METHODS PAPER

Measuring the Diurnal Variation of Root Conductance in Olive Trees Using Microtensiometers and Sap Flow Sensors

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Abstract

Background and aims Understanding the variation of root hydraulic conductance (L_p) is critical for the simulation of the soil–plant-atmosphere continuum (SPAC), but its monitoring remains challenging. In this study, we introduce a new non-destructive method for characterizing L_p dynamics in woody species through the combination of simultaneous determinations of sap flow and xylem water potential. Recent studies indicate that modern microtensiometers provide robust estimates of xylem water potential, but it is unknown whether they allow tracking rapid changes in water potential without significant time lags, which may have implications for the proposed methodology.

Methods The impulse response of microtensiometers was measured in the lab, developing a procedure for correcting sensor data by deconvolution. Then, microtensiometers and compensation heat pulse sensors were used to evaluate the variations in L_p in two well-watered olive trees during the summer of 2022 in Cordoba, Spain.

Results Correcting microtensiometer outputs was critical to analyze our field data as strong stomatal oscillations occurred, with microtensiometers damping xylem water potential variations. By contrast, our results suggest that correction procedures may not be required for many practical applications like irrigation scheduling. The daytime values of L_p were close to those obtained in previous studies, while nighttime values were extremely low. Therefore, a proportionality between L_p and sap flow rate was observed, which

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agrees with previous studies, although it does not prove a causal relationship.

Conclusions The methods proposed here could be applied to studying the temporal dynamics of root hydraulic conductance in other tree species.

Keywords *Olea europaea* L. · Root resistance · Sap flow · SPAC · Xylem water potential

Introduction

Models of the soil–plant-atmosphere continuum (SPAC) are a basic tool for understanding the responses of plants to water supply. Sophisticated models (e.g. García-Tejera et al. 2017a) allow the simulation of stomatal conductance, photosynthesis, transpiration and leaf water potential in response to aerial and soil environmental factors. However, the problem is especially complex in the case of fruit trees with drip irrigation, as water application is usually concentrated in a small fraction of the soil volume, which in turn, favors localized root growth (Fernández et al. 1991).

The general approach for simulating the SPAC requires detailed knowledge of the resistance network to water transport from the soil to the leaves (De Swaef et al. 2022). That includes first the soil resistance, then the radial resistance of roots to water uptake (RR), and finally the resistance of xylem vessels, which is usually much smaller than the other two (Frensch and Steudle 1989). Under well-watered conditions, RR is the major resistance of the catena. Although RR varies in response to changes in the root environment, most SPAC models still use fixed values for this parameter (García-Tejera et al. 2017a).

Root hydraulic conductivity (L_p, m s^{-1} MPa^{-1}) defines the capacity of a root to transport water across a water potential gradient between the root surface and the xylem (Eissenstat 1997). L_p varies in space and time by different factors operating at different temporal scales. Long-term (days to months) L_p variations are mainly regulated by suberization processes affecting internal and external root tissues (Gambetta et al. 2017). These occur naturally as part of root aging, but they can be accelerated by other cues. Drought periods have been shown to modify the anatomical structure of the root, enhancing exodermis suberization and compromising cortex

integrity (North and Nobel 1992, 2000). The intensity and duration of the drought period define the magnitude of L_p reductions and the root capacity to recover (North and Nobel 1992; Martre et al. 2001). Besides, soil temperature also affects L_p. Cold induces modifications in membrane structure with a concomitant reduction in L_p (Kuiper 1964, García-Tejera et al. 2016). This has been associated with observations of low midday water potentials during winter in olive trees, despite the soil water content being close to field capacity (López-Bernal et al. 2015).

At short (hourly) time scales, L_p follows a circadian rhythm with a maximum at noon and a minimum during the night (Henzler et al. 1999; Tyree and Zimmermann 2002). The circadian oscillation has been correlated with variations in the expression of aquaporins (Henzler et al. 1999; Vandeleur 2007; Chaumont and Tyerman 2014), a large family of proteins that serve as gated water-conducting channels. Changes in the circadian oscillation of L_p have been related to different environmental triggers. Caldeira et al. (2014) observed that variations in L_p could be initiated on maize plants under continuous light supply previously submitted to water shortage cycles. These authors showed that the amplitude of the variations could be modified depending on the water stress history of the plant. Plants submitted to water stress presented a larger amplitude in L_p than well-irrigated ones. In grapevines, Vandeleur (2007) observed that depression of the L_p circadian rhythm was genotype-dependent. The *Vitis vinifera* L. 'Grenache' cultivar reduced the diurnal amplitude of L_p in response to water shortage periods whilst those of 'Chardonnay' were barely affected.

Changes in L_p at short time scales might provide an adaptive advantage to plants. In silico and in vivo experiments suggest that L_p circadian oscillations under moderately dry soil allow plants to increase root water uptake and improve their water status, maximizing growth (Caldeira et al. 2014; Gambetta et al. 2017). In this regard, water status maintenance can have a double effect. On the one hand, it may contribute to sustaining high stomatal conductance, which would translate into improvements in photosynthesis rates and leaf evaporative cooling under water stress (Tardieu 2012, Monteith and Unsworth 2013, García-Tejera et al. 2023). On the other hand, it could play a role by sustaining leaf growth and avoiding epinasty and leaf rolling, which would increase

radiation interception and biomass accumulation (Monteith 1977). Growth maintenance through L_p variations can confer advantages in most water deficit scenarios, except for severe terminal water deficits (Tardieu 2012).

Studies on the diurnal variation of L_p have shown much higher values during the daytime than during the night. For instance, Parsons and Kramer (1974) found 2–3 times higher L_p during the day in cotton. Some studies have shown that L_p correlates with transpiration rate (Steudle 2000), preventing an excessive drop in plant water potential during the daytime. A better understanding of diurnal changes in L_p would allow a more realistic simulation of the SPAC in terms of water use and carbon uptake. For instance, the growth simulation model OliveCan has incorporated the response to temperature but not the diurnal variation in L_p (López-Bernal et al. 2018a).

Measuring root L_p is still a challenge today. Existing techniques are often based on the pressurization of small, detached root systems and measuring water flow at different pressures (Gambetta et al. 2017). The use of excised root systems under (non-physiologically relevant) positive hydrostatic pressures usually casts doubts regarding the representativeness of these measurements among researchers. On top of that, the destructive nature of these methods and limitations to the size of root systems poses difficulties for characterizing L_p in tree species and its variations at short temporal scales.

In this study, we propose a new non-destructive method for characterizing L_p dynamics in woody species based on the combination of continuous measurements of sap flow and xylem water potential. While several sap flow methods have been available for some decades (Smith and Allen 1996; Vandegehuchte and Steppe 2013), robust non-destructive determinations of water potential have not been possible until recently. In this regard, modern microtensiometers seem to provide reliable outputs according to the latest scientific reports (Blanco and Kalcsits 2021, Pagay 2022, Blanco and Kalcsits 2023). These devices comprise a microelectromechanical pressure sensor and a nanoporous membrane that hydraulically connects the xylem with the sensor (Pagay et al. 2014). Whether this set up allows tracking rapid changes in water potential without significant delays remains a question mark that may have practical implications for some applications of these devices

in the context of plant-water relation studies, including the precise characterization of temporal changes in L_p.

The objectives of this study were a) to assess the response of microtensiometers to changes in water potential, deriving a correction procedure for estimating the real-time water potential from sensor records and b) to combine sap flow and xylem water potential measurements to characterize the diurnal L_p variations in olive trees.

Materials and methods

Lab experiment

This experiment aimed at evaluating the response of microtensiometers (FloraPulse Co., Davis, CA, USA) to changes in water potential in the lab during January-March 2023. To do so, Florapulse sensors were inserted in a small, hollow metal tube (cartridge). First, the sensor cartridge was filled with coupling paste, taking care of avoiding trapping air and reaching exactly the edge of the cartridge (see Fig. 1). The coupling paste -provided by the manufacturer and made of kaolin- was crucial to achieve an optimal hydraulic connection. To avoid spilling or deformation of the paste block during the following steps, the paste in the cartridge was allowed to dry until a reading of -0.6 MPa was observed from the imbedded sensor. Then the cartridge was introduced into a jar, in a confined atmosphere in equilibrium with a saline solution of -1 MPa. In a separate container, several samples of dry cotton of 0.2 g were previously introduced into small plastic tubes open from both sides (27.5 mm length, 9.5 mm diameter, Fig. 1B), and left in a closed atmosphere with the same saline solution to equilibrate to -1.0 MPa for several days. The saline solution was obtained with 15.3 g L^{-1} of KCl in distilled water. The temperature in the laboratory was always between 19 and 23ºC.

When the water potential in the sensor was stable, i.e. when its variation was less than 0.01 MPa h^{-1}, a plastic tube with the already equilibrated cotton was attached to the sensor (Fig. 1B) and placed again in the -1.0 MPa atmosphere until the sensor reading proved that equilibrium was reached at -1.0 MPa. Then the tip of the plastic tube was immersed in pure water for 5 s to soak the
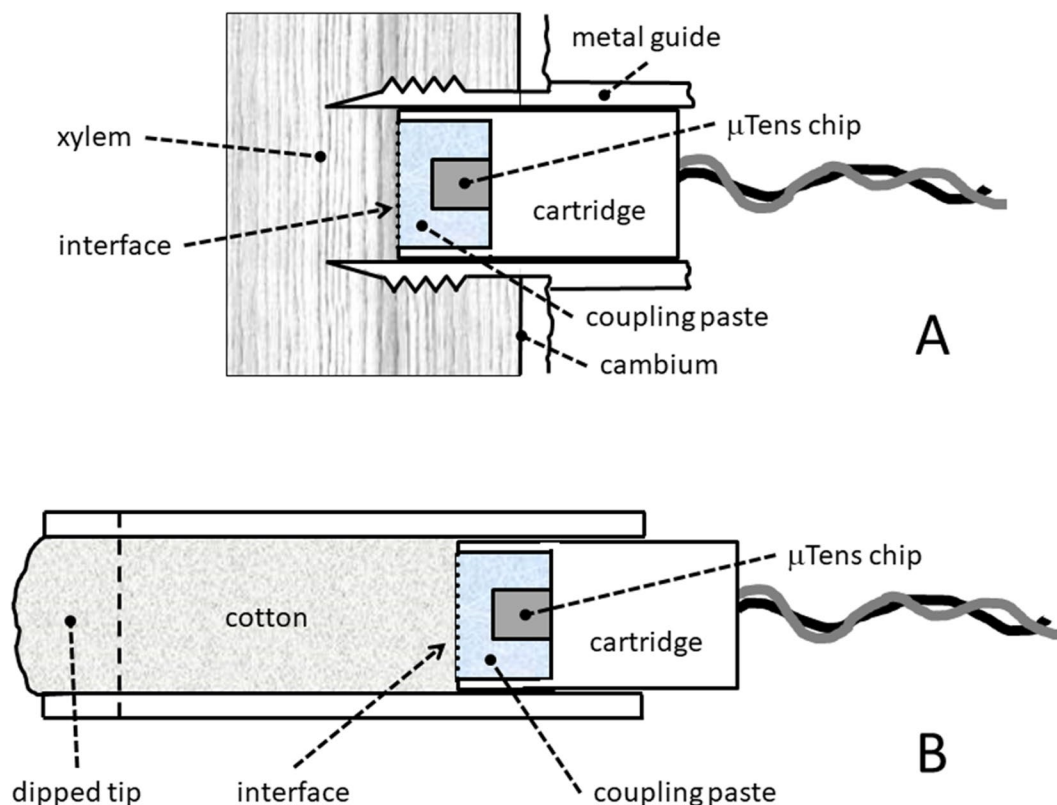
Fig. 1 Installation setup in a standing tree (A) and laboratory setup for the determination of the impulse response (B). The installation in the lab mimics carefully an ideal instant change in water potential of the xylem in a standing tree installation

cotton and then the cartridge with the attached tube was placed in a confined atmosphere, now with pure water, so an abrupt change in water potential from -1.0 to 0 MPa was produced at the interface between the mating paste and the cotton. The time course of the sensor signal (with 1-min intervals) after this moment was used to calculate the impulse response sensu control theory, which is required to correct the sensor measurements (see below). The impulse response was obtained as the time derivative of the step response.

The experiment was repeated twice with a change from -1.0 to 0 MPa and twice from -2.0 to 0 MPa (by changing the equilibrium salt concentration), so we were able to produce 4 impulse responses for the sensor. The average impulse response was analyzed with Matlab (Version R2017A, MathWorks Inc, Natick, MA, USA).

Pot experiment

The experiment was performed at the 'Instituto de Agricultura Sostenible' (Cordoba, Spain, 37.8ºN, 4.8ºW) during spring–summer 2022. Two 9-year-old olive trees growing outdoors in 50-L pots (trunk diameter 4.5 cm, tree height 0.4 m) were selected in January 2022, and pruned to a similar leaf area. The trees were of different cultivars ('Frantoio' and 'Empeltre'). Hereafter, each individual is called after the cultivar name. Pots were filled with a mixture of sand (50%), silt (25%) and peat (25%). Trees were irrigated and fertilized from then on to ensure maximum leaf growth. Irrigation was applied with four 2-L/h drippers on the soil surface. From May 1 to the end of July, the pots were irrigated twice a day. Then, from 27 July to 17 September, the irrigation interval was changed to every 2 h during the daytime, to

keep the pots close to field capacity and thus with soil water potential close to zero. A total of nine 13-min (1.7 L per pot) irrigations were applied each day during the daytime in that period.

On March 15, two soil water potential sensors per tree (model Theros 21; Meter Group, Pullman, WA, USA) were installed at 30 cm depth in each pot. Four 30-cm long soil water content reflectometers (model CS616, Campbell Scientific Inc., Logan, UT, USA) were also installed, two in each pot. Each tree was equipped with a Compensated Heat Pulse (CPH) sensor built at the IAS (Testi and Villalobos 2009) to measure sap flow and a microtensiometer (FloraPulse Co., Davis, CA, USA) inserted in the xylem close to the collar to measure its water potential. The installation of Florapulse sensors followed carefully all the indications of the manufacturer. Sap flow probes were used to estimate the sap flux density at 5- and 15-mm depth from the cambium. Wounding errors were corrected (Green et al 2003) assuming a 2.6 mm wound diameter (Fernández et al. 2006). Sap flow was estimated by integrating sap flux densities first along the trunk radius and then around the azimuth angle. All the xylem was considered functional given the small trunk diameter (4.5 cm). All sensors were controlled by two dataloggers (model CR1000, Campbell Scientific Inc., Logan, UT, USA) which read and stored the measurements at an interval of 300 s.

Additional measurements of stem water potential were performed using a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Values of early morning and midday Ψ were recorded weekly on sunny days at 6:45 and 11:30 UTC in the two trees. Three leaves per tree were selected for the measurements. Leaves were bagged using aluminum foil for a minimum of 30 min prior to the determinations to stop transpiration and allow for equilibration of water potentials between the leaf and shoot (stem).

The leaf area of the two trees was measured on September 25 by counting all the leaves and sampling a group of 100 leaves from each tree, which area was measured using a scanner. The measured plant leaf area was 4.22 and 3.89 m^2 for 'Frantoio' and 'Empeltre' trees, respectively.

In late July, root length density was determined by core sampling in intact pots, but in different specimens, as the damage to the root system would have influenced the experiment. Four cores were obtained from the pots of other 4 trees of the same cultivars (2 per cultivar) potted at the same time, selected by similar canopy sizes and grown under the same management conditions. The cores were collected from the midpoint between the tree trunk and the pot border with a 40-mm diameter auger at a depth of 15 cm. Subsequent treatment and analysis of the sample followed the steps reported by García-Tejera et al. (2017b). The root length density was calculated as the ratio between the total root length of the sampled roots and the core volume. Only roots with less than 2 mm diameter were considered in the calculations in order to exclude non-absorbing structural roots. The root length densities resulted 80,103 and 64,003 m m^{-3} for 'Frantoio' and 'Empeltre', respectively.

Additional weather data were obtained from an automatic weather station located 500 m from the trees.

Correction of xylem water potential measurements

The observed microtensiometer signal $y(t)$ is the result of variations in actual water potential in the xylem $x(t)$ which are distorted by the dynamics of the sensor, as manifested in its response to water potential, on the one hand, and on the transport of water within the paste that connects the sensor and the xylem, on the other. Pagay et al. (2014), characterized the first, reporting a time constant of around 20 min for a similar sensor without the paste covering.

We may write the combined effect as:

$$h(t) * x(t) = y(t) \tag{1}$$

where $h(t)$ is the impulse response and the * operator stands for convolution. Now if $h(t)$ is known (from the Lab experiment), we may obtain the true xylem water potential $x(t)$ by deconvolution of the observed signal $y(t)$. As the impulse response was measured with 1-min interval but the interval in the field measurements was 5 min, we first did a spline interpolation and converted the 5-min field data to 1 min. Then a Wiener deconvolution was applied using the average impulse response (Fig. 1) and produced deconvoluted signals for the water potential in the two trees, taking only the values at the times of the original field signal (every 5 min). The deconvolution was calculated with Numpy in Python. The noise parameter for Wiener was 0.1. It was tuned to produce a signal as clean as

the original one (as measured by the local coefficient of variation of noise).

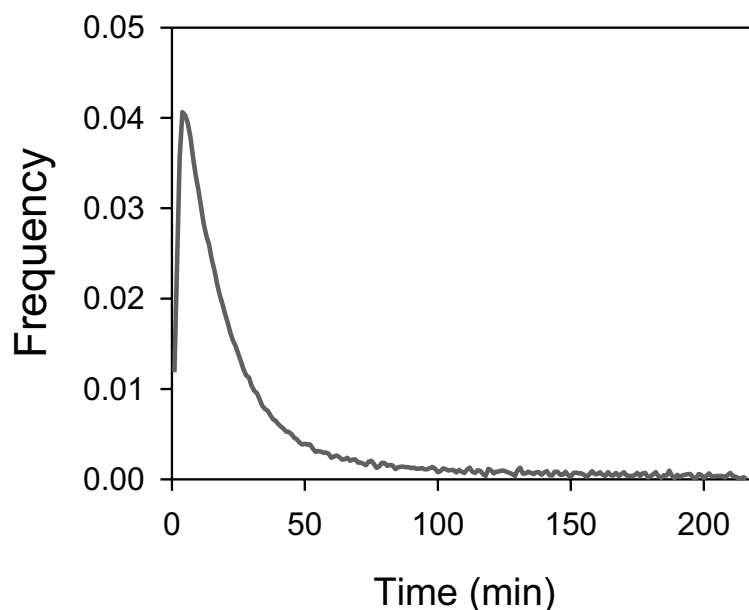## Root hydraulic conductance

As the soil water potential was close to zero during the measurement period (July 27 – September 17), L_p was calculated as the absolute value of the ratio of sap flow and xylem water potential, after deconvolution of the microtensiometer signal. In doing so, we assumed that the xylem root resistance was negligible.

Results

Impulse response

The impulse responses derived from the four experiments were very similar, as the standard deviations of the frequency were always below 0.002. The average impulse response showed a maximum at 4 min and 50 and 90% of the cumulative response was reached at 17 and 79 min, respectively (Fig. 2). The fit with Matlab manifested that the response can be characterized by two exponentials with time constants of 17 and 1.7 min, which indicates a second order model for the system.

Environmental conditions

The experiment was performed under typical summer conditions at Cordoba, with high temperatures, low wind and dry sunny days (Table 1). Between July 27 and September 17 small rainfall events occurred on 13/08 (4.6 mm), 12/09 (0.3 mm), 13/09 (5.6) and 15/09 (3.9 mm). The average reference evapotranspiration (ET_0) was 6.0 mm, which is close to the mean at that time of the year. Soil water potential was close to zero during the whole experiment, with averages of -0.4 and -0.1 kPa in the 'Frantoio' and 'Empeltre' trees, respectively. The average soil water content was 0.50 m^3 m^{-3} in 'Frantoio' and 0.46 m^3 m^{-3} in

Table 1 Average climatic conditions during the experiment (27/07–17/09/2022) measured at an automatic weather station at IAS-CSIC. Reference evapotranspiration was calculated with the Penman–Monteith FAO method

Variable	Units	Mean value
Minimum air temperature	°C	19.6
Maximum air temperature	°C	36.7
Mean air temperature	°C	28.2
Solar radiation	MJ m^{-2} day^{-1}	23.7
Mean relative humidity	%	44.0
Vapor pressure deficit	kPa	2.5
Wind speed	m s^{-1}	1.8
Reference evapotranspiration	mm day^{-1}	6.0

Fig. 2 Impulse response of Florapulse microtensiometers. The frequency refers to the fraction of the response occurring at that particular time. The curve is the average of the results of four experiments performed between 19 and 22°C in a lab at IAS, Cordoba (Spain). The impulse response was derived from changes from -1 to 0 MPa (two experiments) and from -2 to 0 MPa (two experiments)

'Empeltre', with almost no variation during the whole period.

Transpiration

Daily transpiration was slightly higher for 'Frantoio' (average 11.4 L day^{-1}) than for 'Empeltre' (10.3 L day^{-1}) (Fig. 3) and decreased from July to September in parallel to the reduction in ET_0. Maximum transpiration was 15.3 L day^{-1} ('Frantoio', August 2) and the minimum occurred on September 15, a rainy day with transpiration of 5.5 L day^{-1} for both trees.

Daily curves of transpiration rates usually showed large oscillations after noon with amplitudes typically being much higher for the 'Empeltre' tree. An example is shown in Fig. 4 for August 4, a sunny day with maximum and minimum temperatures of 41 and 21.2 ℃, respectively. The oscillations had average

Fig. 3 Time course of transpiration and reference evapotranspiration (ET_0) and transpiration for the two instrumented trees, which were of two different cultivars ('Frantoio' and 'Empeltre')
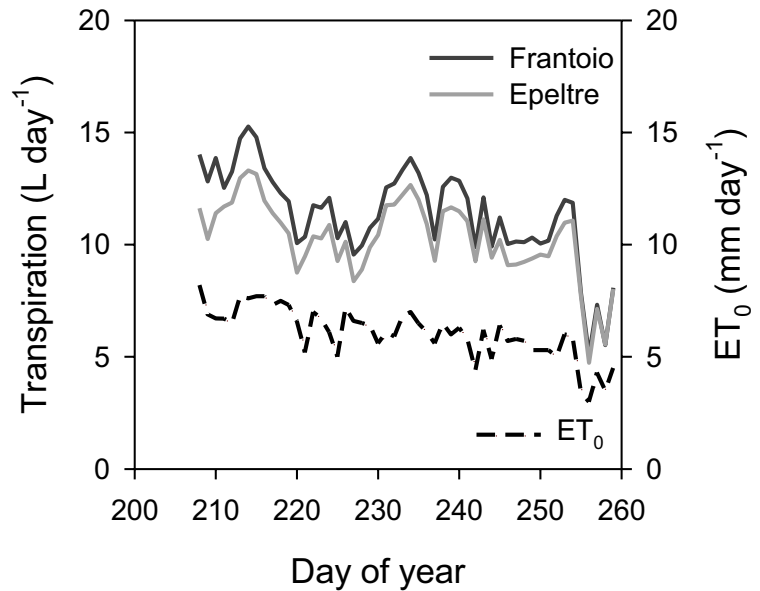
Fig. 4 Time course of transpiration rate on 4 August 2022 for the two instrumented trees, which were of two different cultivars ('Frantoio' and 'Empeltre')
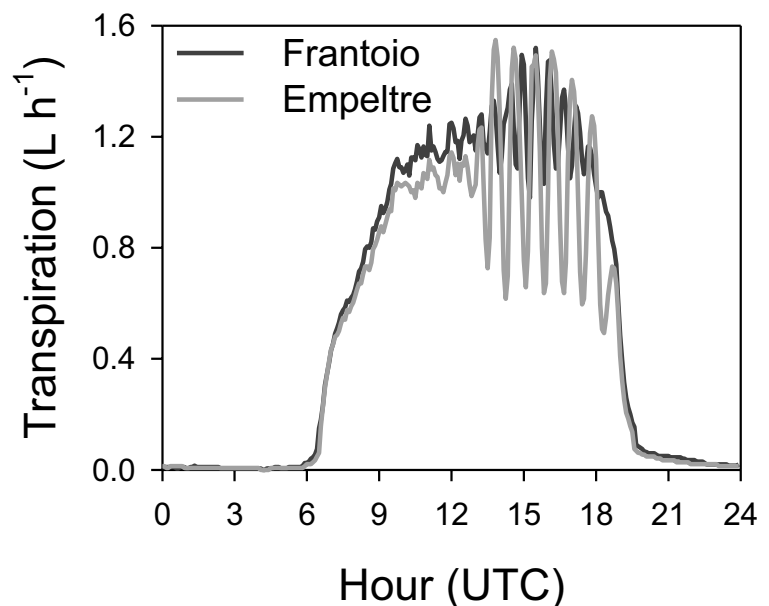
intervals of 32 and 43 min for 'Frantoio' and 'Empeltre', respectively, with oscillations in the range of 1.15–1.45 and 0.7–1.5 L h^{-1}. Data for all other days are provided in the Supplementary Material.

Water potential

The microtensiometers smoothed and delayed the actual variation in xylem water potential obtained after post-processing. An example is shown in Fig. 5 for August 4. In 'Frantoio' the actual (corrected) water potential showed an oscillation that almost did not appear in the sensor (uncorrected) data. In 'Empeltre', oscillations of 0.8 MPa were reduced to less than 0.3 MPa. Maximum xylem water potential occurred around sunrise and then decreased until noon with oscillations occurring during most of the afternoon. Sensor data lagged around 30 min behind the convoluted (corrected) signal. The Supplementary Material includes data for all the other days.

The effect of environmental conditions on the daily curve of xylem water potential is evident in Fig. 6, which includes a sunny (6 August) and a cloudy day with 5.6 mm of rainfall (13 September). In the latter,

oscillations did not occur (except a little flickering in 'Empeltre' registered one hour after solar noon) and the drop in water potential went from a maximum of -0.4 MPa to a minimum of -0.8 ('Empeltre') or -1 MPa ('Frantoio'). On the other hand, the sunny day started at -0.3 ('Empeltre') or -0.45 MPa ('Frantoio') and fell to -1.6 ('Empeltre') or -1.2 ('Frantoio') in the afternoon. Oscillations were evident for 'Empeltre' but almost absent in 'Frantoio'.

Predawn and midday xylem water potentials are shown in Fig. 7 for the whole experiment. Predawn values were 0.15 MPa higher for 'Empeltre' during August, but they became almost equal to those found in 'Frantoio' in September (-0.45 MPa), the latter showing little variation during the experiment. Midday values were more variable, from -1.47 MPa ('Empeltre', August 3) to -0.59 MPa ('Empeltre', September 13). The difference in predawn-midday water potential decreased during the experiment, from 0.8–1 to 0.3–0.4 MPa.

Microtensiometer records of xylem water potential correlated rather well with measurements of stem water potential performed with an Scholander pressure chamber, with the former generally showing

Fig. 5 Time course of corrected and uncorrected xylem water potential on 4 August 2022 for the two instrumented trees, which were of two different cultivars (**A** 'Frantoio' and **B** 'Empeltre')
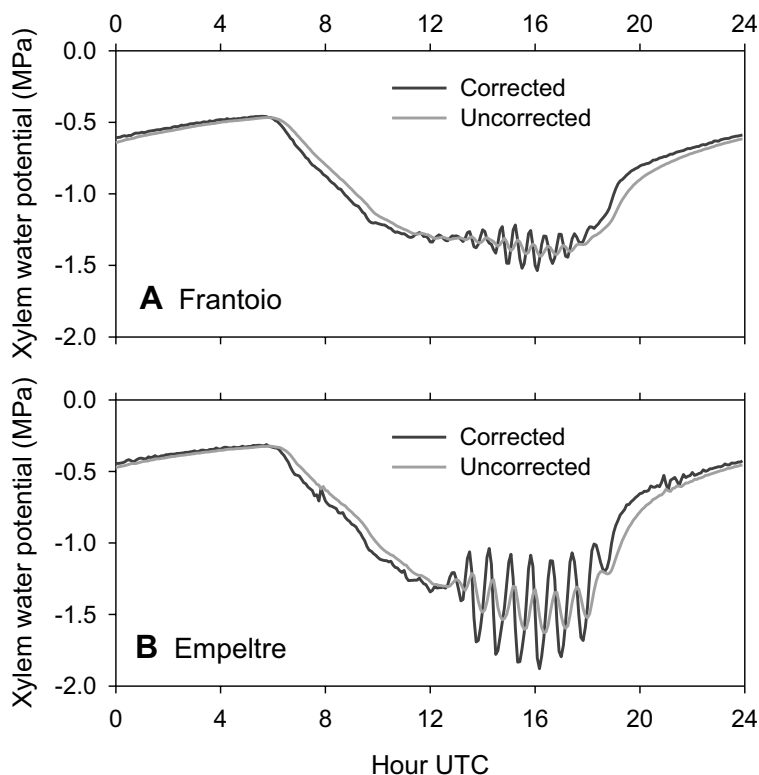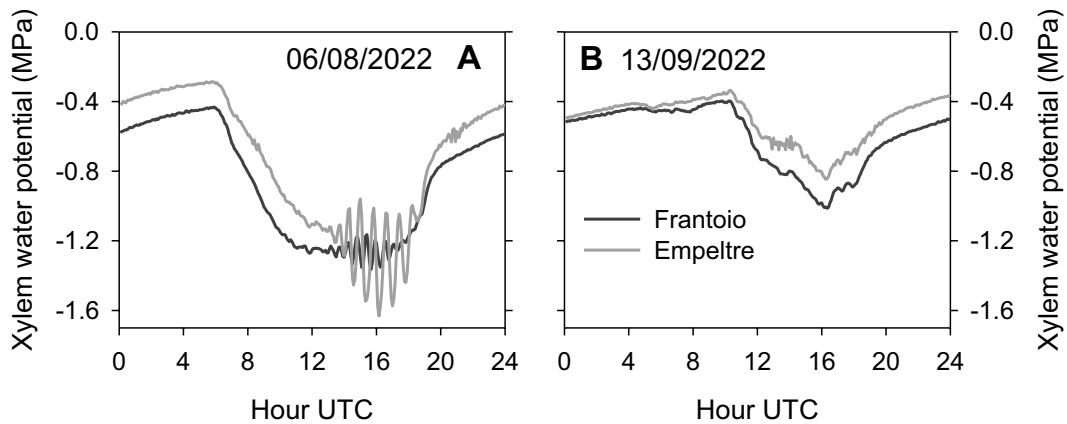
Fig. 6 Time course of corrected xylem water potential on (A) 6/8/2022 and (B) 13/09/2022 for the two instrumented trees, which were of two different cultivars ('Frantoio' and 'Empeltre')

Fig. 7 Time course of predawn and average midday (solar noon ± 4 h) xylem water potential for the two instrumented trees, which were of two different cultivars ('Frantoio' and 'Empeltre'). Plotted data were deduced after correcting microtensiometer outputs
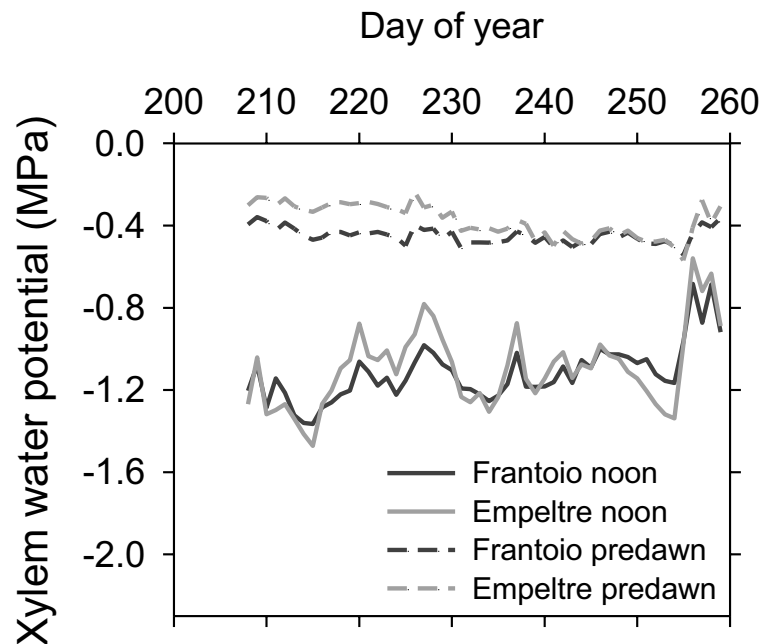
higher values (Fig. 8). A linear regression analysis for the combined dataset of the two trees revealed a slope of 0.99, an intercept of 0.26 MPa and a determination coefficient of 0.88. The intercept is congruent with the existence of a water potential gradient between the trunk and the stem.

Root hydraulic conductance

The root hydraulic conductance was much higher during the daytime. A typical example is shown in

Fig. 9 for August 4. For 'Empeltre', the conductance was maximum (1 L h^{-1} MPa^{-1}) three hours after sunrise and then it decreased with wide oscillations until a fast drop 30 min before sunset. For 'Frantoio' the conductance was fairly constant between 0.8 and 1 L h^{-1} MPa^{-1} during most of the daytime. The fall at the end of the day was equal in both trees and showed a strong decrease in slope at the time when solar radiation became zero (19:45 UTC).

Average midday conductance was almost constant (0.8–1.0 L h^{-1} MPa^{-1}) throughout the experiment

Fig. 8 Relationship between xylem water potential (collected with the Florapulse microtensiometers) and stem water potential (measured with a pressure chamber) for 'Frantoio' and 'Empeltre'). Dotted and solid lines represent 1:1 and regression lines, respectively
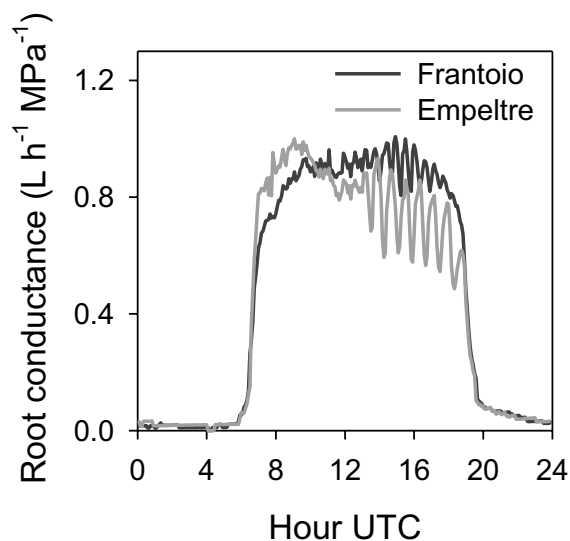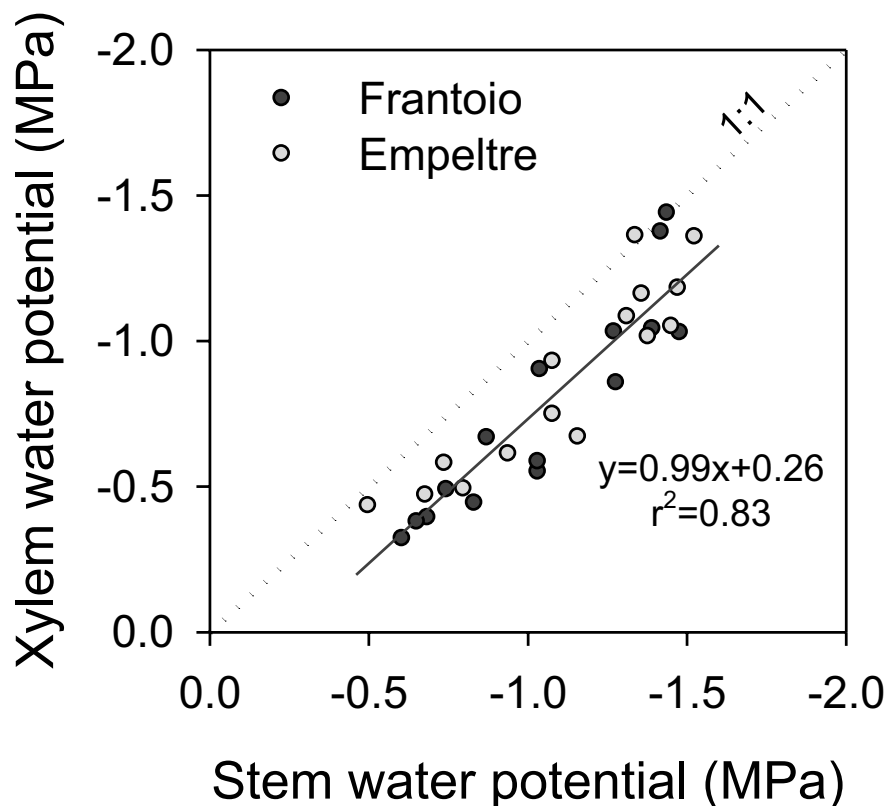

Fig. 9 Time course of root hydraulic conductance on 4 August 2022 for the two instrumented trees, which were of two different cultivars ('Frantoio' and 'Empeltre')
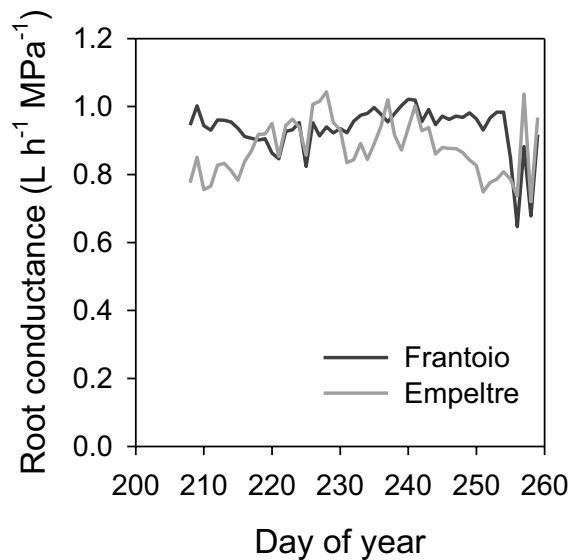
Fig. 10 Time course of midday (noon±4 h) root hydraulic conductance for the two instrumented trees, which were of two different cultivars ('Frantoio' and 'Empeltre')

except for the final days (Fig. 10), with wider variation for the 'Empeltre' tree. The corresponding specific root resistance (considering the estimated root area in the pots) was 15.6•10^3 and 13.3•10^3 MPa s m^2 kg^{-1} for 'Frantoio' and 'Empeltre', respectively, during the central daytime period (Table 2). Nocturnal values were 20–30 times higher.

Discussion

The microtensiometer used in our study was installed in the tree trunk, surrounded by a porous medium that connects it with the xylem (Fig. 1A). Therefore, we needed to characterize its dynamic response to changes in water potential occurring at the boundary of the porous medium, which complicated the experiments in the lab. On the one hand, it took a long time (> 5 h) to equilibrate the sensors to atmospheres of either -1.0 or -2.0 MPa. Changing to zero water potential was also difficult as wetting directly the porous medium caused crumbling which forced us to wet a cotton swab instead, to force nil potential.

The dynamic response of the sensors to steep changes in water potential could not be characterized as a first-order type, which would have shown an exponential decay in the frequency. The response shown in Fig. 2 is more characteristic of a second-order system, characterized by the sum of two exponential functions. Fitting the response to a second order system yielded time constants of 17 and

Table 2 Values of specific root resistance (R_p) in olive trees in the present and previous studies. We show average values for midday (from 8 to 1600 h UTC) and night (2000 to 2400 h) in our study. OGT 2016: García-Tejera et al. (2016), LB 2020: López-Bernal et al. (2020)

Study	Time	Cultivar	R_p (MPa s m^2 kg$^{-1} \times 10^3$)
OGT 2016		Picual	60
LB 2020		Picual	14.4
		Frantoio	18.6
		Arbequina	36.7
This study	Midday	Frantoio	15.6
	Midday	Empeltre	13.3
	Night	Frantoio	472
	Night	Empeltre	276

1.7 min, for the two components of $h(t)$ (Eq. 1). The first would be the dynamical contribution of the microtensiometer while the second would account for the transport of water within the paste surrounding the sensor. Alternatively, the impulse response may indicate a fractional order behavior that would deserve further research. Nevertheless, the deconvolution has been satisfactory in correcting the water potential data in such a complicated experimental situation as the one presented here with strong oscillations in sap flow. In any case, our results suggest that in many practical applications in which a lag of 30 min is unimportant, microtensiometers' outputs may not require correction. That would be the case if only the maximum (predawn) or minimum (midday) xylem water potential is used for irrigation scheduling purposes. On the contrary, research studies in which a detailed time course of water potential is sought would require data post-processing. Another case where a correction may be necessary is under partial cloud cover, which generates oscillations in transpiration, and thus, in water potential. In any case, our measured impulse response may be used to correct by deconvolution any microtensiometer data set.

Oscillations in olive trees are not new in literature. Oscillations in the leaf water potential of young trees with an interval of 25 min and relative amplitudes up to 0.42 were observed by López-Bernal et al. (2018b). This is similar to the values found here for the 'Empeltre' tree, but greater than those observed for 'Frantoio'. Interestingly, oscillations have only been observed in young potted trees but not in mature olive trees in the field so far (López-Bernal et al. 2018b). Further research is needed to disentangle the mechanisms behind this phenomenon.

Our results consistently showed a circadian fluctuation in L_p, with the highest values occurring during the central hours of the day. Knipfer and Fricke (2011) found a ratio of day/night L_p of 9 which is much lower than the values we have found (20–30). However, our nighttime L_p estimates must be taken with caution, since conditions leading to high xylem water potential and low sap flow may increase the error for conductance estimation. This is due to the fact that L_p was calculated from the ratio of sap flow and the absolute value of xylem water potential in this study. In any case, the dynamics of L_p throughout the day observed in this study agree with the results by Meyer and Ritchie (1980), who found that root

conductance was proportional to the transpiration rate. They explained such a response because a larger water flow entering the root favors a larger proportion to follow the apoplastic pathway (higher conductivity) as opposed to the symplastic pathway (lower conductivity, modulated by aquaporins). Alternatively, circadian oscillations in L_p have been attributed to amplified expression of aquaporins during the daytime (Henzler et al. 1999). However, the occurrence of short-term oscillations in our estimates of L_p with similar intervals as those of sap flow supports the idea of a short-term control associated with the sap flow rate. To our knowledge, this is the first attempt at quantifying root conductance in trees showing stomatal oscillations.

In our experiment we kept the soil close to field capacity to maintain the soil water potential close to zero. Soil water deficit partially blocks the apoplastic pathway (Steudle 2000), so L_p decreases and becomes more dependent on aquaporin expression. In this regard, future experiments exploring the daily dynamics of L_p under moderate water deficits may give some light into the mechanisms involved in the regulation of root conductance variations.

Under the conditions of the present study, our results show additional evidence of the existence of an overlooked second regulation mechanism beyond that of stomatal control, which have important implications for the simulation of the SPAC. This control of root conductance seems to be feedforward while stomatal conductance is regulated by a feedback based on leaf water potential. The combination of the two control loops may be helpful in explaining the occurrence of oscillations of stomatal conductance and transpiration in trees and plants in general. In any case, further research is required to elucidate the mechanisms behind the dynamic variation in root conductance at daily or sub-daily time scales.

The trees in our experiment were grown in 50-L pots. Such a confined system may affect root growth patterns, root signaling and water relations as compared to the typical conditions experimented by trees growing in the field (Tardieu 1994). In our experimental trees, the ratio of leaf area to soil volume was 84 and 78 m^2 m^{-3} for 'Frantoio' and 'Empeltre', respectively. Actual values in commercial orchards may be as low as 2–3 m^2 m^{-3} (García-Tejera et al. 2018), but root length density is lower in orchards too.

Our estimates of specific root resistance in the daytime (Table 2) are close to those found by López-Bernal et al. (2020) for 'Frantoio' plants, but much lower than those measured previously by García-Tejera et al. (2016) in cultivar 'Picual'. Our measurements were performed continuously on olive trees growing outdoors while previous data for this species were collected for rooted cuttings, by pressurizing detached root systems with Scholander bombs (García-Tejera et al 2016; López-Bernal et al. 2020). The differences in plant material and the artificiality associated to conventional determinations make the comparisons between present and past studies of limited significance. Besides, we must acknowledge that the reduced sample size in the present study (i.e. only two trees were monitored) sets additional limits to the comparisons. In any case, the proposed methodology seems promising and opens the door for monitoring the dynamics of L_p in field trees, which is impossible with conventional techniques based on pressurizing small, detached root systems. Further research should exploit the methods used in this study to investigate the regulation of L_p in different tree species.

Conclusions

The impulse response of the FloraPulse microtensiometers measured in the lab may be characterized as a second order type. The actual xylem water potential may be obtained by deconvolution of the sensor data using the impulse response. No data correction may be required for many practical applications (e.g. irrigation scheduling) under predominantly sunny weather. The combination of Compensation Heat Pulse sap flow sensors and microtensiometers allowed us to estimate the temporal variations of root hydraulic conductance in standing olive trees. Our calculations revealed similar midday values of root hydraulic conductance as compared with previous data obtained with pressurized root systems. This experimental set up can thus be replicated in further studies to study the dynamics of this variable and its drivers in woody species. In agreement with previous reports, we observed that root hydraulic conductance was proportional to transpiration, but further research is required to identify the actual regulator of these changes.

Declarations

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