

# Influence of the Water Treatment on the Xylem Anatomy and Functionality of Current Year Shoots of Olive Trees

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## Abstract

From May to October 2006 we applied two water treatments in an olive orchard in south Spain with 38-year-old ‘Manzanilla’ trees at 7×5 m spacing: a) rainfed, with rainfall as the only source of water supply; b) FAO, in which the trees were drip-irrigated daily from May to September, to replace the crop water needs. The aim of this work was to evaluate the influence of the water treatments on both the anatomical characteristics and the hydraulic performance of the xylem of current-year shoots. In November, 3 cm long segments were taken at 5 cm from the base of current-year shoots of each treatment. Part of these samples was used for hydraulic conductivity ( $K_h$ ,  $\text{g m MPa}^{-1} \text{s}^{-1}$ ) measurements with a hydraulic conductivity meter designed and built in our group. The rest was cryo-fixed in liquid nitrogen for observation by cryo-scanning electron microscopy (cryo-SEM), which allowed us to determine vessel density (vessels  $\text{mm}^{-2}$ ) and vessel distribution per diameter classes, at 2  $\mu\text{m}$  diameter intervals. The xylem water potential ( $\Psi_{\text{xylem}}$ ) of each sample was also measured, at shoot sampling. The anatomical analysis showed no differences between treatments in vessel density. From the amount of vessel, rainfed trees showed narrower vessels than FAO trees. This may explain the lack of differences in  $K_h$  found between the two studied treatments. In fact, the incidence of cavitation is smaller in vessels of reduced diameter. In addition, any reduction in diameter implies a reduced collective pit area between vessels, which reduces the incidence of embolism due to air-seeding. Therefore, the greater number of vessels with reduced diameter induced by the greater water stress suffered by the rainfed trees may have been the major reason for maintaining similar hydraulic conductivities in those trees than in drip-irrigated olive trees.

## INTRODUCTION

In Mediterranean areas, high atmospheric demand values are common during the irrigation season. This causes low xylem water potential ( $\Psi_{\text{xylem}}$ ) values in many cropped plants, especially if these are in drying soils. As  $\Psi_{\text{xylem}}$  becomes more negative, embolism increases (Tyree and Sperry, 1989), which may reduce the functionality of the vascular system for carrying water from the soil to the leaves (Hubbaret al., 2001; Kramer and Boyer, 1995). Under conditions of severe soil water depletion, this phenomenon may cause substantial losses in xylem functionality due to an increasing number of cavitated vessels (Sperry and Tyree, 1988). The hydraulic conductance of plants under severe water deficit conditions may also decrease because of a reduction in the diameter of the xylem vessels (Lovisolo and Schubert, 1998). Despite the loss in hydraulic conductance, the decrease in the diameter vessel is considered to be one of the adaptation mechanisms to drought, since it reduces the occurrence of cavitation. The olive tree, a plant well adapted

to Mediterranean conditions, has some efficient mechanisms to survive under water scarcity conditions. These are especially a remarkable capacity for taking up water from drying soils (Dichio et al., 2003; Cuevas et al., 2010) and a marked stomatal control (Fernández et al., 2006). A rational water management in olive orchards requires knowledge on how irrigation affects the hydraulic conductance of the trees. Only a few studies about this topic has been published for olive (Bacelar et al., 2007; Ennajeh et al., 2008) but neither of them refers to mature trees under localized irrigation.

The aim of this work was to evaluate possible effects of localized irrigation on both anatomical characteristics and hydraulic conductivity ( $K_h$ ) of the xylem of current-year shoots taken from 38-year-old ‘Manzanilla de Sevilla’ (from now on ‘Manzanilla’) olive trees.

## MATERIALS AND METHODS

### Orchard Characteristics and Water Treatments

Measurements were made during the irrigation season of 2006, in a 0.5 ha olive orchard with 38-year-old ‘Manzanilla’ olive trees at 7×5 m spacing, located near Seville, southwest Spain (37°17' N, 6°3' W, 30 m a.s.l.). The soil is a sandy loam of about 1.6-2.0 m depth. The texture is quite homogeneous, both vertically and horizontally, with average values of 14.8% clay, 7.0% silt, 4.7% fine sand and 73.5% coarse sand. The climate is typically Mediterranean, with a mild, wet season from October to April, being the rest of the year hot and dry.

We established two different water treatments in the orchard: 1) “Rainfed” treatment, with rainfall as the only source of water supply; 2) “FAO” treatment, in which the trees were irrigated daily from May 6 to October 13 with 100% of the crop evapotranspiration ( $ET_c$ ). The  $ET_c$  values were calculated using the crop coefficient approach described by Allen et al. (1998). Basically,  $ET_c$  was calculated as  $ET_c = K_c K_r ET_o$ , with values for the crop coefficient ( $K_c$ ) and for the coefficient related to the percentage of ground covered by the crop ( $K_r$ ) derived by Fernández et al. (2006) for the orchard conditions.  $K_c$  values were 0.76 in May, 0.70 in June, 0.63 in July and August, 0.72 in September and 0.77 in October. The  $K_r$  value was 0.71. The potential evapotranspiration in the area ( $ET_o$ ) was calculated with the FAO56 Penman-Monteith equation, from weather records taken by the meteorological station of the orchard (see below). Water was supplied by a lateral per tree row with five 3 L h<sup>-1</sup> drippers per tree, 1 m apart. This system left part of the roots in drying soil.

### Measurements

On November 14 and 15, at about 12.00 GMT, ten current-year shoots were sampled from representative trees of each treatment, for  $K_h$  measurements. Some two hours before shoot sampling, the leaves of each shoot located immediately before the internode at about 5 cm from the base was covered with aluminium foil for the leaf water potential ( $\Psi_{leaf}$ ) to equilibrate with  $\Psi_{xylem}$ . Just before shoot sampling,  $\Psi_{leaf}$  of each covered leaf was measured with a Scholander chamber (Soilmoisture Equipment Corp., Santa Barbara, CA, USA) following the recommendation by Turner (1981).

The chosen shoots were excised under water, at some 30 cm from the base of the current-year shoot in which the  $K_h$  measurements were going to be performed. This avoided cavitation in their vessels during sampling. The shoots were then placed in a black plastic bag with wet paper, to prevent the loss of water by transpiration during transport to the laboratory. From each current-year shoot, a 3 cm long portion of the internode at 5 cm from the base was taken and connected to a hydraulic conductivity meter designed and built in our laboratory (patent PCT/ES2006/070128).  $K_h$  was measured by perfusing a 50 mol m<sup>-3</sup> KCl solution at 3 kPa using the following equation:

$$K_h = \frac{Q}{\Delta P l} \quad (1)$$

where  $Q$  is the water flow ( $\text{g s}^{-1}$ ),  $\Delta P$  is the pressure gradient between both end of the sample (MPa) and  $l$  is the sample length (m).

The anatomical analysis was made in five of the ten samples used for  $K_h$  measurements. Immediately after the  $K_h$  measurements, these five samples were cryo-fixed in liquid nitrogen for observation by cryo-scanning electron microscope (cryo-SEM). Transversal freeze fractures of the samples were observed with the cryo-SEM (Di Baccio et al., 2010) and images were taken at 500-750x magnification. The images were used to derive data on vessel density (vessels  $\text{mm}^{-2}$ ) and vessel distribution per diameter classes, at 2  $\mu\text{m}$  diameter intervals, with the Adobe Photoshop CS3 software (Adobe Systems Incorporated, USA).

Measurements of volumetric soil water content ( $\theta$ ) in the soil of three trees per treatment were made with a neutron probe Troxler 3300 (Research Triangle Park, North Carolina, USA), every 7-10 days. The access tubes for the probe were located next to the irrigation line, at 0.5, 1.5, 2.5 and 3.5 m from the tree trunk. Measurements of  $\theta$  were made every 0.1 m depth, from 0.2 m down to 2.0 m.  $\theta$  values in the top 0.2 m were determined by gravimetric analysis. These values were used to calculate a depth equivalent of water, expressed as the level of relative extractable water (REW, mm). From the  $\theta$  values we calculated the relative water content (REW) of the soil (Granier, 1987). This was defined as  $\text{REW} = (R - R_{\text{min}}) / (R_{\text{max}} - R_{\text{min}})$ , being  $R$  (mm) is the actual soil water content,  $R_{\text{min}}$  (mm) the minimum soil water content measured during the experiments, and  $R_{\text{max}}$  (mm) is the soil water content at field capacity. Half-hour values of main meteorological variables were recorded by a Campbell automatic weather station (Campbell Scientific Ltd., UK), located some 50 m away from the experimental orchard. The station is under standard conditions, including a well-irrigated, healthy grass surface that permit calculate ET to correctly.

A  $t$ -student test was used to analyze differences in the vessel density,  $\Psi_{\text{xylem}}$  and  $K_h$ . Vessel distributions per diameter classes were analyzed with a  $\chi^2$  test procedure.

## RESULTS AND DISCUSSION

The REW values showed clear differences in soil water availability between the FAO and the rainfed treatment all throughout the experimental period (Fig. 1). In the FAO treatment the soil wetted by drippers (irrigation bulb) was around field capacity during the whole irrigation period, while in the rainfed treatment REW values decreased continuously, down to minimum values of ca. 0.2 recorded from the first fortnight of September to the end of the season. Previous studies on root distribution (Fernández et al., 1991) and size of the irrigation bulbs (Fernández et al., 2003) carried out in the orchard suggest that part of the roots of the FAO trees were in drying soil, outside the wetted irrigation bulbs. This may trigger a root-to-shoot signalling mechanism which may influence stomatal closure. Consequently, the recorded  $\Psi_{\text{xylem}}$  values as well as the water consumption of the FAO trees could have been affected. This was studied for orchard conditions by Fernández et al. (2003, 2006). Still, these authors always found clear differences in the seasonal course of  $\Psi_{\text{xylem}}$  between trees under similar soil water regimes as used in this study. By the time of shoot sampling (November 14-15, day of year, DOY, 318-319),  $\Psi_{\text{xylem}}$  values between treatments were also different (Table 1).

No significant differences were found in xylem vessel density between treatments (Table 1). Significant differences were found, however, in the xylem vessel distribution per diameter classes ( $P < 0.001$ ). From the amount of vessel, rainfed trees showed narrower vessels than FAO trees. In addition, maximum vessel diameters were between 18-20  $\mu\text{m}$  in the rainfed trees and between 22-24  $\mu\text{m}$  in the FAO trees (Fig. 2). Similar results for xylem vessel distribution were found by Bacelar et al. (2007) in 'Cobrançosa', 'Madural' and 'Verdeal Transmontana' young olive trees under different water regimes. Measured  $K_h$  values in the internodes were  $1.8 \cdot 10^{-5} \pm 6.5 \cdot 10^{-6}$  and  $2.0 \cdot 10^{-5} \pm 3.3 \cdot 10^{-6}$   $\text{g m s}^{-1} \text{MPa}^{-1}$  for rainfed and FAO trees respectively. The difference, however, was non-significant (Fig. 3).

These results show that water status of olive trees has an influence on the anatomy

of the xylem of current-year shoots. The lack of water in the soil induced the formation of a greater number of vessels with lower diameter. The lack of difference on  $K_h$  between treatments suggests that, on one hand, these vessels of reduced diameter were effective on avoiding embolism in the rainfed trees, and on the other hand, wider vessel in FAO trees embolized at relatively high  $\Psi_{\text{xylem}}$  which reduced the  $K_h$  considerably. This is not surprising, since it is known that greater tensions are needed for vessels of smaller diameter to cavitate. In addition, vessels with smaller diameter also have a reduced collective pit area between vessels, which reduces the incidence of embolism due to air-seeding (Hacke et al., 2009). The Hagen-Poiseuille Law suggests that greater  $K_h$  values should have been measured in the FAO trees than in the rainfed trees, because of their vessels of greater diameter. The observed anatomical changes induced by the water stress suffered by the rainfed trees, however, were enough to balance this potential advantage of the FAO trees. Effects of water stress, both on anatomical and hydraulic characteristics, have been observed in different species (Limousin et al., 2010; Bacelar et al., 2007; Ladjal et al., 2005). Our results show that the plasticity in vessel diameter induced by changes in water regime could have been efficient on maintaining similar hydraulic conductivities in rainfed than in drip-irrigated olive trees.

## ACKNOWLEDGEMENTS

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## Tables

Table. 1. Mean values  $\pm$  standard errors of vessel density ( $n=5$ ) and xylem water potential ( $\Psi_{\text{xylem}}$ ) ( $n=10$ ).  $\Psi_{\text{xylem}}$  was measured at 12.00 GMT. Mean values with different letters are significantly different according to t-test ( $P \leq 0.05$ ).

	FAO	Rainfed
$\Psi_{\text{xylem}}$ (MPa)	$-0.92 \pm 0.05$ a	$-1.2 \pm 0.07$ b
Vessel density (vessels $\text{mm}^{-2}$ )	$1458 \pm 324$ a	$1582 \pm 176$ a

## Figures

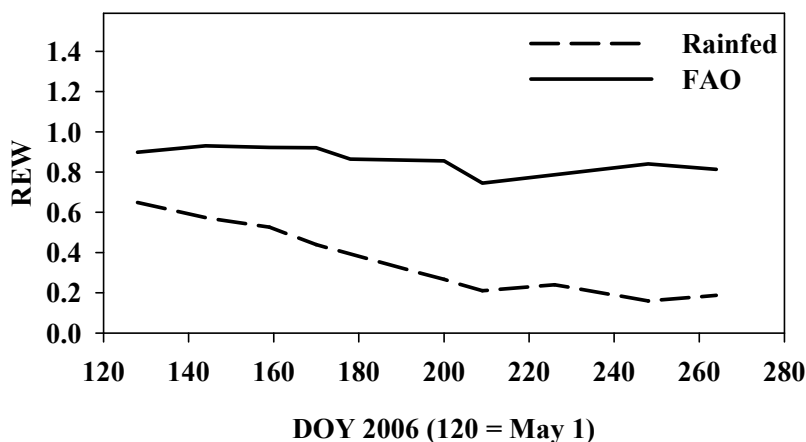


Fig. 1. Values of the relative extractable content (REW) in the soil of each treatment, calculated from volumetric soil water content measurements taken in the orchard during the experimental period. DOY = day of year.

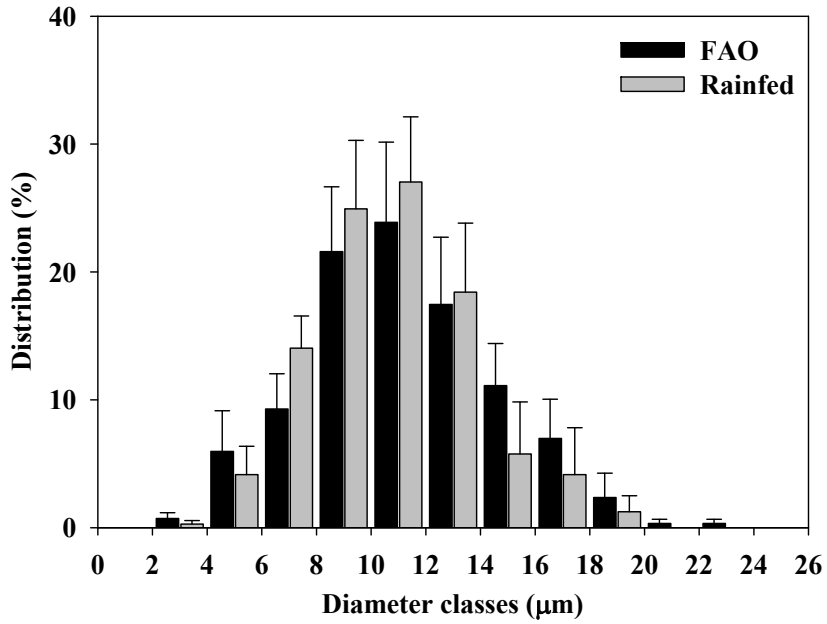


Fig. 2. Xylem vessel distributions per diameter classes in FAO and rainfed treatments.  $\chi^2$  test showed significant differences between the two distributions ( $P < 0.001$ ).

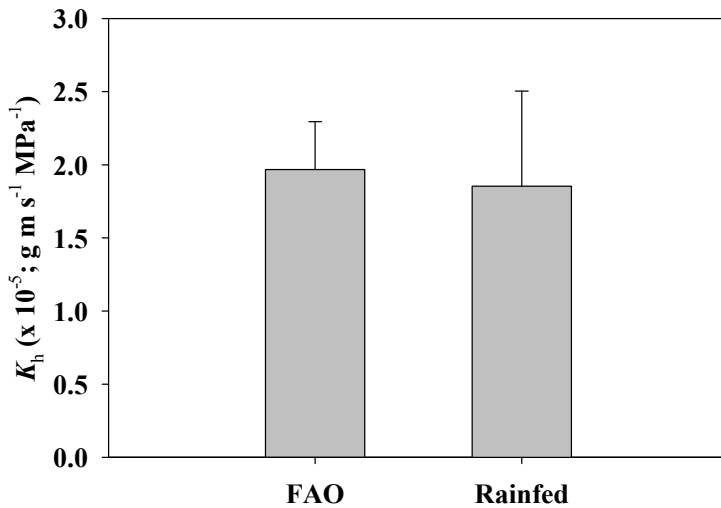


Fig. 3. Hydraulic conductivities ( $K_h$ ) in internodes from current-year shoots. For both treatments  $n=10$ .  $t$ - test did not show significant differences between treatments ( $P=0.131$ ).