Spatial sap flow and xylem anatomical characteristics in olive trees under different irrigation regimes

ÁLVARO LÓPEZ-BERNAL,1,3 ESTEBAN ALCÁNTARA,2 LUCA TESTI1 and FRANCISCO J. VILLALOBOS1,2

1 Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Alameda del Obispo, s/n, 14004 Córdoba, Spain
2 Departamento de Agronomía, Edificio Celestino Matis, Campus de Rabanales, Universidad de Córdoba, 14014 Córdoba, Spain
3 Corresponding author (g42lobea@uco.es)

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Summary  The compensation heat pulse (CHP) method is widely used to estimate sap flow and transpiration in conducting organs of woody plants. Previous studies have reported a natural azimuthal variability in sap flow, which could have practical implications in locating the CHP probes and integrating their output. Sap flow of several olive trees (Olea europaea L. cv. ‘Arbequina’) previously grown under different irrigation treatments were monitored by the CHP method, and their xylem anatomical characteristics were analyzed from wood samples taken at the same location in which the probes were installed. A significant azimuthal variability in the sap flow was found in a well-irrigated olive tree monitored by eight CHP probes. The azimuthal variability was well related to crown architecture, but poorly to azimuthal differences in the xylem anatomical characteristics. Well-irrigated and deficit-irrigated olive trees showed similar xylem anatomical characteristics, but they differed in xylem growth and in the ratio of nocturnal-to-diurnal sap flow (N/D index). The results of this work indicate that transpiration cannot be accurately estimated by the CHP method in olive trees if a small number of sensors are employed and that the N/D index could be used as a sensitive water status indicator.

Keywords: compensated heat pulse, deficit irrigation, transpiration, xylem conductivity.

Introduction

Olive (Olea europaea L.) is the main cultivated tree species of the Mediterranean basin. In the last few years, olive production has been intensified with higher planting densities and drip irrigation (Moriana et al. 2003). However, irrigation resources are limiting because of water scarcity and increased competition with non-agricultural uses (Fereres and Soriano 2007). This context has driven the development of deficit irrigation strategies with promising results (Moriana et al. 2003, Iniesta et al. 2009). Besides, important efforts have been made to develop irrigation scheduling techniques based on the plant’s actual water requirements (Orgaz et al. 2006, Testi et al. 2006).

Deficit irrigation might lead to a reduction in plant transpiration, which needs to be quantified in order to derive the net water savings due to water restrictions. Tree transpiration can be estimated by lysimeters, gas exchange chambers or sap flow sensors, which are the most practical. The compensated heat pulse (CHP) (Swanson and Whitfield 1981) is a method that has been used widely to determine the dynamics of transpiration in trees. In addition, that technique could be the basis for automatic control of irrigation (Fernández et al. 2001, 2008).

Nevertheless, the CHP method presents some difficulties such as those related to the heterogeneity of sapwood functioning. Fernández et al. (2001) and Nadezhdina et al. (2007) found that radial profiles of sap velocity in olive trees either decreased with depth or showed a maximum close to the cambium. Besides, Fernández et al. (2001) reported a great variability between CHP probes inserted in the same trunk at different azimuthal positions. Azimuthal variations are related to sectoriality and the lateral transport of sap, which may be affected by anatomical characteristics of the xylem tissue, such as intervessel pitting, vessel density (VD) and vessel grouping (Orians et al. 2005, Ellmore et al. 2006). As a consequence of the large radial and azimuthal variability, the estimates of sap flow by the CHP method require a large number of probes per tree for an adequate monitoring of absolute transpiration, increasing the instrumentation complexity and its cost.

Sap flow ($Q$) through a stem segment is equal to the product between water potential gradient ($\psi$), which is related to the water supply and demand conditions, and xylem-specific conductivity (Tyree and Ewers 1991).
Specific conductivity depends mainly on xylem anatomical characteristics and also on the fraction of embolized vessels (Tyree and Sperry 1989). Several models based on the Hagen–Poiseuille equation have been developed for estimating the theoretical specific conductivity \( (k) \) from histological observations of VD and vessel diameter (Lewis and Boose 1995).

According to Tyree and Ewers (1991), water stress produces a reduction in xylem-specific conductivity, which may be a consequence of a major proportion of embolized vessels. However, little research has been done on the effect of water stress on xylem anatomical characteristics such as theoretical specific conductivity, VD or vessel diameter. Lovisolo and Schubert (1998) found that water stress caused a decrease in vessel diameter in container-grown grapevine plants, diminishing vulnerability to xylem embolism. On the other hand, analyzing shoot samples of 1-year-old olive plants with different water status, Bacelar et al. (2007) observed an increase in VD in water-stressed plants, whereas vessel diameter did not change.

Night-time sap flow has been observed in a large number of studies and plant species (Snyder et al. 2003, Dawson et al. 2007). Nocturnal sap flow could be related to plant water status as it partially contributes to the refilling of internal water stores depleted during the previous day when the available water in the soil is enough (Daley and Phillips 2006). As a result, we hypothesize an increase in the amount of nocturnal sap flow versus diurnal sap flow when deficit irrigation strategies are employed. However, this topic is still under debate, as nocturnal sap flow is also associated with night-time transpiration processes (Daley and Phillips 2006).

In the present study, we investigated the radial and azimuthal variabilities of sap flow in the trunk of a mature olive tree, relating this variability to changes in theoretical specific conductivity or to crown architecture, in order to establish rational criteria for the placement of CHP probes. Besides, we analyzed the effect of different irrigation strategies on sap flow patterns, xylem anatomical characteristics and xylem growth.

### Materials and methods

#### Experimental site

Experiments were conducted in an experimental olive (cv. 'Arbequina') orchard located at the CIFA Experimental Station, Córdoba, Spain (37.8°N, 4.8°W, 110 m altitude). The climate is Mediterranean, with an average annual rainfall of 600 mm concentrated from spring to summer and potential evapotranspiration of 1390 mm (Pérez-Priego et al. 2005). The olive trees were planted in 1997, tree spacing was 7 m × 3.5 m and irrigation was applied 5 days a week by drip, with seven 41 h⁻¹ drippers per tree (Iniesta et al. 2009). The soil is a Typic Xerofluvvent of sandy loam texture exceeding 1.5 m in depth, with upper drained soil water content limit of 0.23 m³ m⁻³ and lower soil water content limit of 0.07 m³ m⁻³ (Testi et al. 2004).

The reference daily evapotranspiration (ET₀) was calculated following Allen et al. (1998) from standard daily meteorological data recorded in an automated weather station placed 500 m from the orchard.

#### Sap flow measurements

Sap flow was estimated by the CHP method (Swanson and Whittfield 1981). The probes used were designed and produced in the IAS-CSIC laboratory in Córdoba, Spain and consist of a 4.8 W stainless steel heater of 2 mm diameter and two temperature sensors of the same diameter (Testi and Villalobos 2009) located 10 and 5 mm down- and upstream of the heater, respectively. Each temperature probe has four embedded Type E (chromel–constantan wire) thermocouple junctions, spaced 10 mm along the needle, that were sampled separately to obtain heat-pulse velocities at 5, 15, 25 and 35 mm below the cambium (Testi and Villalobos 2009). Sensors were installed at 30 cm height from the soil, and the system was controlled by a datalogger (CR10X, Campbell Scientific Inc., Logan, UT, USA).

Heat-pulse velocities were corrected for wounding effects according to Green et al. (2003). Finally, sap velocities \( (v, \text{ cm} \text{ h}^{-1}) \) and sap flow \( (Q, \text{ l} \text{ h}^{-1}) \) were calculated, and the calibrated average gradient procedure (Testi and Villalobos 2009) was used to obtain low sap velocities. Measurements were made at 15 min intervals. Water fractions needed for sap flow calculations were determined for each tree monitored just before the experiments using a 5 mm diameter increment borer (Suunto, Finland). The nomenclature for sap flow and velocities suggested by Edwards et al. (1996) has been adopted.

#### Analysis of anatomical characteristics

Wood samples were collected at the end of each experiment and consisted of a basal portion of each trunk that was taken at the same height in which CHP sensors had been inserted. The width of the rings was measured at the same azimuthal positions in which CHP probes had been inserted.

Wooden blocks with a cross-section of 1 cm² were obtained for each depth of each probe. Thin slices were cut with a sliding microtome, non-permanent preparations were mounted with water and without employing any stain and finally they were examined in an optical microscope (Nikon, Eclipse 80i, Japan). Nine digital images with 2.22 mm × 1.66 mm were taken of each wood slice using the program NIS-Elements D 2.30 (Nikon). Images were analyzed using ImageJ (Rasband 2002), a public domain Java image-processing program developed at the National Institutes of Health (USA). The following wood
characteristics were measured: minor and major vessel inner diameters, estimated as lengths of the minor and major axes of the best-fitting ellipse ($a_i$ and $b_i$); the proportion of xylem occupied by vessel lumen ($%A$); VD and mean vessel area (VA).

Theoretical specific conductivity of the vessel lumen ($k_i$, kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$; sensu Tyree and Ewers 1991) was calculated according to Lewis and Boose (1995):

$$k_i = \frac{\pi \rho}{64 \eta A_x} \sum_{i=1}^{n} \frac{a_i b_i}{a_i^2 + b_i^2} \quad (1)$$

where $\rho$ is the density of water (kg m$^{-3}$), $A_x$ the sampled cross-sectional area of xylem (m$^2$), $\eta$ the dynamic viscosity of the xylem (MPa s), $a_i$ and $b_i$ the minor and major perpendicular lumen diameters of the $i$th vessel (µm) and $n$ the number of measured vessels. The theoretical hydraulic characteristics were calculated for a reference temperature of 20 °C.

**Experiment I**

The aim of Experiment I was to study the variability in sap flow and anatomical characteristics within the sapwood. To achieve this goal, eight probes (P1, P2, P3, P4, P5, P6, P7 and P8) were installed at uniform spacing along the trunk perimeter (65 cm) of a well-irrigated olive tree. Sap flow measurements were made from 15 to 25 July 2008, a dry period with high evaporative demand ($ET_0$ ranged between 5.9 and 7.9 mm day$^{-1}$). The tree crown consisted of four main branches (B1, B2, B3 and B4, Figure 1) that were cut sequentially on 18, 21, 22 and 23 July 2008 at dawn (0600 Coordinated Universal Time, UTC). On the same dates, the total leaf area of the corresponding branch was estimated from the total leaf weight and the relationship between leaf weight and leaf area of a sample of leaves. The sample’s leaf area was measured using an area meter (model Li-Cor LI-3100). Anatomical characteristics were analyzed from wood samples obtained on 25 July 2008, after the tree was cut down.

**Analysis of the variability in anatomical characteristics and sap flow**

Mean sap velocity ($v_s$) and sap flow ($Q$) values were calculated from 1100 to 1700 UTC (when maximum sap flow occurred). A map of $v_s$ values in a 3.2 µm grid was obtained by kriging (block kriging, with an isotropic variogram model) using the program ‘GS+ Geostatistics for Environmental Sciences’ (version 5.1.1 by Gamma Design Software, EEUU) for the outer 4 cm of the sapwood cross-section. Besides, variability in the xylem anatomical characteristics was analyzed in the same positions in which sap velocities were estimated. Finally, sap velocity within lumens ($v_i$, cm h$^{-1}$) was calculated by dividing sap velocity ($v_s$) by the proportion of xylem occupied by vessel lumen ($%A$).

A value of total sap flow was calculated as a mean of the sap flow estimated by our 8 real probes and by another 24 fictitious ones, installed uniformly along the trunk perimeter. The sap flow estimated by each fictitious probe was calculated using the map of $v_s$ values. The resulting sap flow was assumed to be the actual value of the total ascending sap flow ($Q_m$). The deviation in sap flow estimation ($D_i$) by each one of the real probes ($Q_i$) with respect to the assumed actual value ($Q_m$) was calculated as:

$$D_i = \frac{Q_i - Q_m}{Q_m} \times 100 \quad (2)$$

The mean deviation due to installation of only one probe in the experimental tree trunk was estimated as the mean of the $D_i$ values corresponding to the eight real probes. Following a similar methodology, the mean deviation from the $Q_m$ value due to the symmetrical installation of two, three, four, five, six, seven or eight probes along the trunk perimeter was calculated. For example, in the case of two probes, we assumed that they were installed on opposite sides of the trunk perimeter, so Eq. (2) was used to calculate the deviations from the $Q_m$ value for the combinations of probes S1/S4, S2/S5, S3/S6 and S4/S8. Finally, the mean deviation due to the installation of two opposite probes was obtained as the mean of the four mentioned combinations.

**Analysis of the relations between sap flow and crown architecture**

A mean sap flow value per sensor ($Q$) was calculated from 1100 to 1700 UTC for each day of the measurement period. These data were normalized using daily $ET_0$. 
A connectivity index ($\Phi_j$) between each probe and each branch was calculated by:

$$\Phi_j = \frac{\Delta Q_j}{\sum \Delta Q_j}$$

where $\Delta Q_j$ is the reduction in ET$_0$-normalized sap flow caused by the elimination of branch $j$ and $\Sigma \Delta Q_j$ is the total reduction in ET$_0$-normalized sap flow caused by the elimination of the four branches. Besides, sap supply from each xylem sector (probe) to each branch ($S_j$, 1 h$^{-1}$) was calculated by:

$$S_j = \Phi_j \cdot Q$$

where $Q$ is the sap flow estimated by the probe from 1100 to 1700 UTC on 16 July 2008 and $\Phi_j$ is the connectivity index calculated for the branch and sensor considered.

The Huber value (HV; cm$^2$ m$^{-2}$; sensu Tyree and Ewers 1991) was calculated by dividing the sapwood cross-section by total leaf area. As at first the sapwood cross-section was unknown, HV was calculated considering three different values of sapwood depth (25, 40 and 55 mm).

**Experiment II**

The aim of Experiment II was to assess the effect of water deficit on xylem anatomical characteristics and sap flow. To achieve this goal, we used olive trees from an experiment testing different irrigation treatments, which started in 2004 (Iniesta et al. 2009):

(i) control treatment (C), applying enough irrigation to keep maximum evapotranspiration;

(ii) continuous deficit irrigation (CDI), which applied 25% of water applied in the control, distributed throughout the irrigation season (typically from the start of May to the beginning of October);

(iii) regulated deficit irrigation (RDI), which applied the same total amount of irrigation as CDI, but with a midsummer (1 July to 10–15 September) deficit period without irrigation. So, in the rest of the irrigation season, more water was applied in RDI than in CDI treatment.

The experimental field was arranged as a randomized complete block with three replications, and each plot consisted of 12 olive trees in three adjacent rows.

Six olive trees (two per irrigation treatment) were monitored with one CHP probe per tree from 29 September 2008 (day of the year (DOY) = 273) to 9 October 2008 (DOY = 283). During that period, no rainfall took place and ET$_0$ ranged between 2.8 and 3.7 mm day$^{-1}$. In addition, the irrigation season ended on 5 October 2008 (DOY = 279), so no water was applied from that date to the end of the experiment. Every day, mean sap flow values were calculated from 1030 to 1630 UTC and from 2100 to 0600 UTC for each tree and for each irrigation treatment. After the end of sap flow measurements, wood samples were taken from the same height at which the probes had been installed to analyze anatomical characteristics. Mean values of $k_t$, VD and VA were obtained for each irrigation treatment.

Besides, 20 olive trees (8 C, 6 CDI and 6 RDI) were sawn off on 28 October 2009. Trunk cross-section samples were taken to measure growth ring width and to calculate xylem area produced in 2004, 2005 and 2006.

**Results**

**Experiment I**

**Variability in anatomical characteristics and sap flow** The main anatomical characteristics showed a large variability that was observed in both radial and azimuthal directions. The VD ranged between 20 and 80 mm$^{-2}$, with a mean value of 51.9 mm$^{-2}$ (CV = 33.0%), whereas mean VA ranged between 800 and 1200 μm$^2$ (CV = 11.8%) with a mean value of 968 μm$^2$. On the other hand, theoretical specific conductivity of the vessel lumen ($k_t$) ranged between 0.5 and 3.2 kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$ (CV = 51.3%, Figure 2) with a mean value of 1.53 kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$. Variability in $k_t$ was in good agreement with differences in VD ($r^2 = 0.92$) and VA ($r^2 = 0.82$), but the best fit was found for the linear relationship with the proportion of xylem occupied by vessel lumen (% A) ($r^2 = 0.98$).

In addition, the width of the growth rings also presented azimuthal variability (data not shown), with the highest values corresponding to the locations of probes P6 and P7 and the lowest to those of probes P1 and P8. Consequently, the age of the wood corresponding to the inner depths of each probe was different. For example, the inner depth of probe P6 was inserted in 5-year-old wood, whereas that of probe P1 was inserted in 6-year-old wood (data not shown).

Sap velocity at central hours of the day showed a great variability, but it was poorly correlated with anatomical characteristics, as is shown for $k_t$ ($r^2 = 0.23$, Figure 2). Sap velocity ranged between 0 and 120 cm h$^{-1}$ with a mean value of 38.9 cm h$^{-1}$ (CV = 58.2%), and both radial and

![Figure 2](http://www.treephys.oxfordjournals.org)

Figure 2. Sap velocity ($v_s$) averaged from 1100 to 1700 UTC on 16 July 2008 versus theoretical specific conductivity of xylem ($k_t$). Data corresponded to 32 points of measurement in a well-irrigated olive tree (Experiment I).
azimuthal variability was found (Figure 3). Thus, the lowest sap velocities were obtained in the inner depths of probes P1, P4 and P5, and the highest values appeared in the outer depths of probe P6. On the other hand, the sap velocities within lumens at midday ($v_l$) ranged between 0 and 22 m h$^{-1}$ (CV = 46.3%) with a mean value of 11.6 m h$^{-1}$, showing a large radial and azimuthal variability (data not shown).

Both the mean value of $k_t$ and the total sap flow at central hours of the day were calculated for each probe, showing large azimuthal variations (Table 1, CV = 54.1%). The highest value of $k_t$ was found in probe P6, whereas the lowest one was observed in probe P8. On the other hand, total sap flow ranged between 11.34 (probe P6) and 3.29 l h$^{-1}$ (probe P5) with a mean value of 5.23 l h$^{-1}$. Azimuthal variations in sap flow were partly associated with variations in $k_t$ ($r^2 = 0.49$). In addition, the large azimuthal variability of sap flow led to high deviations from the actual value of transpiration when only a small number of symmetrically installed probes were considered (Figure 4).

### Table 1

<table>
<thead>
<tr>
<th>Probe</th>
<th>$k_t$ (kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$)</th>
<th>$Q$ (l h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1.53</td>
<td>3.31</td>
</tr>
<tr>
<td>P2</td>
<td>1.18</td>
<td>5.07</td>
</tr>
<tr>
<td>P3</td>
<td>1.32</td>
<td>4.38</td>
</tr>
<tr>
<td>P4</td>
<td>1.14</td>
<td>3.78</td>
</tr>
<tr>
<td>P5</td>
<td>1.82</td>
<td>3.29</td>
</tr>
<tr>
<td>P6</td>
<td>2.37</td>
<td>11.34</td>
</tr>
<tr>
<td>P7</td>
<td>1.99</td>
<td>6.43</td>
</tr>
<tr>
<td>P8</td>
<td>0.89</td>
<td>4.22</td>
</tr>
<tr>
<td>Mean</td>
<td>1.53</td>
<td>5.23</td>
</tr>
<tr>
<td>SD</td>
<td>0.50</td>
<td>2.83</td>
</tr>
</tbody>
</table>

### Relations between sap flow and crown architecture

Total sap flow (calculated as mean of the eight probes) decreased in parallel with the reduction in leaf area after cutting the branches. However, the elimination of each branch had a distinct effect on sap flow estimates made by the different probes (Figure 5). For example, when branch B2 was eliminated, sap flow decreased significantly in probes P1 and P2 and no important reductions were observed in probes P4, P5, P6 and P7. On the other hand, when branches B3 and B4 were cut, sap flow in probes P4, P5, P6 and P7 decreased more than that in P1 and P2.

A xylem connections map was obtained from the analysis of variations in sap flow after the branches were removed (Figure 6), indicating that 1.07 l h$^{-1}$ of sap flow reaching branch B2 was mainly supplied by the xylem sectors corresponding to probes P8, P1 and P2. On the other hand, 4.15 l h$^{-1}$ of sap flow reaching branches B1, B3 and B4 was mainly supplied by the remaining sectors, especially the one corresponding to probe P6.

The HV was 1.92, 2.81 and 3.49 cm$^2$ m$^{-2}$, assuming a sapwood depth of 25, 40 and 55 mm, respectively.

### Experiment II

Xylem area produced from 2004 to 2006 was higher in the control (C), followed by RDI and finally by CDI (Table 2). On the other hand, the irrigation treatments produced no differences in $k_t$, VD or VA (Table 2).

Sap flow diurnal patterns showed a steep morning increase leading to maximum rates achieved at about midday followed by a steep decrease until late in the afternoon (Figure 7). The control (C) treatment presented higher maximum values of sap flow than the CDI. On the other hand, one of the RDI olive trees showed a pattern similar to that of the control treatment, whereas the second one was more similar to the CDI patterns.

Nocturnal sap flow was detected for all treatments. The ratio of nocturnal-to-diurnal sap flow ($N/D$ index) was affected by irrigation treatments, with higher values in CDI than in RDI and control treatments (Figure 8). The irrigation

### Figure 4

Deviation from the assumed actual total sap flow ($Q_m$) calculated by 8 real probes and by 24 fictitious ones simulating a variable number of symmetrically located probes in the olive trunk of Experiment I.
season terminated on 5 October 2008 (DOY = 279); after that, the N/D index showed a clear increase in the three treatments (Figure 8).

**Discussion**

Variability in anatomical characteristics and sap flow

This is the first study estimating the theoretical specific conductivity of the vessel lumen ($k_t$) in olive trunks using the Lewis and Boose (1995) methodology. Mean $k_t$ values were 1.53 and 2.74 kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$ for Experiments I and II, respectively (Tables 1 and 2). Using a similar methodology, Sellin et al. (2008) found higher values of $k_t$ (~3.5 kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$) in Betula pendula trunks.

$k_t$ values showed a great variability between the analyzed wood samples in Experiment I. According to Sellin et al. (2008), differences in $k_t$ can be divided into two components: VA and VD. The values of $k_t$ were correlated with both characteristics, but the variation coefficient was lower for mean VA. Hence, differences in $k_t$ were more related to

![Figure 5: Sap flow relative estimates (%) in each probe from 1100 to 1700 UTC on 16 July 2008 (crown complete), 18 July 2008 (after cutting branch B1), 21 July 2008 (after cutting branch B2), 22 July 2008 (after cutting branch B3) and 23 July 2008 (after cutting branch B4) for the well-irrigated olive tree monitored in Experiment I. Values are referred to the initial measurements (16 July 2008) and normalized by ET$_{p}$.

![Figure 6: Sap flow ($1\text{h}^{-1}$) supplying south/north branches (respectively) in a well-irrigated olive tree (Experiment I) from the sectors of xylem corresponding to each probe and the total amount of sap flow reaching south and north branches. Sap flow values correspond to measurements made from 1100 to 1700 UTC on 16 July 2008.

![Figure 7: Diurnal patterns of sap flow for the six monitored olives of Experiment II on 4 October 2008.

Table 2. Mean values of xylem area produced from 2004 to 2006 (both years inclusive), theoretical specific conductivity ($k_t$), VD and mean VA for each irrigation treatment. Means tagged with the same letter within a column are not significantly different at $P < 0.01$.

<table>
<thead>
<tr>
<th>Irrigation treatment</th>
<th>Xylem produced (cm$^2$)</th>
<th>$k_t$ (kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$)</th>
<th>VD (mm$^{-2}$)</th>
<th>VA ($\mu$m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>88.1a</td>
<td>2.75a</td>
<td>52.2a</td>
<td></td>
</tr>
<tr>
<td>RDI</td>
<td>77.8b</td>
<td>2.85a</td>
<td>53.3a</td>
<td>1067a</td>
</tr>
<tr>
<td>CDI</td>
<td>62.0c</td>
<td>2.63a</td>
<td>49.8a</td>
<td>1063a</td>
</tr>
<tr>
<td>Mean</td>
<td>76.0</td>
<td>2.74</td>
<td>51.8</td>
<td>1062</td>
</tr>
</tbody>
</table>
proportion of non-conducting vessels (impossible to discriminate by microscopy), or to an increase in the radial resistance to water flow as xylem ages (Gartner and Meinzer 2005). However, substantial values of sap velocity were still estimated by inner depths of the probes, which were placed in 5- or 6-year-old wood. These results suggest that olive evolution has led to a conservative strategy in the water supply/demand ratio, as the life span of olive leaves is ~2 years only.

Enormous azimuthal variability was found in diurnal sap flow (CV = 54.1%). Once again, the correlation between sap flow and mean \( k_t \) values per probe was not very high \( (r^2 = 0.49, \text{Table 1}) \), which suggests also azimuthal heterogeneity in xylem functioning.

Irregularities in the azimuthal distribution of sap flow have been observed in olive trees (Palomo et al. 1998, Fernández et al. 2001) and in other tree species (Loustou et al. 1998, Oliveras and Llorens 2001). In spite of that, the magnitude of that phenomenon or its implication in the estimation of transpiration by the CHP method is often underestimated. Thus, Fernández et al. (2001) proposed that only two to four probes per trunk would be required to obtain a good estimate of tree transpiration, while our results show deviations >10% if fewer than six probes are installed for our experimental tree trunk (Figure 4). Therefore, under the condition of the present experiment (mature olive trees under severe pruning regimes), the estimates of plant transpiration obtained by a single or even a pair of uncalibrated sap flow probes could be incorrect.

Relations between sap flow and canopy architecture

High azimuthal differences in sap flow estimates (Table 1) made evident that sap ascent was sectorial. In addition, our results show that each branch is supplied by xylem sectors placed near the azimuthal point of trunk insertion (Figures 5 and 6). Thus, the xylem sectors corresponding to probes P3, P4, P5, P6 and P7 were supplying the north branches and those of probes P8, P1 and P2 were supplying the south branch. On the other hand, total sap flow supplying north branches was higher than that calculated for the south branch (Figure 6), in which a lower total leaf area was found (Figure 1). Consequently, xylem sectoriality was mainly influenced by crown architecture (particularly by the point of branch insertion and by the leaf area of each branch), which is in agreement with the observations of Palomo et al. (1998). Therefore, the high azimuthal variability in sap flow (and the high associated deviations in the estimation of tree transpiration) could be a result of the particular crown architecture in our experimental olive tree. We should expect lower sap flow azimuthal variability in olive trees with a larger number of branches of similar size inserted into the trunk at different azimuthal positions.

Root architecture could also play a role in the azimuthal variability of sap flow in olive trees as it could reflect heterogeneity of water uptake by roots, which requires further
research. Anyway, studying the sap ascent pattern from roots to the crown may help in predicting sap flow azimuthal variability. Several old studies established up to seven patterns of sap ascent (Kozlowski and Winget 1963, Waisel et al. 1972). Only three of those patterns (‘sectorial winding’, ‘sectorial turning into a ring’ or ‘spiral turning clockwise’) fit with our observations.

Radial profiles of sap velocity at the central hours of the day apparently indicated that sapwood was deeper than 40 mm for most of the probes. These results suggest that our HV calculations from assumed sapwood depths of either 25 or even 40 mm were underestimated. Therefore, the actual HV should be >2.8 cm² m⁻². Anyway, these observations suggest that the HV corresponding to olive tree trunks is similar to that found in Quercus ilex stems (HV = 3.3 cm² m⁻², Villar-Salvador et al. 1997) and higher than that in B. pendula trunks (HV = 2 cm² m⁻², Sellin and Kupper 2006).

Effects of water deficit on xylem anatomical characteristics and sap flow

The three irrigation treatments had a different effect on xylem growth. The well-irrigated control treatment (C) showed the highest xylem area production during the 2004–06 period (Table 2). Besides, xylem growth was significantly higher in RDI than in CDI (Table 2) despite receiving the same seasonal amount of irrigation, indicating that water availability during the hotter part of the season has a lower impact on xylem growth.

On the other hand, the control treatment (C) showed values of midday water potential (ψ) of between –0.5 and –1.7 MPa between 2004 and 2006, whereas in the deficit treatments, ψ decreased during the irrigation seasons reaching values of –2.9 and –3.6 MPa in CDI and RDI, respectively, according to Iniesta et al. (2009). In spite of these large differences in water status, kₜ, mean VA and VD did not differ significantly between irrigation treatments (Table 2). These results are in disagreement with those of Bacelar et al. (2007), who found that water stress induced an increase in xylem VD, but they worked with 1-year-old olive plants, whose histological characteristics may differ from those of adult trees. On the other hand, it may be the xylem in the shoots and other portions closer to the outer parts of the canopy, the one most influenced by the water treatments.

Similar values of the N/D index were found between the olive trees with the same irrigation treatment (Figure 8). Even the pair of olive trees with RDI treatment satisfied this condition, despite the differences in the diurnal patterns of sap flow (Figure 7), which might have been a consequence of differences in leaf area between them. On the other hand, the treatment with the lowest irrigation (CDI) showed the highest values of the N/D index (Figure 8). RDI treatment showed values of the N/D index similar to those of the control, which is in agreement with the fast recovery from midsummer stress reported by Iniesta et al. (2009). Finally, the N/D index showed a continuous increase in the 4 days following the end of the irrigation season on 5 October 2008 (DOY = 279) (Figure 8), probably as a result of the decrease in soil water content (in that period the average reference evapotranspiration was 3.5 mm day⁻¹ and no rainfall took place). These observations suggest that the N/D index may serve as a sensitive water status indicator and that the refilling of depleted water stores is a significant component of nocturnal sap flow, which is in agreement with Daley and Phillips (2006).

Conclusion

The present study is the first systematic one analyzing and quantifying simultaneously xylem anatomical characteristics and sap flow patterns within sapwood in olive trees. The results suggest that olive xylem remains active for >5 years.

Our experimental evidence demonstrates that sap flow azimuthal variability in mature olive trees could be very high, making absolute transpiration estimates by the CHP method a challenging task.

On the other hand, the study has shown that deficit irrigation does not affect xylem VD or vessel dimensions, but reduces trunk growth rate. Besides, we have presented preliminary evidence on the validity of the ratio of nocturnal-to-diurnal sap flow as a water status indicator, which deserves further research.

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Res. Serv. Branch, National Institutes of Health, Bethesda, MD.


