### Osmotic adjustment

For each group of water-stressed plants,  $\Delta\Psi_{\pi}$  during the last day of the drought treatment was calculated. The  $\Delta\Psi_{\pi}$  at a specified hour of the day (0500, 0700, 1200, 1500 and 1800 h) was defined as the difference between osmotic potential measured at predawn in control plants ( $\Psi_{\pi(\text{predawn})}$ ) and osmotic potential measured at the specified hour in water-stressed plants ( $\Psi_{\pi(\text{hour})}$ ), according to Girma and Krieg (1992).

For each group of water-stressed plants,  $A\Delta\Psi_{\pi}$  during the last day of the drought treatment was calculated. The  $A\Delta\Psi_{\pi}$  at a specified hour of the day (0500, 0700, 1200, 1500 and 1800 h) was defined as the difference between estimated osmotic potential at full turgor measured at predawn in control plants ( $\Psi_{\pi 100(\text{predawn})}$ ) and estimated osmotic potential at full turgor measured at the specified hour in water-stressed plants ( $\Psi_{\pi 100(\text{hour})}$ ).

The contribution of the loss of symplastic water to the decline in  $P\Delta\Psi_{\pi}$  was determined as:

$$P\Delta\Psi_{\pi} = \Delta\Psi_{\pi} - A\Delta\Psi_{\pi} \quad (6)$$

#### Statistical analysis

All measurements were expressed as means of three measurements ( $\pm$  SE) from three plants per treatment with similar predawn  $\Psi_w$ . Significant differences were detected at  $P = 0.05$ , according to the Student's  $t$  test.

#### Results

During the last day of the 13-day drought treatment, VPD ranged between 0.9 (0500 h) and 4.1 kPa (1300 h), and the highest PPF was  $1640 \mu\text{mol m}^{-2} \text{s}^{-1}$  (1100 h; Figure 1A). During the first day of rewatering, VPD values fluctuated from 1.4 (0500 h) to 4.2 kPa (1300 h) and the PPF pattern peaked at  $1680 \mu\text{mol m}^{-2} \text{s}^{-1}$  (1300 h; Figure 1B). The decline in RWC in the olive plants during the drought treatment was paralleled by a substantial decrease in  $\Psi_w$  (Figure 2A). Soil water content during soil drying progressively declined with time (Figure 2B). Values of  $\Psi_w$  decreased during the day and subsequently recovered and re-equilibrated at night, showing a pattern of progressive decline during the drought treatment (Figure 2B). During the last day of the drought treatment,  $\Psi_w$  decreased in all plants subjected to drought stress, whereas control plants showed a recovery of  $\Psi_w$  between 1200 and 1800 h (Figure 3A). Values of  $\Psi_{\pi}$  declined with increasing water deficit (Figure 3B). Predawn  $\Psi_{\pi}$  ranged between  $-2.9$  MPa in control plants and  $-5.4$  MPa in high-stress-treated plants (Figure 3B). In the three groups of drought-stressed plants, diurnal variations in  $\Psi_{\pi}$  showed a pattern similar to that of  $\Psi_w$ , whereas in control plants, diurnal variations in  $\Psi_{\pi}$  and  $\Psi_w$  differed (Figures 3A and 3B). The values of  $\Psi_p$  fluctuated between 2.5 MPa in control plants to zero turgor in high-stress-treated plants (Figure 3C).

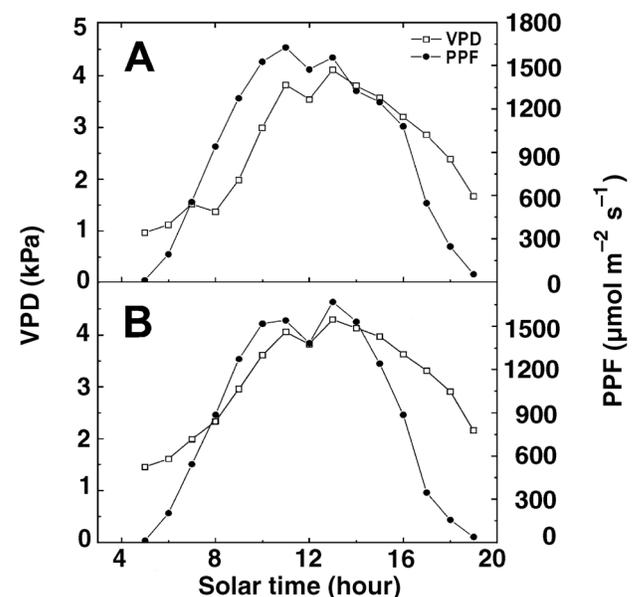


Figure 1. Vapor pressure deficit (VPD) and photosynthetic photon flux (PPF) at the field site during the last day of the drought treatment (A) and the first day of the rewatering phase (B).

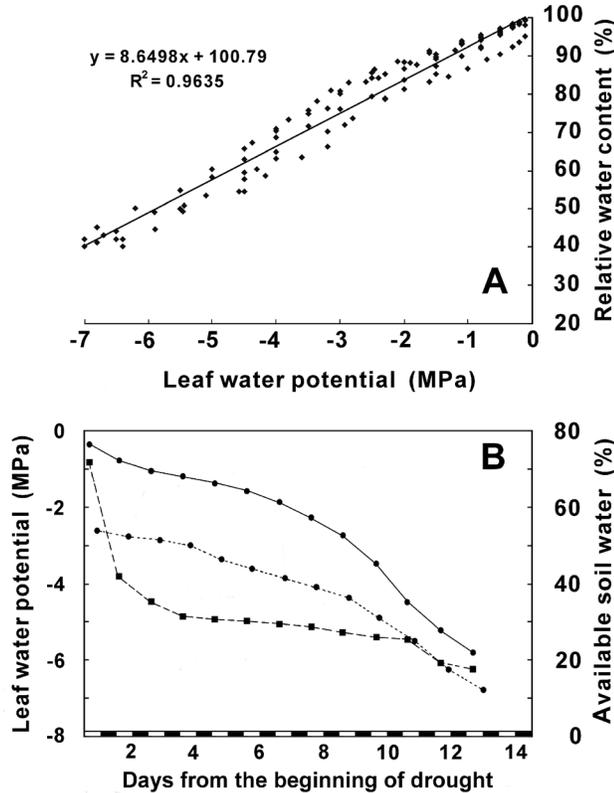


Figure 2. Relationship between leaf water potential and relative water content in drought-stressed olive plants (A). Patterns of predawn leaf water potential (●, solid line), midday leaf water potential (●, dotted line) and soil available water (■) measured in olive plants during the drought treatment (B).

During the last day of the drought treatment,  $P\Delta\Psi_{\pi}$ ,  $A\Delta\Psi_{\pi}$  and  $\Delta\Psi_{\pi}$  increased with decreasing  $\Psi_w$  in all drought-stressed plants (Figure 4). Values of  $\Delta\Psi_{\pi}$  in high-stress plants varied from 2.4 MPa at 0500 h to 3.8 MPa at 1800 h (Figure 4A).

Values of  $P\Delta\Psi_{\pi}$  in high-stress-treated plants ranged between 1.4 MPa at 0500 h and 2.2 MPa at 1800 h, corresponding to 57 and 58% of total osmotic adjustment, respectively (Figure 4C). Values of  $A\Delta\Psi_{\pi}$  increased in drought-stress plants, particularly in high-stress-treated plants, ranging from 1.0 MPa at 0500 h to 1.6 MPa at 1800 h, corresponding to 43 and 42% of total osmotic adjustment, respectively (Figure 4B). Absolute values of stomatal conductance in control plants ranged from 0.128 to 0.149 mol m<sup>-2</sup> s<sup>-1</sup> at 0700 h and from 0.086 to 0.095 mol m<sup>-2</sup> s<sup>-1</sup> at 1300 h. Absolute values of net photosynthetic rate in control plants ranged from 20.3 to 22.7 μmol m<sup>-2</sup> s<sup>-1</sup> at 0700 h and from 14.2 to 16.1 μmol m<sup>-2</sup> s<sup>-1</sup> at 1300 h. Stomatal conductance in drought-stressed plants ranged between 18 and 78% of control values at 0700 h, and between 3 and 55% at 1300 h (Figure 5A), whereas photosynthetic rates in drought-stressed plants varied from 13 to 78% of control values at 700 h, and from 5 to 40% at 1300 h (Figure 5B).

Values of  $\Psi_{\pi 100}$  and  $A\Delta\Psi_{\pi}$  measured predawn during the rewatering phase increased in all drought-stressed plants (Ta-

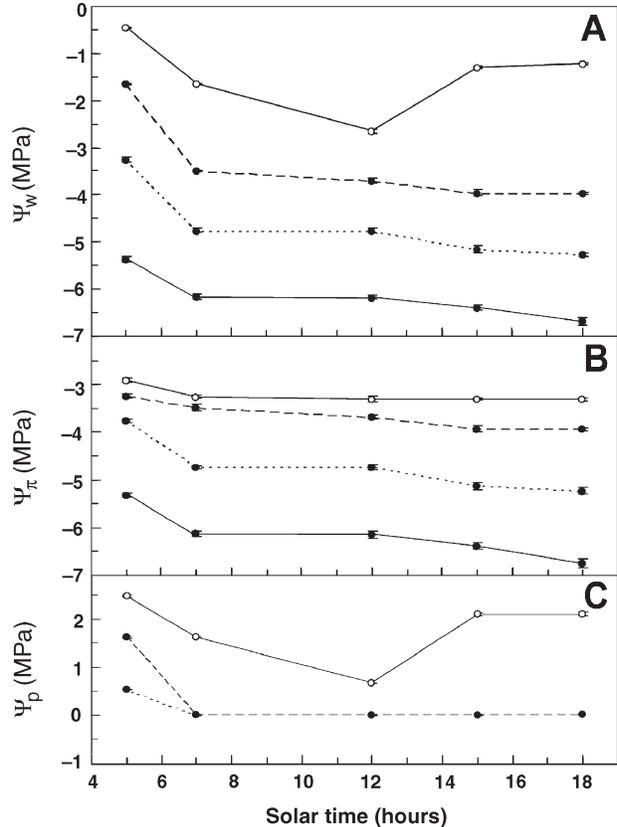


Figure 3. Diurnal patterns of (A) leaf water potential ( $\Psi_w$ ), (B) osmotic potential ( $\Psi_{\pi}$ ) and (C) turgor potential ( $\Psi_p$ ) in olive plants subjected to different severities of drought stress (●, dashed line = -1.65 MPa; ●, dotted line = -3.25 MPa; ●, solid line = -5.35 MPa) and in control plants (○ = -0.45 MPa) during the last day of the drought period. Each value is the mean  $\pm$  SE of three measurements on three plants per drought treatment.

ble 1). Even on Day 29 of the rewatering period, leaves of plants that had been subjected to drought stress did not fully regain pretreatment values of  $\Psi_{\pi 100}$  and  $A\Delta\Psi_{\pi}$ . On days 6 and 29 of the rewatering period, high-stress-treated plants had higher values of  $\Psi_{\pi 100}$  and  $A\Delta\Psi_{\pi}$  than low- and medium-stress-treated plants (Table 1). In roots, rewatering caused predawn  $\Psi_{\pi 100}$  and  $A\Delta\Psi_{\pi}$  to increase and the patterns of  $\Psi_{\pi 100}$  and  $A\Delta\Psi_{\pi}$  to vary depending on the severity of the water stress previously experienced by the plants. On Day 29 of the rewatering period, roots in all the water-stress treatments had higher values of  $\Psi_{\pi 100}$  and  $A\Delta\Psi_{\pi}$  than control plants (Table 2).

## Discussion

The ability of the olive tree to transfer water from its tissues to the xylem sap, both under well-watered and drought conditions, causes a greater lowering of  $\Psi_w$  compared with that observed in most other tree species (Xiloyannis et al. 1988). Under drought conditions, olive leaves can release about 60% of the water stored in their tissues to transpiration (Tombesi et al. 1986), thus contributing to transpiration requirements as

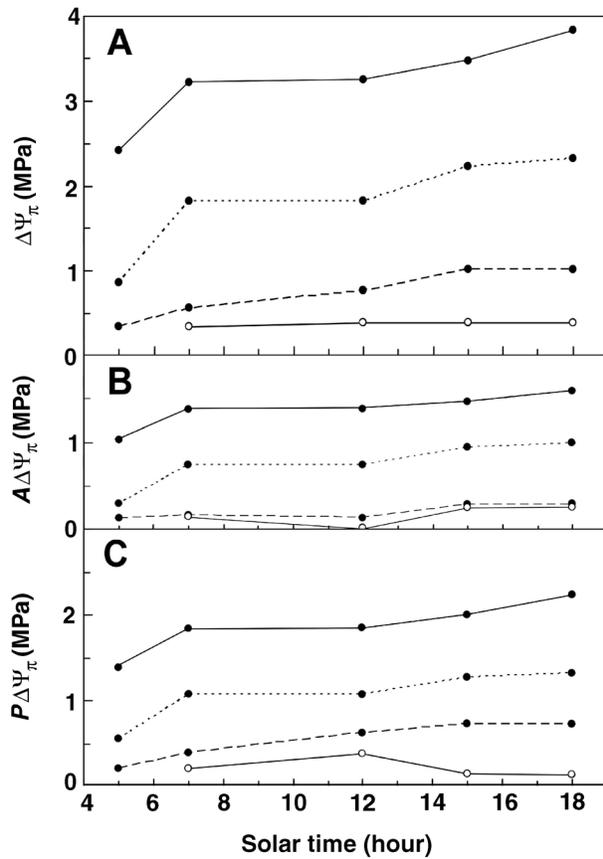


Figure 4. Diurnal patterns of (A) total osmotic adjustment ( $\Delta\Psi_{\pi}$ ), (B) active osmotic adjustment ( $A\Delta\Psi_{\pi}$ ) and (C) passive osmotic adjustment ( $P\Delta\Psi_{\pi}$ ) in olive plants subjected to different severities of drought stress (●, dashed line =  $-1.65$  MPa; ●, dotted line =  $-3.25$  MPa; ●, solid line =  $-5.35$  MPa) and in control plants (○ =  $-0.45$  MPa) during the last day of the drought period. Each value is the mean  $\pm$  SE of three measurements on three plants per drought treatment.

water stress increases to extreme values of  $-7.0$  MPa, when RWC reaches 40% (Figure 2A). The amount of water released from olive tissue for transpiration is high compared with that released by leaves of other fruit species (e.g., about 9% in

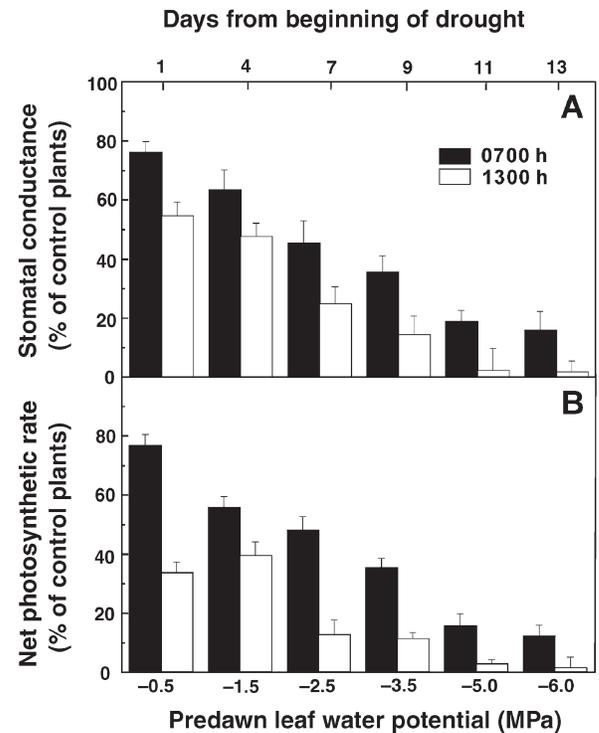


Figure 5. Stomatal conductance (A) and net photosynthetic rate (B) during the drought treatment measured at 0700 h (black columns) and 1300 h (gray columns). The histograms represent the mean  $\pm$  SE of three measurements on three plants per drought treatment.

kiwifruit) under conditions of severe water deficit (Nuzzo et al. 1997).

The leaf-to-air VPD is the driving force for transpiration (Figure 1) and the water flux in plants reflects the rate of water movement in the soil–plant–atmosphere continuum as a function of this gradient. Kiwifruit and grapevine—species with large conducting vessels—exhibit only slight water potential fluctuations during the day ( $-0.2$  to  $-1.2$  MPa) and a limited water potential gradient between leaves and roots (Salleo et al. 1985, Sperry et al. 1987). By contrast, olive trees have a low hydraulic conductivity (Salleo et al. 1985) because of their narrow conducting vessels (diameter =  $30$ – $50$   $\mu\text{m}$ ) with lumina of about 8% of the total xylem cross-sectional area.

Table 1. Osmotic potential at full turgor ( $\Psi_{\pi 100}$ ; MPa) and active osmotic adjustment ( $A\Delta\Psi_{\pi}$ ; MPa) measured at 0500 h in leaves of well-irrigated and drought-stressed olive plants at 0, 6 and 29 days after the beginning of the rewatering period. Each value is the mean  $\pm$  SE of three measurements on three plants per drought treatment. Within a column, values of  $\Psi_{\pi 100}$  followed by different letters are significantly different ( $P = 0.05$ , Student's  $t$ -test,  $n = 3$ ).

Treatments	Last day of drought		Day 6 of rewatering		Day 29 of rewatering	
	$\Psi_{\pi 100}$	$A\Delta\Psi_{\pi}$	$\Psi_{\pi 100}$	$A\Delta\Psi_{\pi}$	$\Psi_{\pi 100}$	$A\Delta\Psi_{\pi}$
Control	$-2.93 \pm 0.04$ a	–	$-2.68 \pm 0.04$ a	–	$-2.41 \pm 0.07$ a	–
Low stress	$-3.06 \pm 0.05$ b	0.13	$-2.82 \pm 0.09$ b	0.14	$-2.70 \pm 0.07$ b	0.29
Medium stress	$-3.23 \pm 0.03$ c	0.30	$-2.80 \pm 0.09$ b	0.12	$-2.64 \pm 0.08$ b	0.23
High stress	$-3.97 \pm 0.02$ d	1.04	$-3.20 \pm 0.03$ c	0.52	$-3.19 \pm 0.01$ c	0.78

Table 2. Osmotic potential at full turgor ( $\Psi_{\pi 100}$ ; -MPa) and active osmotic adjustment ( $A\Delta\Psi_{\pi}$ ; MPa) measured at 0500 h in roots (diameter = 4–5 mm) of well-irrigated and drought-stressed olive plants at 0, 6 and 29 days from the beginning of the rewatering period. Each value is the mean  $\pm$  SE of three measurements on three plants within a drought treatment. Within a column, values of  $\Psi_{\pi 100}$  followed by different letters are significantly different ( $P = 0.05$ , Student's  $t$ -test,  $n = 3$ ).

Treatments	Last day of drought		Day 6 of watering		Day 29 of watering	
	$\Psi_{\pi 100}$	$A\Delta\Psi_{\pi}$	$\Psi_{\pi 100}$	$A\Delta\Psi_{\pi}$	$\Psi_{\pi 100}$	$A\Delta\Psi_{\pi}$
Control	$-1.34 \pm 0.08a$	–	$-1.40 \pm 0.07 a$	–	$-1.38 \pm 0.07a$	–
Low stress	$-1.88 \pm 0.05 b$	0.54	$-1.43 \pm 0.05 a$	0.04	$-1.40 \pm 0.07 ab$	0.02
Medium stress	$-2.65 \pm 0.03 c$	1.31	$-1.94 \pm 0.05 b$	0.55	$-1.43 \pm 0.09 bc$	0.05
High stress	$-3.01 \pm 0.02 d$	1.67	$-2.11 \pm 0.05 b$	0.71	$-1.58 \pm 0.07 c$	0.20

During the drought treatment, available soil water decreased from field capacity to near the permanent wilting point (Figure 2B), which is equal to  $-2.5$  MPa in olive trees (Dichio et al. 2003). The ability of olive tissues to lose water to transpirational flux (Figure 2A) caused the concentration of cell solutes to increase and  $\Psi_{\pi}$  to decrease with increasing drought stress (Figure 3B) (cf. Chartzoulakis et al. 1999). Lo Gullo and Salleo (1988) showed that predawn  $\Psi_{\pi 100}$  in *Olea oleaster* Hoffmgg. et Link plants grown in a semi-arid environment fluctuated from  $-1.95$  to  $-2.50$  MPa. These values are higher than those measured in our investigation (Table 1) and this difference is probably associated with the different mechanisms of osmotic adjustment adopted by cultivated (*Olea europaea*) and wild (*Olea oleaster*) olive trees. Three cultivars of *Ceratonia siliqua* L. subjected to seasonal drought also had higher midday  $\Psi_{\pi}$  values than our olive trees (Correia et al. 2001). Furthermore,  $\Psi_{\pi 100}$  in two species of *Eucalyptus* (Ngugi et al. 2003) and in cherry trees (Ranney et al. 1991) subjected to drought were higher than in our olive plants. These findings confirm the greater ability of olive trees to tolerate severe water deficits through regulation of  $\Psi_{\pi}$  compared with other tree species.

The accumulation of compatible solutes in olive trees allows the maintenance of cell turgor and thus the opening of the stomata during periods of drought (Chartzoulakis et al. 1999). It is known that the lowering of cell  $\Psi_{\pi}$  in olive is responsible for a high  $\Psi_w$  gradient between leaves and roots, allowing plants to extract water from the soil even at soil  $\Psi_w$  values as low as  $-2.5$  MPa (Xiloyannis et al. 1999). In olive, the turgor loss point was at a  $\Psi_w$  of  $-3.5$  MPa (Figure 3A–3C), which is in agreement with the data of other authors (Lo Gullo and Salleo 1988, Rieger 1995). Although loss of turgor can compromise cell metabolism and, in particular, the reaction of photosynthetic systems (Chaves et al. 2003), olive plants are able to maintain transpiration and photosynthetic activity below the turgor loss point to  $\Psi_w$  values of  $-6.0$  to  $-7.0$  MPa (Xiloyannis et al. 1988, Angelopoulos et al. 1996). Our results confirm the capacity of olive tissues to continue photosynthesis during a prolonged drought and to lose large amounts of tissue water to transpiration, ensuring some photosynthesis during a drought not only in the early morning but also during the hottest hours of the day (Figure 5B). This capacity may depend on osmotic adjustment, changes to the photosynthetic apparatus

(Moriani et al. 2002) and stomatal control (Fernández et al. 1997). In kiwifruit plants, which are sensitive to water deficit and exert instantaneous stomatal control of transpiration, stomatal conductance sharply decreases as leaf water potential falls below  $-0.3$  MPa (Gucci et al. 1996).

The value of  $P\Delta\Psi_{\pi}$  increased with  $\Psi_w$  in all groups of drought-stressed plants, but particularly in high-stress-treated plants (Figure 4C). The contribution of  $P\Delta\Psi_{\pi}$  to total osmotic adjustment was 57–58% and the corresponding value for  $A\Delta\Psi_{\pi}$  was 42–43%. The finding that ex novo synthesis of compatible solutes, such as sugars (Dichio et al. 2003) and proline (Sofa et al. 2004b), can occur in both leaves and roots of drought-stressed olive plants highlights the important role of  $A\Delta\Psi_{\pi}$  in olive plants (Figure 4B; Tables 1 and 2). The values of  $A\Delta\Psi_{\pi}$  in Figure 4B are similar to those reported by Rieger (1995) who found a difference of 1.4 MPa in  $\Psi_{\pi 100}$  values between water-limited and well-irrigated olive plants. Larcher et al. (1981) found  $A\Delta\Psi_{\pi}$  values of 0.4 and 0.6 MPa at  $\Psi_w$  values of  $-6.0$  and  $-6.6$  MPa, respectively, in cv. 'Leccino' of olive. As indicated by the values of  $\Psi_{\pi 100}$  and  $A\Delta\Psi_{\pi}$  in Table 2, osmotic adjustment in roots enabled plant growth during periods with low soil water availability (Chaves et al. 2003) and contributed to drought tolerance. Furthermore, at 6 and 29 days after the beginning of the rewatering period, the olive plants that had been subjected to water deficit had high values of  $\Psi_{\pi 100}$  and  $A\Delta\Psi_{\pi}$  both in leaves and roots (Tables 1 and 2).

In conclusion, our results highlight the roles of active and passive osmotic adjustment in the olive tree during drought. Osmotic adjustment in leaves and roots of olive is an important mechanism enabling plants to tolerate low water potentials and to maintain photosynthetic activity during periods of water stress.

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