Using the compensated heat pulse method to monitor trends in stem water content in standing trees

Álvaro López-Bernal1,3, Luca Testi1 and Francisco J. Villalobos1,2

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

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Studying the dynamics of stem water content ($\theta$) in living trees has an outstanding physiological interest but all the available techniques to measure $\theta$ exhibit major drawbacks. In this work, we present a new methodology to estimate variations in $\theta$ along with sap velocity using the compensated heat pulse (CHP) technique. One lab experiment was performed on several wooden blocks obtained from three different tree species. Samples were slowly dried and their moisture loss was monitored by both gravimetric approaches and time-domain reflectometry (TDR) or CHP probes in order to contrast the validity of our methodology (volumetric specific heat (VSH)-CHP) over a range of water contents. In addition, a field experiment was conducted to monitor $\theta$ fluctuations in standing olive trees (Olea europaea L. cv. ‘Arbequina’) growing under three different irrigation regimes. In the lab test, the actual $\theta$ values deduced gravimetrically differed from the estimates yielded by the VSH-CHP method. However, it could successfully track relative changes in the water stored for the range of $\theta$ expected in living wood. Furthermore, the field experiment showed a seasonal change in $\theta$, which was similar in shape and magnitude to those reported in the literature for olive and other Mediterranean tree species. On the other hand, differences in the seasonal patterns of $\theta$ between irrigation treatments strongly corresponded with those of sap flow and some leaf water potential measurements. The results of this work suggest that the CHP technique could be employed to monitor the dynamics of both $\theta$ and sap flow simultaneously in standing trees and evidence that seasonal changes in $\theta$ might be used as a long-term water status indicator.

Keywords: compensated heat pulse, Olea europaea, stem water content, TDR, water relations.

Introduction

The stem water content ($\theta$) of trees has been the subject of numerous studies for decades (Waring and Running 1978, Tyree and Yang 1990, Goldstein et al. 1998, Phillips et al. 2003). There is ample evidence that trees store water in sapwood during times of low evaporative demand and consume this water in transpiration when the evaporative demand exceeds root water uptake. Therefore, the water stored is withdrawn and replenished in both daily and seasonal cycles, although the magnitude of the first is small compared with the latter. Thus, several studies have reported that the annual percentage change in $\theta$ ranges from 8% (0.50–0.46 cm$^3$ cm$^{-3}$, Pinus sylvestris L.; Irvine and Grace 1997) to 67% (0.61–0.20 cm$^3$ cm$^{-3}$, Aesculus californica Nutt.; Constantz and Murphy 1990) depending on species and environmental conditions (Constantz and Murphy 1990, Wullschleger et al. 1996, Irvine and Grace 1997, Hernández-Santana et al. 2008, Nadler and Tyree 2008), whereas it rarely exceeds 10% on a daily basis (Nadler and Tyree 2008).

Water extraction from stems occurs when the soil water potential becomes progressively negative during a drought. Under these conditions the water stored allows the tree to maintain both higher transpiration and photosynthesis rates holding up stomatal closure, so it has been contended that $\theta$ plays a biologically significant role (Goldstein et al. 1998, Cermák et al. 2007). Furthermore, some studies have proposed that measurements of $\theta$ could be employed to detect water
stress (Nadler et al. 2003, 2006, Hernández-Santana et al. 2008) as the close contact between the bole and the ground led \( \theta \) to track changes in soil water potential more closely than leaf water potential (Tyree and Ewers 1991). However, measuring water content in standing trees still remains a challenging task.

The simplest technique of measuring stem water content is to collect stem cores and directly measure the water content by weighing tissue samples before and after drying. Such traditional gravimetric measurement is labor intensive, difficult to automate and harmful for the tree after repeated sampling. These shortcomings led to the development of new approaches such as gamma-ray attenuation (Edwards and Jarvis 1983), nuclear magnetic resonance (Byrne et al. 1986, Van As et al. 2009), electrical conductivity (Nadler and Tyree 2008, Nadler et al. 2008) or time-domain reflectometry (TDR). The first one is not currently used because of the risk of exposure to radiation while nuclear magnetic resonance is expensive and remains difficult to apply in the field and electrical conductivity measurements present some drawbacks due to the need to wait for a long curing period after probe installation and the fact of being affected by salinity. Constantz and Murphy (1990) were the first to use TDR in living trees and noted that the technology provided a rapid, automatable and accurate mean of measuring \( \theta \). Since then, some authors have employed TDR methodology with different species (Wüschleger et al. 1996, Irvine and Grace 1997, Hernández-Santana et al. 2008). On the contrary, the TDR method is expensive, complicated and requires calibration, although Wüschleger et al. (1996) produced an empirical relationship which was valid for a wide range of species. During the revision process of this article, we have become aware of a work by Vandegheuvelt and Steppe (2012a) developing a non-empirical heat-pulse-based method to determine simultaneously both sap flux density and the sapwood water content using four-needle sap flow sensors (referred to as Sapflow+). The results of both finite-element modeling and lab experiments show that Sapflow+ can afford accurate estimates of sap flux density and also of water content (at least during periods of low velocities), but it has not yet been tested in living trees.

In this paper, we describe a new methodology to estimate \( \theta \) in standing trees using the CHP technique. The CHP has been widely used to determine the dynamics of transpiration by measuring sap flow in conductive organs of woody plants (Swanson and Whitfield 1981) and presents a great potential for irrigation scheduling (Ferrández et al. 2001, 2008). In addition, this technique can also be used to detect water stress as the decline in tree transpiration (Ferrández et al. 2001, Tognetti et al. 2004, 2005) and, more recently, López-Bernal et al. (2010) have suggested that the proportion of nocturnal to diurnal sap flow (N/D index) could also be another sensitive water status indicator.

The main goals of this study are to develop a simple methodology based on the calculation of volumetric specific heat (VSH-CHP) that allows estimating \( \theta \) along with sap velocity using CHP sensors and to test its feasibility for monitoring fluctuations in \( \theta \) of the woody parts of trees. In addition, we investigate if the \( \theta \) monitored by that method could be employed as a suitable indicator of water status in olive trees growing under different irrigation regimes.

**Material and methods**

**Theoretical framework of VSH-CHP**

The CHP method is based on the measurement of the temperature difference between sensors located above and below a heater inserted in the tree trunk (Swanson 1962). Marshall (1958) established the theoretical basis for this technique by deriving an analytical solution to the diffusion equation with coupled convective transport by sap within an infinite medium which has been recently adapted for anisotropic conditions by Vandegheuvelt and Steppe (2012b). That equation may be written as

\[
\Delta T = T_1 - T_2 = k \frac{H}{4\pi D p c} \left[ \exp \left( -\frac{x_1 - v_s t}{4D t} \right) - \exp \left( -\frac{x_2 + v_s t}{4D t} \right) \right]
\]

(1)

where \( \Delta T \) is the temperature difference between the down- and the up-stream sensor (K), \( x_1 \) and \( x_2 \) are the distances from down- and up-stream to the heater, respectively (m), \( H \) is the heat input from heat pulse (J m\(^{-1}\)), \( D \) is the thermal diffusivity in the axial direction (m\(^2\) s\(^{-1}\)), \( t \) is the time since heat pulse applied (s), \( v_s \) is the heat pulse velocity (m s\(^{-1}\)), \( \rho \) and \( c \) are density and specific heat of the wood (i.e., the volumetric specific heat, J m\(^{-3}\) K\(^{-1}\)) and \( k \) is the square root of the quotient between axial and tangential thermal conductivities (\( K_{ax} \) and \( K_{tg} \), respectively; W m\(^{-1}\) K\(^{-1}\)):

\[
k = \sqrt{\frac{K_{ax}}{K_{tg}}} \quad \text{(2)}
\]

Swanson and Whitfield (1981) showed that the heat pulse velocity is linearly related to the inverse of the time from the heat pulse emission until the temperature difference returns to its initial value (\( t_0 \)):

\[
v_s = \frac{x_1 + x_2}{2t_0} \quad \text{(3)}
\]

To avoid underestimations, heat pulse velocities should be corrected for wounding effects (Swanson and Whitfield 1981, Green et al. 2003) before further calculations.
The function $\Delta T (t)$ given by Eq. (1) reaches a minimum value before $t_0$ as the upstream sensor is heated faster than the downstream one. We can calculate this minimum using the derivative of Eq. (1):

$$\frac{\partial \Delta T}{\partial t} = \frac{K_w}{K_h} \frac{H}{4\pi D c t^2} \left[ \exp \left( -\frac{(x_1 - v_h t)^2}{4D t} \right) \left( \frac{x_1^2 - v_h^2 t^2}{4Dt} - 1 \right) \right]$$

Equation (4) is equal to zero at the time of the minimum ($t_n$), so we arrive at the next equation:

$$\exp \left( \frac{x_1^2 - x_2^2 + 2v_h t_n (x_1 - x_2)}{4Dt_n} \right) = \frac{x_1^2 - t_n^2 v_h^2}{x_1^2 - t_n^2 v_h^2 - 4Dt_n}$$

Knowing $v_h$—from Eq. (3)—we can compute $D$ numerically from Eq. (5), and inverting Eq. (1) we arrive at

$$\rho c = \frac{k H}{4\pi D t_n} \left( \frac{1}{t_1 - t_2} \right) \ln \left[ \frac{\exp \left( \frac{(x_1 - v_h t)^2}{4D t_n} \right) - \exp \left( \frac{(x_2 + v_h t)^2}{4D t_n} \right)}{\exp \left( \frac{(x_1 - v_h t)^2}{4D t_n} \right) - \exp \left( \frac{(x_2 - v_h t)^2}{4D t_n} \right)} \right]$$

At this point some remarks should be made. First, any error in $V_h$ is taken up in Eqs. (5) and (6) so the correction for wounding effects becomes a crucial point for the accuracy of the methodology. Second, under isotropic conditions (i.e., $K_w = K_h$ and so $k = 1$) all the variables in Eq. (6) are known and $\rho c$ can be deduced. Finally, for zero heat pulse velocity the product $4Dt_n$ depends only on geometrical characteristics (i.e., $x_1$ and $x_2$) as deduced from Eq. (5), so, for that condition, Eq. (6) can be simplified to

$$\rho c = k \frac{H}{4\pi D t_n} \left( \frac{1}{t_1 - t_2} \right)$$

where $\gamma$ can be calculated from $H$ and the product $4Dt_n$, which is a constant that depends only on $x_1$ and $x_2$ (see Eq. (5) for $v_h = 0$). For instance, when $x_1 = 10$ mm and $x_2 = 5$ mm, $4Dt_n = 22.35$ mm$^2$.

The last step of the VSH-CHP methodology is the estimation of $\theta$, which can be determined from $\rho c$ as there is a relationship between both variables (Edwards and Warwick 1984):

$$\theta = \frac{\rho c - \rho_s \rho_w}{\rho_s c_w}$$

where $c_w$ is the water specific heat (J kg$^{-1}$K$^{-1}$), $c_s$ is the solid matrix specific heat and $\rho_s$ and $\rho_w$ are the basic densities of wood and water, respectively (kg m$^{-3}$).

The presented framework is limited as there is no basis to estimate $k$, which is a prerequisite to deduce $\rho c$ in Eq. (6). In contrast, the methodology allows the monitoring of $\theta$ from $\rho c$ estimates for isotropic conditions. In this work, all the calculations of $\theta$ assume $k = 1$ and the errors arising from that assumption are discussed below.

**Lab experiments**

Lab experiments were conducted employing wood samples from several plant species including olive (*Olea europaea* L.), plum (*Prunus domestica* L.) and fig (*Ficus carica* L.) trees. Plant materials, consisting of tree stem segments of, at least, 20 cm diameter, were collected from different locations and dates (always in winter) near the city of Córdoba, Spain (37.8°N, 4.8°W, 110 m altitude). These segments were cut using an industrial saw to obtain wooden blocks with cubic shapes of about 10 cm × 10 cm × 10 cm preserving the bark on one of their sides. In order to prevent moisture loss during transportation from the field to the lab, the samples were kept in plastic bags within isothermal boxes and, once they reached the lab, they were kept in cold conditions (4 °C). The time from sample collection to the beginning of experiments was short (4 days) for plum and fig tree samples and, long (several months) for those of olive, which were immersed in water for 3 days before the experiment to ensure a high initial $\theta$.

Lab experiments consisted of a drying process in which the moisture loss of the wooden blocks was simultaneously monitored employing gravimetric procedures ($\theta_3$) and either the VSH-CHP methodology as detailed above ($\theta_1$) or the TDR technique (see Table 1). The water-displacement method (based on the Archimedes principle) was employed for volume measurements before installing one sensor per wooden block on the side where the bark had been preserved.

The CHP sensors 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 probes of the same diameter that are placed in holes drilled in the wood 10 and 5 mm down- and up-stream of the heater, respectively. Accurate vertical spacing and parallel drilling are achieved using a steel drill-bit guide. Each temperature probe has four embedded Type E (chromel-constantan wire) thermocouple junctions, spaced 10 mm along the needle that were sampled separately to estimate heat pulse velocity and volumetric specific heat at 5, 15, 25 and 35 mm below the cambium. However, only the mean of the two intermediate depths

<table>
<thead>
<tr>
<th>Blocks</th>
<th>Species</th>
<th>Technique</th>
<th>Temperature (°C)</th>
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<tbody>
<tr>
<td>1–4</td>
<td>Olive</td>
<td>CHP</td>
<td>30</td>
</tr>
<tr>
<td>5–8</td>
<td>Fig</td>
<td>CHP</td>
<td>30</td>
</tr>
<tr>
<td>9–11</td>
<td>Plum</td>
<td>CHP</td>
<td>30</td>
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<tr>
<td>12–15</td>
<td>Olive</td>
<td>CHP</td>
<td>21</td>
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<tr>
<td>16–19</td>
<td>Olive</td>
<td>TDR</td>
<td>21</td>
</tr>
</tbody>
</table>
was taken into account to monitor the moisture content ($\theta$) as a precautionary measure to avoid possible heterogeneities in the heat applied. The system was controlled by a datalogger (CR1000, Campbell Scientific Inc., Logan, UT, USA), which performs measurement cycles at 15-min intervals (for more details see Testi and Villalobos 2009). On the other hand, the TDR system (TDR 100) included 5 cm length probes and was also controlled by a datalogger (CR1000, Campbell Scientific Inc.), measuring the apparent dielectric constant every 30 min.

The wooden blocks were dried for at least 1 week in either an oven at 30°C or in the lab at a room temperature of 21°C (see Table 1). The mass of each block (with the sensors always kept in place) was measured frequently during the tests using a precision balance of ±0.1 g resolution, especially at the beginning, when $\theta$ loss was higher. Finally, CHP and TDR sensors were removed and weighted and the wooden blocks were dried at 105°C until they achieved constant mass ($M_d$). The sapwood water content $\theta_g$ was then calculated for every mass measurement as

$$\theta_g = \frac{M - M_d}{V \times \rho_w}$$

(9)

where $V$ is the fresh sample volume (m$^3$) and $M$ the block mass after subtracting the sensor mass (kg). These $\theta_g$ data were fitted to the best polynomial trend to obtain values each 15 min and they were compared with those of sapwood water content estimated from the VSH-CHP methodology ($\theta_c$).

Field experiments

The feasibility of using VSH-CHP to monitor changes in moisture storage in living trees was examined by monitoring $\theta_c$ values during a long-term experiment which was 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) during 2006. The climate is Mediterranean and the soil is a Typic Xerochrept of sandy loam texture extending 1.5 m in depth, with upper drained soil water content limit of 0.23 m$^3$ m$^{-3}$ and lower soil water content limit of 0.07 m$^3$ m$^{-3}$ (López-Bernal et al. 2010). 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 4 l h$^{-1}$ drippers per tree. From 2004 to 2006 Iniesta et al. (2009) conducted an experiment testing three different irrigation treatments, which started in 2004 and finished in 2006:

1. Control treatment (C), which applied enough irrigation to keep the maximum estimated evapotranspiration.
2. Continuous deficit irrigation (CDI), which applied 25% of water applied in the control, distributed throughout the irrigation season (typically from the end of May to the beginning of October).
3. Regulated deficit irrigation (RDI), which applied the same total amount of irrigation as CDI, but with a midsummer deficit period without irrigation (typically from the beginning of July to the middle of September).

Particularly, in 2006, the irrigation season started on 1 June (DOY 152) and finished on 17 October (DOY 290) and the midsummer period with no irrigation for RDI trees covered from 28 June (DOY 179) to 12 September (DOY 255).

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. Both the two central trees of each plot (except for two in C treatment) were instrumented with two CHP sensors per tree, at a height of 30 cm over the soil (4 C, 6 CDI and 6 RDI trees) in order to monitor the daily sap flow and $\theta_c$ dynamics. As in the lab experiments, only the CHP outputs corresponding to the intermediate thermocouple junctions were taken into account in the $\theta_c$ calculations. Irregularities associated with daily variations in the moisture content were avoided considering only the averaged $\theta_c$ values from 03:00 to 05:00 GMT for each day. Mean values of $\theta_g$ and sap flow were calculated for each irrigation treatment. Besides, the mean relative sap flow was calculated for deficit treatments (i.e., the ratio between either CDI or RDI sap flow and that of C) in order to contrast differences in water status. Weather data were recorded by an automated weather station placed 500 m from the orchard and reference daily evapotranspiration was calculated following Allen et al. (1998).

Results

Lab experiments

Stem segments used in all the experiments showed initial $\theta_g$ ranging from 0.590 (Block 4) to 0.447 cm$^3$ cm$^{-3}$ (Block 9) and a clear curvilinear decay throughout the tests. However, values of $\theta_g$ initially ranged from 0.642 (Block 15) to 0.391 cm$^3$ cm$^{-3}$ (Block 4) and later they were found to decrease only during a variable period of time after the beginning of the experiments (from 1 to 4 days) until a stabilization occurred. As a consequence, both values and trends of $\theta_g$ did not match closely with those of $\theta_c$ in the drying experiments as exemplified in Figure 1 for one of the fig wooden blocks (Block 6).

The similarities and differences between the gravimetical and the calculated values of moisture content are presented in Figure 2 for all samples plotting $\theta_g$ against the interpolated $\theta_c$. Although most of the sensors showed deviations from the 1/1 line (dashed line), all of them presented clear parallelisms to it for higher moisture contents. This indicates that the relative reduction in $\theta_g$ measured during the first hours of drying was in good agreement with that detected by the VSH-CHP methodology. Figure 1 also illustrates those results showing similar
reductions in $\theta_g$ and $\theta_c$ (0.102 and 0.106 cm$^3$ cm$^{-3}$, respectively) if only the first 45 h of drying test are considered.

These findings were supported by a linear regression analysis between $\theta_c$ and $\theta_g$ which was performed for each sample using only pairs of data with $\theta_g > 75\%$ of the initial value of $\theta_g$ (Table 2). All correlations had statistically significant results ($P < 0.0001$) with determination coefficients $>0.850$ (except for Block 7). The slopes and the intercepts of the linear regression equations showed mean values of 0.993 and 0.003, respectively, which were very close to the theoretical optimum. On the other hand, the slope and intercept standard deviations were 0.229 and 0.107, respectively, indicating a large sensor variability which was not apparently related either to species or to experimental conditions.

Finally, the wooden blocks equipped with TDR sensors presented a curvilinear pattern between the monitored apparent dielectric constant ($\epsilon$) and $\theta_g$ (Figure 3). The averaged $\epsilon$ values decreased from 22.93 to 10.77 while those of $\theta_g$ ranged from 0.547 to 0.282 cm$^3$ cm$^{-3}$. Most of the variations in $\epsilon$ occurred when $\theta_g$ decreased from 0.55 to 0.40 cm$^3$ cm$^{-3}$.

Field experiments

The mean patterns of the calculated $\theta_c$ along the year 2006 are shown in Figure 4. In general, the dynamics of $\theta_c$ followed a sinusoidal trend with a spring maximum and a late summer minimum by the end of the dry season. The mean $\theta_c$ values for these extremes ranged between 0.548 and 0.488 cm$^3$ cm$^{-3}$, respectively, and the magnitude of this interval was slightly influenced by irrigation treatment. Thus, RDI showed the highest seasonal $\theta_c$ differences (0.546–0.478 cm$^3$ cm$^{-3}$) followed by CDI (0.548–0.488 cm$^3$ cm$^{-3}$) and C trees (0.550–0.497 cm$^3$ cm$^{-3}$).

A deeper analysis of the annual changes in moisture storage revealed decreasing trends in $\theta_c$ for all irrigation treatments during summertime which were sharper for the deficit treatments (especially in RDI). In contrast, similar patterns were found in winter, spring and fall. Thus, from 28 June (DOY 179) to 12 September (DOY 255) the mean $\theta_c$ values were 0.523, 0.506 and 0.498 cm$^3$ cm$^{-3}$ for C, CDI and RDI, respectively, whereas, in the rest of the season they averaged 0.524, 0.521 and 0.519 cm$^3$ cm$^{-3}$.

On the other hand, sap flow trends were in good agreement with those of $\theta_c$, with differences among treatments concentrated during the irrigation season when control trees maintained high values of sap flow whereas those of deficit treatments showed a clear decline which was sharper during midsummer. The similarities between $\theta_c$ and sap flow patterns...
are illustrated in relative terms in Figure 5. Thus, from 28 June (DOY 179) to 12 September (DOY 255) CDI and RDI, the mean sap flow values were 0.46 and 0.33 times lower in relation to C, respectively. Finally, the short and steep recovering trends found for RDI and CDI from 17 August (DOY 229) were associated with a rainfall event of 47 mm.

Discussion

Lab experiments

The trends in wood water stored calculated by VSH-CHP methodology did not match with the actual values of $\theta$. The main difference between these trends occurred as a consequence of an unexpected stabilization in $\theta$ values (Figure 1). In fact,
the $\theta_c - \theta_g$ trends (Figure 2) draw an analogy with the curvilinear relationships obtained by TDR between the apparent dielectric constant and the $\theta_c$ found in both our lab experiments (Figure 3) and the literature (Constantz and Murphy 1990, Wullschleger et al. 1996, Irvine and Grace 1997). Thus, both the $\theta_c$ and the apparent dielectric constant show little changes when the actual moisture contents are low. This may have been the result of heterogeneities in the drying process after losing most of the free water. Under these circumstances (fiber saturation point), the more strongly bound water could be leaving the outer portions of the wooden blocks faster than the inner parts (where the sensor measurements were made). However, both the outer- and the innermost thermocouple junctions of the probes (not taken into consideration in further analysis to avoid heterogeneities in the heat applied) revealed identical $\theta_c$ patterns to those of the intermediate depths (data not shown), suggesting that the drying process was spatially homogeneous throughout the volume of the blocks and, therefore, rejecting that hypothesis. An alternative explanation could be that the stabilization in $\theta_c$ values might be due to changes in the local conditions of contact between wood tissue and CHP probes as wood shrinks with water withdrawal. Air may have replaced the air in the minuscule gaps between CHP probes and wood after the depletion of free water. Such a scenario would reduce the amount of heat reaching the thermocouple junctions resulting in a decrease in the maximum temperature difference between the down- and the upstream probes ($T_D - T_U$) and in an overestimation of $\rho c$ and $\theta_c$ according to Eq. (7). As a final remark, some small cracks originated during the drying processes indicating that changes in wood volume had taken place which may also be playing a role in the stabilization of $\theta_c$. Anyway, more research efforts are required on the subject if the causes of this phenomenon are to be clarified.

The stabilization in $\theta_c$, values resulted in an underestimation of the actual drop in moisture content (given by $\theta_g$) during the second part of the drying tests. In contrast, the VSH-CHP methodology was able to track accurately relative variations in $\theta_g$ during the first hours of the trials which was supported by the similarity between $\theta_c$ and $\theta_g$ slopes in Figure 1, by the parallelisms with the 1/1 line in Figure 2 and by the results of the linear regression analysis. Moreover, the slopes of the regression lines were close to 1 (Table 2), indicating that a decrease in the actual water content results in a reduction in $\theta_c$ of the same magnitude. As mentioned in the previous section, the regression analysis included only those data with $\theta_g > 75\%$ of the initial value to avoid the stabilization in $\theta_c$. This range of $\theta_g$ variation (i.e., 25%) should cover the seasonal changes of $\theta$ in most living trees. Standing olive trees, for example, present a seasonal range of $\theta$ from 0.45 to 0.39 cm$^3$ cm$^{-3}$ (i.e., 13.3%) according to Nadler and Tyree (2008). As a consequence, it can be concluded that the VSH-CHP methodology might be useful to monitor relative $\theta$ changes in living trees although its validity remains uncertain for those species and conditions presenting a seasonal range of $\theta > 25\%$.

On the other hand, the variability found in the regression coefficients in Table 2 was not found to be influenced by either tree species, trial conditions (summarized in Table 1) or basic density (Table 2). In other words, even samples under the same experimental conditions presented differences in the slope and intercept of the linear regression between $\theta_c$ and $\theta_g$. Differences in the heat applied by each sensor ($H$ in Eq. (6)) were analyzed as a possible cause for those results. For this purpose, the electrical power absorbed by each probe was individually determined by precisely measuring the voltage and current passing through them during the firing of a heat pulse. Although these measurements revealed some differences among probes (standard deviation of 0.11 W around a mean power of 4.7 W at 23°C) their magnitude was insufficient to explain all the variability found in laboratory experiments. Small deviations with regard to the theoretical position of the thermocouple junctions may also be a source of sensor variability, although the sensors were installed using a steel drill-bit guide to ensure parallelism. Again, differences in the interface conditions between wood and sensors may be playing a major role as a source of variability in $\theta_c - \theta_g$ relationships, but more research efforts are required to address that point. Consequently, calibration is almost impossible, as it would require a different equation to convert the $\theta_c$ estimates in actual values of $\theta$ for each scenario of sensor–wood. Hence, the feasibility to obtain absolute estimates of $\theta$ by the VSH-CHP methodology is questionable.

**Influence of anisotropy on VSH-CHP methodology**

All the calculations of $\theta$ presented in this study were made assuming isotropic conditions. Such an assumption is unrealistic as thermal conductivity is higher along than across the grain ($K_{ax} > K_{ag}$) (Maku 1954, Steinhagen 1977). The origin of anisotropy lies in the histology of wood or, in other words, in the size, shape and orientation of the fibers, vessels and other cells that compose wood (Maku 1954). As a result, the degree of anisotropy should differ between species, individuals or even between stem portions of the same tree.

Considering anisotropy introduces several implications for the VSH-CHP method. First of all, the coefficient $k$ in Eq. (6) is unknown and should present values $>1$. Consequently, the methodology is theoretically unable to determine precisely the actual $\theta$, and omitting anisotropy results in an underestimation of $\rho c$ and $\theta_c$. Secondly, the degree of anisotropy depends on the moisture content with $k$ increasing when wood dries (Siau 1971). Both issues should affect the feasibility of VSH-CHP to monitor the exact magnitude of changes in $\theta$.

In an attempt to assess the impact of anisotropy on the results of our lab experiments the model of Siau (1971) with the corrected equations proposed by Vandegehuchte and
Steppe (2012c) was applied to estimate the values of $k$ from $\theta_g$ data corresponding to the extremes considered in the linear regression analysis for each sample. These $k$ values were employed to deduce corrected wood water contents ($\theta_c$). As expected, the results of these rough calculations (summarized in Table 3) show that $\theta_c$ values are consistently higher than those of $\theta_g$ and $\theta_k$. Conversely, variations in $\theta_k$ in all samples are slightly higher than those of $\theta_g$ and $\theta_k$ (on average 0.15 versus 0.12 and 0.12 cm$^3$ cm$^{-3}$, respectively). Accordingly, if anisotropy is taken into account, the VSH-CHP method fails to accurately estimate the change in $\theta_g$ but still can afford useful information in the relative monitoring of water content variations to some extent. On the other hand, Table 3 shows minimal differences in $k$ between samples of the same species and experimental conditions indicating that the sensor variability found in regression analysis (Table 2) is not a consequence of assuming $k = 1$ in $\theta_k$ calculations. As a final remark, conclusions arising from the above analysis should be taken with caution, as it is founded on indirect estimations of $k$. Therefore, it is obvious that anisotropy adds some uncertainty about the reliability of VSH-CHP, which deserves further research.

### Field experiments

Field experiments showed the occurrence of substantial seasonal changes in $\theta_k$ for all monitored trees. Annual variations in $\theta_k$ revealed a typical pattern for all irrigation treatments, with maximum values at the end of spring followed by a progressive decline during the summer and a latter recovery associated with the end of the dry season and the occurrence of autumn rainfalls (Figure 4; Iniesta et al. 2009). This pattern bears a close resemblance to those previously reported in the literature and determined by TDR under similar climatic conditions (Constantz and Murphy 1990, Hernández-Santana et al. 2008). The first found an annual percentage change in $\theta$ ranging from 15 to 70% depending on species whereas the latter obtained from 10 to 17% of $\theta$ seasonal variation for three consecutive years in melojo oak trees (*Quercus pyrenaica* Willd.). In addition, Nadler and Tyree (2008) reported the annual $\theta$ variation for different species including olive, which ranged from 0.06 cm$^3$ cm$^{-3}$ with a maximum value of 0.45 cm$^3$ cm$^{-3}$ (i.e., a 13.33% of percentage change). Similarly, our results showed an average annual $\theta_k$ variation of 0.060 cm$^3$ cm$^{-3}$ with a maximum average value of 0.548 cm$^3$ cm$^{-3}$ (i.e., a 9.5% of percentage change). The good agreement found in the magnitude of the annual change in $\theta$ is very encouraging as it indicates that the VSH-CHP methodology can be successfully employed in order to monitor $\theta$ seasonal variations in living trees. On the contrary, the aforementioned absolute values of $\theta_c$ gave higher results than those reported by Nadler and Tyree (2008), stressing again that the ability of the VSH-CHP methodology to provide actual values of $\theta$ is uncertain at our actual stage of understanding.

Large differences were found in $\theta_c$ between irrigation treatments throughout the dry season which were in good agreement with tree sap flow patterns. During the summer, $\theta_c$ showed a sharper decline in CDI and RDI than in C trees due to water shortening (Iniesta et al. 2009), RDI being the treatment which reached the lowest values and shows the widest $\theta_c$ seasonal range (Figures 4 and 5). For the same period, sap flow followed a similar trend, with significant differences between C and deficit treatments and RDI being the one reaching the lowest values (Figure 5). In addition, Iniesta et al. (2009) conducted some
midday leaf water potential (Ψ) measurements for the same olive trees in 2006. Despite the scarce available data (only two measures in summer), differences between irrigation treatments can be clearly detected again, RDI showing the lowest Ψ values followed by CDI and C. Finally, the three irrigation treatments showed no significant differences in both θc, Ψ and sap flow values either before or after the irrigation season (Figure 4, Iniesta et al. 2009). Both the decline in sap flow, Ψ and θc patterns during the dry season indicate differences in water status between irrigation treatments which were in good agreement with the irrigation applied and thus with water availability. All this body of evidence indicates that θc could be employed as a long-term water status indicator as has previously suggested by other authors (Nadler et al. 2003, 2006, Hernández-Santana et al. 2008).

Conclusions

The present study shows a new alternative to track θ trends in living trees by using the CHP technique. Our lab and field experiments prove that the VSH-CHP method can successfully monitor relative changes in water stored in a given sampling depth. The technique is rapid, easy to automate, provides simultaneous information about sap velocity and tree transpiration and, like TDR, is not too harmful for the tree. Nevertheless, it does not obtain actual values of θc, calibration is not apparently possible and its feasibility is uncertain for trees with high seasonal changes in θ which deserves further research. In addition, the methodology assumes that wood acts as an isotropic medium which is unrealistic and may slightly affect the accuracy of the technique. On the other hand, this work has presented new evidence on the validity of θ as a long-term water stress indicator and has stressed that the CHP technique is a valuable tool to obtain information about the water status of trees.

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Conflict of interest

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