

Shoot hydraulic characteristics, plant water status and stomatal response in olive trees under different soil water conditions

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Abstract

Aims To evaluate the impact of the amount and distribution of soil water on xylem anatomy and xylem hydraulics of current-year shoots, plant water status and stomatal conductance of mature ‘Manzanilla’ olive trees.

Methods Measurements of water potential, stomatal conductance, hydraulic conductivity, vulnerability to embolism, vessel diameter distribution and vessel density were made in trees under full irrigation with non-limiting soil water conditions, localized irrigation, and rain-fed conditions.

Results All trees showed lower stomatal conductance values in the afternoon than in the morning. The irrigated trees showed water potential values around -1.4 and -1.6 MPa whereas the rain-fed trees reached lower

values. All trees showed similar specific hydraulic conductivity (K_s) and loss of conductivity values during the morning. In the afternoon, K_s of rain-fed trees tended to be lower than of irrigated trees. No differences in vulnerability to embolism, vessel-diameter distribution and vessel density were observed between treatments.

Conclusions A tight control of stomatal conductance was observed in olive which allowed irrigated trees to avoid critical water potential values and keep them in a safe range to avoid embolism. The applied water treatments did not influence the xylem anatomy and vulnerability to embolism of current-year shoots of mature olive trees.

Keywords Cavitation · Olive · Irrigation · Vulnerability to drought-induced embolism · Water stress · Xylem anatomy

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Introduction

According to the cohesion theory (Dixon and Joly 1894; Askenasy 1895) water ascends plants in a metastable state. The driving force is generated by the negative pressure at the evaporating surfaces of the leaf. The tension is transmitted through a continuous water column from leaves to roots and lowers their water potential below the potential of the surrounding soil. This causes water uptake from the soil and its upward movement to the aerial part of the plant. The decrease in water potential as the soil gets dry may cause cavitation of

conduits and the resulting embolism to loss of hydraulic conductivity (K) (Tyree and Zimmermann 2002). Stomatal closure is one of the most effective mechanisms to avoid critical water potential values (Choat et al. 2012). Accordingly, also olive trees minimize water losses under high water demand conditions by stomata regulation (Fernández et al. 1997; Tognetti et al. 2009; Boughalleb and Hajlaoui 2011). Stomatal closure is known to be especially advantageous in environments with wide fluctuations both in evaporative demand and soil moisture (Franks et al. 2007), as those in which the olive tree is widely grown (Fernández and Moreno 1999).

The relationships between stomatal conductance (g_s), leaf water potential (Ψ_l), K and environmental variables are complex. Feedback mechanisms between these variables (Chaves et al. 2003; Lovisolo et al. 2010) and differences between cultivars (Winkel and Rambal 1990; Fernandez et al. 2008) have been reported. In addition, irregular distribution of water in the rootzone may trigger root-to-shoot signals inducing stomatal closure (Dry and Loveys 1999; Dodd 2005). This is caused by some irrigation systems, as localized irrigation, in which a fraction only of the rootzone is wetted by irrigation. In olive, Fernández et al. (2003) reported restricted transpiration in trees under localized irrigation, but they could not unravel whether the stomatal closure was induced by a chemical signal involving abscisic acid (ABA) generated in the roots remaining in the drying soil or by a hydraulic signals, as e.g. the drop of hydraulic conductance, caused by soil drying. Bacelar et al. (2007) analyzed the effect of the soil water regime on gas exchange and xylem hydraulic properties of olive cultivars. They reported that water stress caused a marked decline of g_s and an increase in xylem vessel density in all cultivars. Some of those cultivars also showed a reduction in vessel diameter. They worked, however, with 1-year-old plants in pots and their experiments did not include localized irrigation. Tognetti et al. (2009) reported that the loss of hydraulic conductance is an important signal for the stomatal control of transpiration in olive trees under drying soil conditions. A better understanding of how soil water regime and plant water status influence stomata regulation and hydraulics of olive cultivars is important for a efficient management of water used in irrigated orchards. The interest in this area of research is considerable given that 2.3 out of the 10.5 Mha of olive surface are irrigated, mostly with

localized irrigation systems (International Olive Council, www.internationaloliveoil.org; Pastor 2005).

We evaluated the effect of different soil water regimes on xylem anatomy, xylem hydraulics and gas exchange in current-year shoots of mature ‘Manzanilla’ olive trees. Measurements of xylem anatomical parameters, Ψ_l , g_s , specific hydraulic conductivity (K_s) and percentage loss of conductivity (PLC) and analysis of the vulnerability to embolism were made in 41-year-old ‘Manzanilla’ olive trees along a full irrigation season. The trees were under three different soil water regimes: rain-fed conditions, localized irrigation in which part of the rootzone remained in drying soil, and full irrigation that kept the whole rootzone under non-limiting soil water conditions. We hypothesized that differences in soil water conditions imposed by the mentioned water treatments limit plant hydraulics and lead to stomatal regulations as well as to acclimation in xylem anatomical traits in olive trees.

Materials and methods

Experimental site and water treatments

This study was carried out at ‘La Hampa’ experimental farm, 15 km from Seville, southwest Spain (37° 17' N, 6° 3' W, 30 m a.s.l.). Climate in the area is Mediterranean with a wet, mild season from October to April and a hot, dry season from May to September. Trees in the orchard were 41-year-old ‘Manzanilla de Sevilla’ (from now on ‘Manzanilla’) olive trees at 7 m × 5 m spacing (286 trees ha⁻¹). The soil of the orchard is a sandy loam (Xerochrept) of 1.6–2.0 m depth. The texture is quite homogeneous, both vertically and horizontally (Moreno et al. 1988), with mean values of 14.8 % clay, 7.0 % silt, 4.7 % fine sand and 73.5 % coarse sand. Laboratory measurements showed a volumetric soil water content (θ_v) of 0.33 and 0.09 m³ m⁻³ for a soil matric potential of -0.01 and -1.5 MPa respectively. Field measurements showed $\theta_v=0.22$ m³ m⁻³ at field capacity conditions.

Experiments were made during the irrigation season of 2009, from May 6, day of year (DOY) 126, to October 2 (DOY 275). Three adjacent 0.2 ha plots were used in the project, each under a different water treatment: 1) a rain-fed treatment (R), in which rainfall was the only source of water supply until a recovery irrigation was applied from September 8 (DOY 251) to the end of the season. This consisted on supplying daily

three times more water than to the LI trees (described below), in a circle of ca. 2 m radius around the trees; 2) Localized irrigation (LI), in which trees were irrigated daily throughout the irrigation season with enough water to replace 100 % of the crop evapotranspiration (ET_c). The irrigation system consisted of a lateral pipe per tree row with five 3 L h⁻¹ drippers per tree, 1 m apart. This system leaves part of the roots under soil drying conditions during the irrigation season. The irrigation dose was calculated with the crop coefficient approach (Allen et al. 1998), with coefficients adjusted for the orchard conditions by Fernández et al. (2006); and 3) Full irrigation (FI), in which trees were irrigated with a 0.4 m×0.4 m grid of pipes with a 2 L hour⁻¹ dripper in every node. The grid covered a surface of 8 m×6 m, with the tree in the middle, enough to keep non-limiting soil water conditions in the whole rootzone throughout the irrigation season. The FI trees were irrigated every other day, to avoid hypoxia.

Soil water and weather measurements

Soil water profiles were measured every 7–10 days during the experimental period. We used a PR2-Profile probe (Delta-T Devices Ltd, Cambridge, UK) with three access tubes per tree in three trees per treatment, at 0.50 m, 1.50 m and 2.25 m from the trunk to cover possible heterogeneities in soil water distribution. In each access tube θ_v values were measured at 0.1, 0.2, 0.3, 0.4, 0.6 and 1.0 m depth. These values were used to calculate the relative extractable water (REW) according to Granier (1987). Simultaneous measurements of root distribution and soil water content in the orchard down to 2.4 m depth made by Fernández et al. (1991) showed that the explored top meter of soil was enough for a reliable average value of θ_v in the rootzone of the experimental trees.

Main weather variables were recorded every 30 min with a weather station (Campbell Scientific Ltd., Leicestershire, UK) under standard conditions, located at ca. 50 m from the orchard. These data were used to calculate the FAO56 Penman-Monteith potential evapotranspiration, ET_o (Allen et al. 1998).

Leaf water potential and stomatal conductance

Values of Ψ_l at predawn, in the morning (9–10 GMT) and in the afternoon (14–15 GMT) were measured on June 23 (DOY 175), July 28 (DOY 209), August 25

(DOY 237) and October 1 (DOY 274). We sampled two fully expanded leaves of the current year per tree in three trees per treatment. Measurements were made with a Scholander-type pressure chamber (Soilmoisture Equipment Corp., Santa Barbara, California, USA), following recommendations by Turner (1988) and Koide et al. (1989). We measured g_s at the same time as Ψ_l both in the morning and in the afternoon. We used a LI-6400 portable photosynthesis system (Licor Inc., USA) and sampled the same type and number of leaves as for Ψ_l .

Hydraulic conductivity and native embolism

Five branches per treatment of 1.2 m in length with at least one 0.4 m long current-year shoot, respectively, were cut under water (to avoid air entering into the vessels), wrapped in plastic bags with wet paper towel inside (to prevent transpiration) and transported to the laboratory. To avoid any possible influence of the sampling position on the results, all branches were sampled from similarly-oriented parts of the canopy, at ca. 1.5 m above ground. The sampling was made at the same time, in the same trees and from the same part of the canopy as the g_s and Ψ_l measurements. Once in the laboratory, we submerged one current-year shoot of each branch in a container with perfusion solution (see below). From each shoot we sampled one ca. 30 mm long segment under water, removed its bark and trimmed sample ends with a razor blade. All the sampled segments were collected from the mid-part of the shoots. The native K of each segment was then determined with a XYL'EM[®] apparatus (Bronkhorst, Montigny-les-Cormeilles, France) as:

$$K = \frac{F}{\Delta P} L \quad (1)$$

where F mass flow rate of a solution through the segment, ΔP is the applied pressure gradient driving the flow and L is the sample length. We used a filtered (0.22 μ m) 50 mM KCl perfusion solution made with distilled water, and applied a pressure gradient of 3 kPa until a steady-state native K was attained. Previous experiments in olive showed that this water head is below the threshold at which embolized vessel opened at both ends are flushed and artificially contribute to K (data not shown). Flushing was obtained after initial K measurements by perfusion at 150 kPa for 20 min. The pressure was then lowered again down to 3 kPa to

determine maximum K . Percent loss of conductivity (PLC) (i.e. native embolism) was calculated as:

$$\text{PLC} = 100 \times \left(1 - \frac{\text{native } K}{\text{maximum } K}\right) \quad (2)$$

The K_s was calculated by dividing native K by the mean cross-sectional area of the sample.

Vulnerability curves

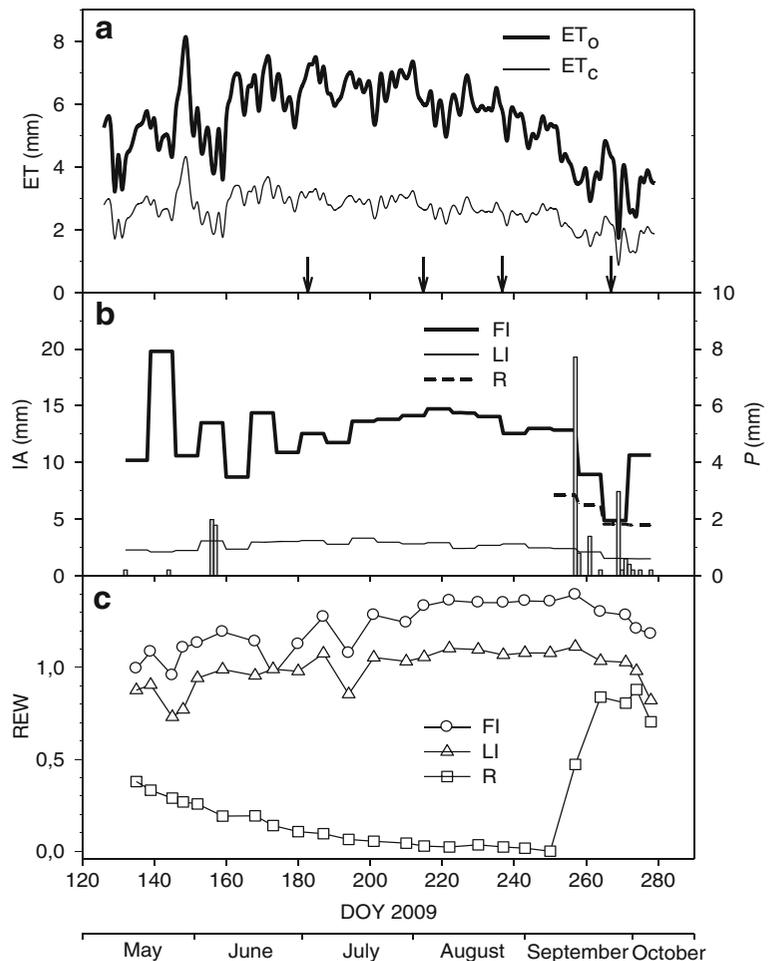
On November 2 (DOY 306), twenty 1.5 m long branches with 3–4 current-year shoots, respectively were sampled under water from 3 to 5 representative trees of each treatment, and transported to the laboratory as described above. The branches were used to determine vulnerability to drought-induced embolism by the bench-top drying technique (Tyree and Dixon 1986; Sperry and Tyree 1988). During dehydration, xylem water potential (Ψ_x) was measured in intervals until the desired values down to -10 MPa were reached. Branches then were stored for

1.5 h in a plastic bag with a wet paper towel inside to allow the equilibrium between Ψ_i and Ψ_x . Measurements of Ψ_x were made in two leaves per branch and afterwards, one segment of ca. 30 mm length per current-year shoot was sampled, following the procedure described above for native K measurements and PLC determinations. We generated one vulnerability curve per treatment by plotting PLC versus Ψ_x . For fitting the vulnerability curves obtained from each treatment we used a Weibull function (Neufeld et al. 1992) with an additional independent factor to consider the levels of embolism measured at $\Psi_x \approx 0$:

$$\text{PLC} = (100 - y_0) - (100 - y_0)e^{-\left(\frac{\Psi_x}{b}\right)^c} + y_0 \quad (3)$$

being x the Ψ_x , b the Ψ_x for a PLC of 63 %, c a dimensionless parameter controlling the shape of the curve and y_0 the PLC at $\Psi_x = 0$ MPa. These points were fitted to Eq. 3 using Excel's solver function. The Ψ_x

Fig. 1 Time courses of (a) reference (ET_o) and crop evapotranspiration (ET_c), (b) collected precipitation (P) and irrigation amounts (IA) supplied to trees of each treatment, and (c) relative extractable water (REW) for each treatment. DOY day of year. Arrows indicate sampling dates for variables shown in Figs. 2, 3 and 4



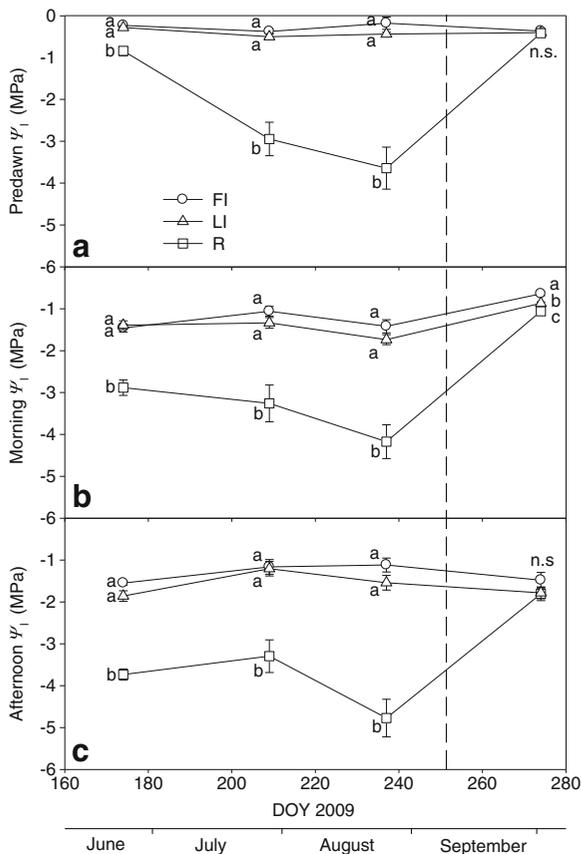


Fig. 2 Time courses of leaf water potential (Ψ_l) measured at (a) predawn, b at 9.00–10.00 GMT (morning Ψ_l), and (c) at 14.00–15.00 GMT (afternoon Ψ_l). Data points are average of six values; vertical bars represent \pm the standard error. Different letters indicate statistically significant difference ($p < 0.05$) between treatments. *n.s.* no significant difference. The dashed line indicates the beginning of the recovery irrigation applied to the R trees. DOY day of year

values corresponding to 50 % loss of hydraulic conductivity (P_{50} , MPa) due to embolism formation and its 95 % confidence intervals were calculated from each vulnerability curve.

Anatomical measurements

On November 2 (DOY 306), current-year shoots of similar characteristics than those used for K measurements were collected from four different representative trees per treatment. One 10 mm segment was sampled from each shoot, dehydrated in an acetone series and embedded in SPURR resin. Sections were cut with an ultramicrotome, stained with toluidine blue

(1 %) for 3 min, rinsed in water and photographed with a digital camera attached to a light microscope (Olympus BX61). Images from each section were divided into four parts of similar area. Vessel density (number of vessels in a given area) was determined in two of these parts using Adobe Photoshop CS3 software (Adobe Systems Incorporated, USA). The vessel diameter was calculated for each vessel from its surface area, previously determined with the mentioned software, assuming a circularity of 1. The diameter distribution per treatment was determined after classifying the vessels into bin diameters (diameter size classes of 2 μm width). The resulting values were expressed as percentage of vessel number from total in each class. Some 300 to 500 vessels were measured per section.

Statistical analysis

Data sets were tested for normality with the Kolmogorov-Smirnov test and homogeneity of variances was determined by Brown & Forsythe test. Differences in Ψ_l , g_s , K , PLC, vessel density and percentage of vessel in each bin diameter between water treatments were evaluated by a one-way analysis of variance (ANOVA). When the differences were significant, a multiple comparison of means (post hoc Tukey honest significant difference test) was carried out. Statistical comparisons were considered significant at $p < 0.05$. P_{50} values calculated from the vulnerability curves obtained for trees of each water treatment were considered significantly different when their 95 % confidence intervals did not overlap.

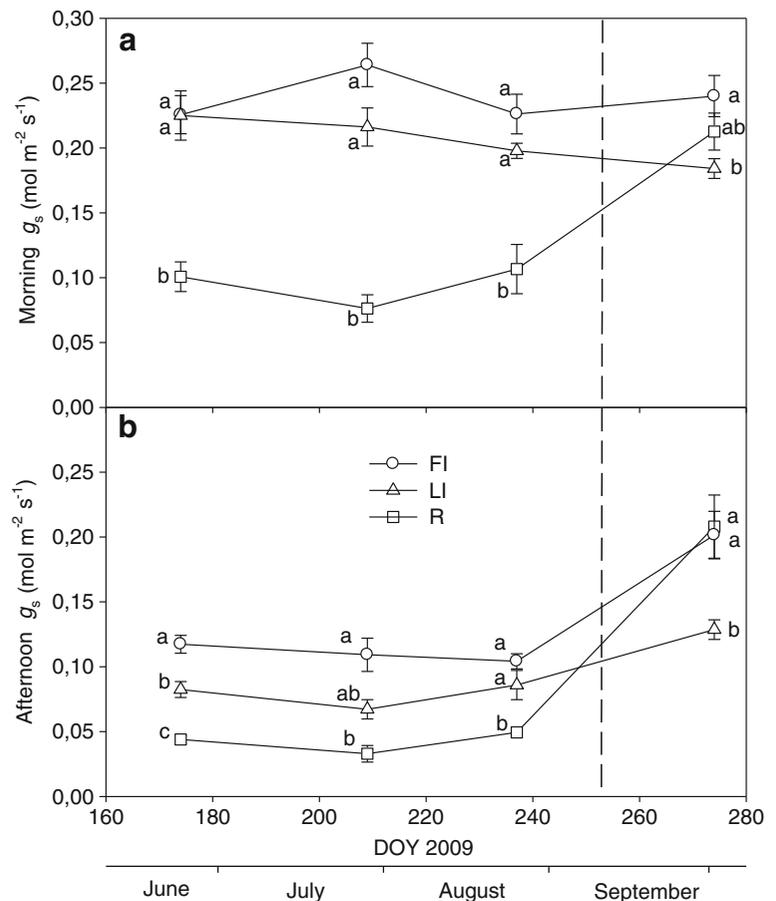
Repeated measures analyses of variance (ANOVA) over time were carried out to test differences in Ψ_l , g_s , K and PLC between FI and LI trees during the season. All analyses were performed by using STATISTICA software (StatSoft, Inc., USA) and Sigmaplot (SPSS Inc., USA).

Results

Weather conditions and soil moisture

Except for peak values of ET_0 recorded at the end of May, the time course of the atmospheric demand during the experimental period was as usual in the area, with high values in the middle of the summer and decreasing values from the end of August (Fig. 1a).

Fig. 3 Time courses of stomatal conductance (g_s) measured at (a) ca. 9.00–10.00 GMT and (b) 14.00–15.00 GMT. Data points are average of six values; vertical bars represent \pm the standard error. Different letters indicate statistically significant difference ($p < 0.05$). The dashed line indicates the beginning of the recovery irrigation applied to the R trees. DOY day of year



The irrigation amounts applied to the LI trees (Fig. 1b) were enough to maintain REW values close to field capacity throughout the irrigation season (Fig. 1c). In the FI plot, REW values showed conditions close to saturation. Total amounts of water supplied to LI and FI trees were 3361.8 m³ ha⁻¹ (88 % ET_c) and 16121 m³ ha⁻¹ (420 % ET_c), respectively. The high irrigation supplies in the FI treatment were justified by the need of ensuring non-limiting soil water conditions in the whole rootzone. REW values in the R plot decreased during the irrigation season, until the recovery irrigation applied from DOY251 to DOY275, when 1482.3 m³ ha⁻¹ of water were supplied. This, together with the first rainfall events after the summer dry period (Fig. 1b) increased REW values to ca. 0.9 (Fig. 1c).

Plant water status and stomatal conductance

Similar values of Ψ_1 at predawn were recorded in the LI and FI trees during the dry season (Fig. 2a). In

contrast, R trees showed decreasing Ψ_1 values at predawn throughout the summer, in agreement with the gradual decrease in REW (Fig. 1c). In the LI and FI trees, Ψ_1 values were close to -1.5 MPa both in the morning and the afternoon throughout the dry season (Figs. 2b and c), indicating no increase in water stress during the season. Although slightly, the Ψ_1 was

Table 1 Values of the vapour pressure deficit of the air (D_a) recorded in the morning (9–10 GMT) and in the afternoon (14–15 GMT) of the days when main physiological variables were measured

DOY	D_a (kPa)	
	Morning	Afternoon
174	1.47	3.73
209	2.10	4.66
234	1.85	3.42
274	0.30	1.65

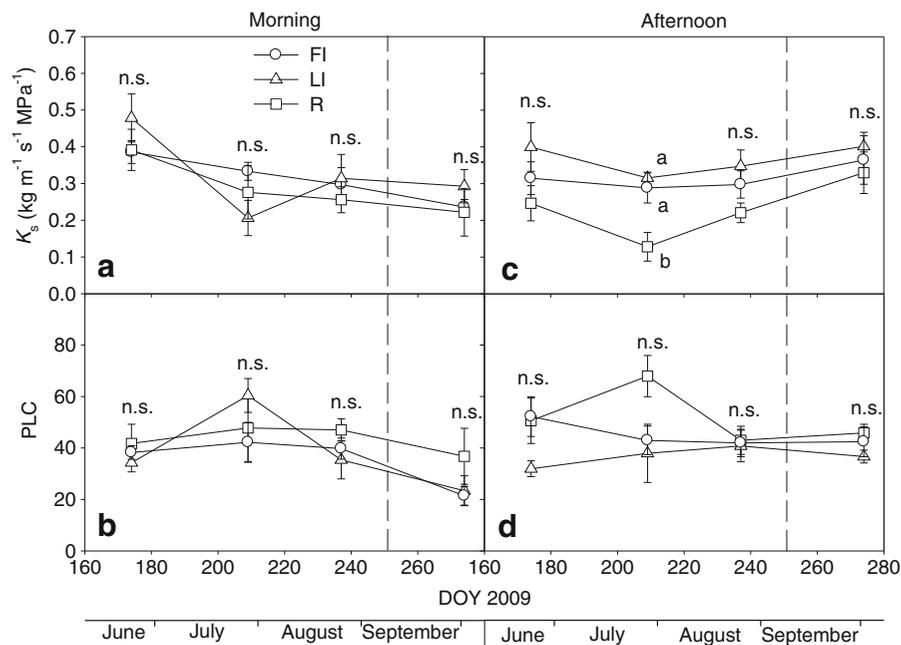


Fig. 4 Average values of specific hydraulic conductivity (K_s) and percentage loss of hydraulic conductivity (PLC) at 9.00–10.00 GMT (i.e. morning) (**a**, **b** respectively) and at 14.00–15.00 GMT (i.e. afternoon) (**c**, **d** respectively) (i.e. afternoon). Data points are average of three to five values; vertical bars represent \pm the

standard error. Different letters indicate statistically significant difference ($p < 0.05$); n.s. no significant difference. The dashed lines show the beginning of the recovery irrigation applied to the R trees. DOY day of year

consistently lower in LI trees than in the FI trees during the whole season (Table 3) but not on a day-by-day basis (Fig. 2). In the R trees, Ψ_1 values during the dry period were significantly lower than in LI and FI trees. Similarly to predawn, Ψ_1 decreased in the R trees both in the morning and in the afternoon throughout the season as the soil water was depleted. The most negative values were recorded at the end of August (morning $\Psi_1 = -4.2 \pm 0.4$ MPa; afternoon $\Psi_1 = -4.8 \pm 0.4$ MPa), when lowest REW were recorded (Fig. 1c). The water supplied by the recovery irrigation and the rainfall events in autumn (Fig. 1b) caused Ψ_1 of R trees to raise to values similar to LI and FI trees (Fig. 2).

In LI and FI trees, g_s values in the morning were over $0.2 \text{ mol m}^{-2} \text{ s}^{-1}$ during the entire dry season (i.e. until the first rainfall events), while R trees showed much lower g_s values (Fig. 3a). In all trees, g_s in the afternoon was lower than in the morning during the drought season but similar at the end of the study period (Fig. 3). Both in the morning and in the afternoon, the LI trees used to show lower g_s values than the FI trees although differences were not always significant day by day. But, when the entire season is considered, the g_s was consistently lower in LI than in

FI (Table 3). Marked differences in D_a between morning and afternoon were recorded during the whole season (Table 1).

Hydraulic and anatomical characteristics

No pronounced differences or trends were observed between treatments in K_s or PLC of morning samples (Fig. 4a, b). In the afternoon, K_s in the R trees tended to be lower than in the irrigated trees with significant differences at DOY 209. In the FI and LI trees, no significant differences or trends in K_s or PLC were found, either day by day or during the season (Fig. 4; Table 3). Corresponding to the course in K_s , PLC of R trees peaked at DOY 209 (ca. 60 %), while PLC values were similar on all other sampling days (Fig. 4c, d).

Trees from all treatments showed a similar vulnerability to embolism (Fig. 5a) and no difference between P_{50} (Table 2). Figure 5a shows that native PLC at Ψ_x close to 0 MPa was 15–30 % in all treatments. Accordingly, anatomical analysis revealed similar vessel diameter distributions in all treatments (Fig. 6). Vessel density tended to be highest in R trees, followed by LI and FI trees, but differences were not significant (Table 2).

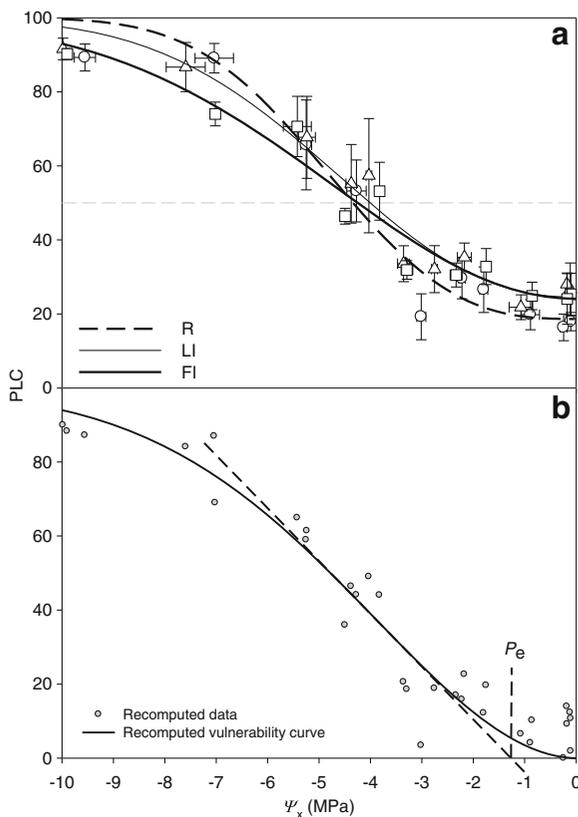


Fig. 5 **a** Xylem vulnerability to embolism curves of current-year shoots of FI (\circ), LI (white triangle) and R (white square) trees, represented as the percentage loss of conductivity (PLC), as a function of decreasing xylem water potential (Ψ_x). Data points are the average of five to seven samples; vertical bars represent \pm the standard error. The dashed grey line indicates 50 % loss of hydraulic conductivity; **b** All data represented in Fig. 5a were recomputed considering PLC=0 at $\Psi_x=0$, and the resulting vulnerability curve fitted and plotted. The black dashed line represents the tangent through the midpoint of the vulnerability curve and its x-intercept represents the air entry pressure (P_e) following Meinzer et al. (2009)

Discussion

Stomatal control of transpiration and embolism formation

Results of the present study suggest a tight control of Ψ_1 in olive (Fig. 3a, b). Despite of the two-fold increase in D_a between morning and afternoon (Table 1), Ψ_1 was maintained fairly constant and around 1.4–1.6 MPa, which allowed FI and LI trees to avoid critical Ψ_1 values. Critical thresholds in olive have been reported to be around -1.5 MPa (Sofó et al. 2008). Differences in g_s between morning and afternoon were proportional to

Table 2 Values both of the xylem tension inducing 50 % loss of hydraulic conductivity (P_{50}) and their 95 % confidence interval (95 % CI). Also shown are the mean vessel densities ($n=4$) found in current-year shoots from trees of each treatment. P_{50} values which 95 % CI do not overlap are considered significantly different. No significant differences were found on vessel density between treatments ($p<0.05$); SE = standard error

Treatment	P_{50} (MPa)	Vessel density (vessels mm^{-2})
R	-4.35 (-3.67 ; -4.97)	618 ± 63 SE
LI	-4.02 (-3.50 ; -4.52)	555 ± 69 SE
FI	-4.26 (-3.59 ; -4.91)	499 ± 75 SE

those observed in D_a (Fig. 4), assuring a good balance between the increase in water demand and supply. This behaviour is typical for olive, a species well adapted to drought, with stomatal closure from early in the morning as an efficient strategy to reduce water losses and thus decreases in water potential (Fernández et al. 1997; Cuevas et al 2010). Despite restrictive stomatal closure recorded also in the R trees, these trees reached Ψ_1 values more than two-fold lower than irrigated trees (Figs. 2 and 3).

Interestingly, similar PLC values (Fig. 4) were observed in all treatments although only R trees were exposed to low Ψ_1 during the study. Since vulnerability curves also reflected some native PLC, a recomputed vulnerability curve based on data of all treatments was constructed (Fig. 5b). This curve enabled to compute the air entry pressure (P_e), indicating the threshold xylem

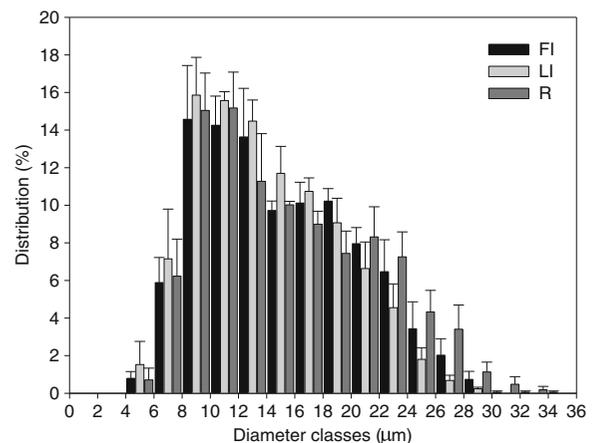


Fig. 6 Xylem vessel diameter distribution measured in four cross-sections per treatment from current-year shoots. From 300 to 500 vessels were measured per section. Vertical bars represent \pm the standard error. No significant differences were found between treatments

Table 3 Significances (p) of the differences in leaf water potential (Ψ_1), stomatal conductance (g_s), specific hydraulic conductivity (K_s) and percentage loss of hydraulic conductivity

(PLC) during the season between full irrigated (FI) and localized irrigated (LI) trees calculated by using repeated measures (ANOVA) over time tests

	Ψ_1		g_s		K_s		PLC		
	Predawn	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
p	0.025*	0.006*	0.019*	0.003*	0.000*	0.741	0.128	0.445	0.053

*Differences were considered significant when $p < 0.05$

pressure at which loss of conductivity begins to increase rapidly (Meinzer et al. 2009). In our case, P_e was around -1.3 MPa, demonstrating that water potentials observed in irrigated trees (i.e. FI and LI trees) were too high for any relevant induction of embolism. We thus conclude that their native embolisms were formed prior to our first measurements, maybe in an early stage of xylogenesis. During the study period, irrigated trees were obviously able to keep their water potential in a safe range and to avoid further embolism. It is well known that under non-extreme conditions, g_s is regulated to keep Ψ_1 within a safety margin avoiding the development of embolism (Brodrribb and Holbrook 2003; Cochard et al. 2002; Meinzer et al. 2009; Choat et al. 2012). In contrast, R trees were not able to balance water deficits: observed minimum Ψ_1 of ca. -4.8 MPa and maximum PLC of ca. 60 % (Figs. 2 and 3) correspond relatively well with the vulnerability curve. The full recovery of Ψ_1 and g_s in the R trees observed on DOY 274, after the water supplies of September (Fig. 1b), agrees with the well known capacity of the olive tree to recover quickly from water stress after the summer dry period (Lavee and Wodner 1991; Moriana et al. 2007).

Heterogeneity in soil water distribution within the rootzone of the LI trees can promote changes in the hydraulic conductance of the soil–leaf pathway, which may indirectly drive changes in g_s and transpiration (Sperry et al. 2002). This, therefore, would explain the fact that the g_s values recorded in trees with localized irrigation systems (i.e. LI trees) tended to be lower than in FI trees, which had access to water homogeneously distributed in the soil (Fig. 3; Table 3). Indeed, an additional chemical root-to-shoot signalling mechanism triggered in the LI trees by roots remaining under soil drying conditions could also have influenced the stomatal behaviour (Dodd et al. 2008), although this mechanism is still to be proven in olive. From a similar study carried out on the same trees and under the same treatments, Morales-Sillero et al. (2013)

recently reported that the reductions in g_s observed in LI trees also reduced the net photosynthesis rate with regard the FI trees. They also reported that localized irrigation improved oil quality but reduced fruit yield as compared to an irrigation system able to wet the whole rootzone. Although they did not evaluate water productivity in the orchard, they suggested that it would be higher in orchards with localized irrigation than in those with irrigation systems that wet the whole rootzone.

Acclimation in xylem anatomy and function

The applied water treatments did not influence the vulnerability to embolism (Fig. 5a, Table 2), although a significant fraction of the new wood in shoots was formed under the influence of the water treatments. Intra-specific differences in xylem resistance induced by soil moisture conditions have been reported for some species (Choat et al. 2007; Beikircher and Mayr 2009; Fichot et al. 2010), while other studies found no effects (Maherali et al. 2002; Cornwell et al. 2007). Lacking differences in hydraulic safety in studied olive trees corresponded well with similarity in vessel-diameter distribution and vessel density across water treatments. Only the number of vessels per mm^{-2} tended to increase in trees with lower irrigation doses (Table 2). Contrasting results on the effect of the water regime on the xylem characteristics in olive have been previously reported for different olives varieties (Bacelar et al. 2007; Lopez-Bernal et al. 2010). These discrepancies could be due, apart from differences between cultivars, to the different age of the studied material, since histological characteristics may differ between young and adult olive trees (Lopez-Bernal et al. 2010). In each case, our results, reject our hypothesis about the influence of soil water conditions on the xylem anatomy and vulnerability to embolism of current-year shoots.

Conclusions

Our results showed a tight control of g_s in olive which allowed irrigated trees to avoid critical Ψ_1 values and keep them in a safe range to avoid embolism. Marked stomatal closure was also observed in the R trees, but this did not impede Ψ_1 values being lower in those trees than in the FI and LI trees. Values of K_s tended to be lower in the R than in the irrigated trees. All treatments, however, showed similar PLC values. The applied water treatments did not affect the vulnerability to embolism, vessel-diameter distribution and vessel density of current-year shoots of mature ‘Manzanilla’ olive trees.

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References

- Allen R, Pereira LS, Raes D, Smith M (1998) Crop evapotranspiration. Guidelines for computing crop water requirements. Irrigation and Drainage Paper, No. 56. FAO, Rome
- Askenasy E (1895) Über das Saftsteigen. Verhandl Naturhist Med Ver Heidelberg, NF 5:325–345
- Bacelar EA, Moutinho-Pereira JM, Goncalves BC, Ferreira HF, Correia CA (2007) Changes in growth, gas exchange, xylem hydraulic properties and water use efficiency of three olive cultivars under contrasting water availability regimes. Environ Exp Bot 60:183–192
- Beikircher B, Mayr S (2009) Intraspecific differences in drought tolerance and acclimation in hydraulics of *Ligustrum vulgare* and *Viburnum lantana*. Tree Physiol 29:765–775
- Boughalleb F, Hajlaoui H (2011) Physiological and anatomical changes induced by drought in two olive cultivars (cv Zalmati and Chemlali). Acta Physiol Plant 33:53–65
- Brodribb TJ, Holbrook NM (2003) Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. Plant Physiol 132:2166–2173
- Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought—from genes to the whole plant. Funct Plant Biol 30:239–264
- Choat B, Sack L, Holbrook NM (2007) Diversity of hydraulic traits in nine *Cordia* species growing in tropical forests with contrasting precipitation. New Phytol 175:686–698
- Choat B, Jansen S, Brodribb TJ, Cochard H, Delzon S et al (2012) Global convergence in the vulnerability of forests to drought. Nature 491:752–755
- Cochard H, Coll L, Le Roux X, Ameglio T (2002) Unraveling the effects of plant hydraulics on stomatal closure during water stress in walnut. Plant Physiol 128:282–290
- Comwell WK, Bhaskar R, Sack L, Cordell S, Lurch CK (2007) Adjustment of structure and function of Hawaiian *Metrosideros polymorpha* at high vs. low precipitation. Funct Ecol 21:1063–1071
- Cuevas MV, Torres-Ruiz JM, Álvarez R, Jiménez MD, Cuerva J, Fernández JE (2010) Assessment of trunk diameter variation derived indices as water stress indicators in mature olive trees. Agric Water Manage 97:293–302
- Dixon HH, Joly J (1894) On the ascent of sap. Ann Bot London 8:468–470
- Dodd IC (2005) Root-to-shoot signalling: Assessing the roles of ‘up’ in the up and down world of long-distance signalling in planta. Plant Soil 274:251–270
- Dodd I, Egea G, Davies W (2008) Abscisic acid signalling when soil moisture is heterogeneous: decreased photoperiod sap flow from drying roots limits abscisic acid export to the shoots. Plant Cell Environ 31:1263–1274
- Dry PR, Loveys BR (1999) Grapevine shoot growth and stomatal conductance are reduced when part of the root system is dried. Vitis 38:151–156
- Fernández JE, Moreno F (1999) Water use by the olive tree. J Crop Prod 2:101–162
- Fernández JE, Moreno F, Cabrera F, Arrúe JL, Martín-Aranda J (1991) Drip irrigation, soil characteristics and the root distribution and root activity of olive trees. Plant Soil 133:239–251
- Fernández JE, Moreno F, Girón IF, Blázquez OM (1997) Stomatal control of water use in olive tree leaves. Plant Soil 190:179–192
- Fernández JE, Palomo MJ, Diaz-Espejo A, Giron IF (2003) Influence of partial soil wetting on water relation parameters of the olive tree. Agronomie 23:545–552
- Fernández JE, Diaz-Espejo A, Infante JM, Durán P, Palomo MJ, Chamorro V, Girón IF, Villagarcía L (2006) Water relations and gas exchange in olive trees under regulated deficit irrigation and partial rootzone drying. Plant Soil 284:273–291
- Fernandez JE, Diaz-Espejo A, D’Andria R, Sebastiani L, Tognetti R (2008) Potential and limitations of improving olive orchard design and management through modelling. Plant Biosyst 142:130–137
- Fichot R, Barigah TS, Chamaillard S, Le Thiec D, Laurans F, Cochard H, Brignolas F (2010) Common trade-offs between xylem resistance to cavitation and other physiological traits do not hold among unrelated *Populus deltoides* x *Populus nigra* hybrids. Plant Cell Environ 33:1553–1568
- Franks PJ, Drake PL, Froend RH (2007) Anisohydric but isohydrodynamic: seasonally constant plant water potential gradient explained by a stomatal control mechanism incorporating variable plant hydraulic conductance. Plant Cell Environ 30:19–30
- Granier A (1987) Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. Tree Physiol 3:309–320
- Koide RT, Robichaux RH, Morse SR, Smith CM (1989) Plant water status, hydraulic resistance and capacitance. In: Percy RW, Ehleringer J, Mooney HA, Rundel PW (eds) Plant physiological ecology. Chapman & Hall, London, pp 161–179

- Lavee S, Wodner M (1991) Factors affecting the nature of oil accumulation in fruit of olive (*Olea europaea* L) cultivars. *J Hortic Sci* 66:583–591
- Lopez-Bernal A, Alcantara E, Testi L, Villalobos FJ (2010) Spatial sap flow and xylem anatomical characteristics in olive trees under different irrigation regimes. *Tree Physiol* 30:1536–1544
- Lovisol C, Perrone I, Carra A, Ferrandino A, Flexas J, Medrano H, Schubert A (2010) Drought-induced changes in development and function of grapevine (*Vitis* spp.) organs and in their hydraulic and non-hydraulic interactions at the whole-plant level: a physiological and molecular update. *Funct Plant Biol* 37:98–116
- Maherali H, Williams BL, Paige KN, Delucia EH (2002) Hydraulic differentiation of *Ponderosa pine* populations along a climate gradient is not associated with ecotypic divergence. *Funct Ecol* 16:510–521
- Meinzer FC, Johnson DM, Lachenbruch B, McCulloh KA, Woodruff DR (2009) Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. *Funct Ecol* 23:922–930
- Morales-Sillero A, García JM, Torres-Ruiz JM, Montero A, Sánchez-Ortiz A, Fernández JE (2013) Is the productive performance of olive trees under localized irrigation affected by leaving some roots in drying soil? *Agric Water Manage* 123:79–92
- Moreno F, Vachaud G, Martín-Aranda J, Vauclin M, Fernández JE (1988) Balance hídrico de un olivar con riego gota a gota. Resultados de cuatro años de experiencias. *Agronomie* 8:521–537
- Moriana A, Perez-Lopez D, Gomez-Rico A, Salvador MDD, Olmedilla N, Ribas F, Fregapane G (2007) Irrigation scheduling for traditional, low-density olive orchards: water relations and influence on oil characteristics. *Agric Water Manage* 87:171–179
- Neufeld HS, Grantz DA, Meinzer FC, Goldstein G, Crisota GM, Crisosto C (1992) Genotypic variability in vulnerability of leaf xylem to cavitation in water-stressed and well-irrigated sugarcane. *Plant Physiol* 100:1020–1028
- Pastor M (2005) Cultivo del olivo con riego localizado. Junta de Andalucía, Consejería de agricultura y pesca & Mundi-Prensa.
- Sofo A, Manfreda S, Fiorentino M, Dichio B, Xiloyannis C (2008) The olive tree: a paradigm for drought tolerance in Mediterranean climates. *Hydrol Earth Syst Sci* 12:293–301
- Sperry JS, Tyree MT (1988) Mechanism of water stress-induced xylem embolism. *Plant Physiol* 88:581–587
- Sperry JS, Hacke UG, Oren R, Comstock JP (2002) Water deficits and hydraulic limits to leaf water supply. *Plant Cell Environ* 35:251–263
- Tognetti R, Giovannelli A, Lavini A, Morelli G, Fragnito F, d'Andria R (2009) Assessing environmental controls over conductances through the soil-plant-atmosphere continuum in an experimental olive tree plantation of southern Italy. *Agric Forest Meteorol* 149:1229–1243
- Turner NC (1988) Measurement of plant water status by the pressure chamber technique. *Irrigation Sci* 9:289–308
- Tyree MT, Dixon MA (1986) Water stress induced cavitation and embolism in some woody plants. *Physiol Plantarum* 66:397–405
- Tyree MT, Zimmerman MH (2002) Xylem structure and the ascent of sap. Springer, New York
- Winkel T, Rambal S (1990) Stomatal conductance of some grapevines growing in the field under a Mediterranean environment. *Agric For Meteorol* 51:107–121

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