Postprint of: New Phytologist (2019) doi:10.1111/nph.16177

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Declining root water transport drives stomatal closure in olive under moderate water stress

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Summary

• Efficient water transport from soil to leaves sustains stomatal opening and steady-state photosynthesis. The above-ground portion of this pathway is well-described, yet the roots and their connection with the soil are still poorly understood due to technical limitations.

• Here we used a novel rehydration technique to investigate changes in the hydraulic pathway between roots and soil and within the plant body as individual olive plants were subjected to a range of water stresses.

• Whole root hydraulic resistance (including the radial pathway from xylem to the soil-root interface) constituted 81% of the whole-plant resistance in unstressed plants, increasing to >95% under a moderate level of water stress. The decline in this whole root hydraulic conductance occurred in parallel with stomatal closure and contributed significantly to the reduction in canopy conductance according to a hydraulic model.

• Our results demonstrate that losses in root hydraulic conductance, mainly due to a disconnection from the soil during moderate water stress in olive plants, are profound and sufficient to induce stomatal closure before cavitation occurs. Future studies will determine whether this core regulatory role of root hydraulics exists more generally among diverse plant species.

Key words: hydraulics, olive, rehydration kinetics, root hydraulic conductance, shoot hydraulic conductance, soil-root interface, water stress.

Introduction

Dynamic regulation of water use in plants has an enormous global impact since more than half of the global precipitation per year is returned to the atmosphere as transpiration (Jackson *et al.*, 2000; Hetherington & Woodward, 2003). Understanding plant water use requires a detailed knowledge of the plant vascular system because stomatal valves controlling transpiration are directly or indirectly regulated by changes in leaf water potential, produced by either drying soil or friction (hydraulic resistance) in the water transport system (Buckley, 2005). Roots form the critical gateway between a plant and its water source in the soil and constitute a major component of whole plant hydraulic resistance (Frensch & Steudle, 1989; Steudle & Peterson, 1998; Personne *et al.*, 2003; McCormack *et al.*, 2015), but the hydraulics of these below-ground structures are poorly

understood due to a lack of methodologies that can quantify soil, xylem and whole-root resistances in intact plants (Sperry *et al.*, 1998).

Daytime stomatal closure is a major limiter of plant productivity, and has most recently been modelled as a mechanism to avoid dehydration damage to water transport tissues (Sperry *et al.*, 2016; Deans *et al.*, 2017; Martin-StPaul *et al.*, 2017). In terms of xylem damage by air-embolism, there is clear evidence that stomatal closure during dehydration always precedes the onset of cavitation, thereby substantially delaying irreversible xylem damage (Brodribb & McAdam, 2017; Hochberg *et al.*, 2017; Martin-StPaul *et al.*, 2017; Choat *et al.*, 2018). This stomatal behaviour should allow plant hydraulic conductance (K) to remain static under all but the most extreme/damaging water deficit conditions, meaning that stomatal regulation would be entirely independent of changes in K. However, other studies propose that during dehydration K in leaves can decline without cavitation, influencing stomatal closure due to changes in the outside-xylem hydraulic path between the end of the xylem and the sites of evaporation in the leaf (Scoffoni *et al.*, 2017). This suggestion is important because it implies that leaf K is dynamic at a diurnal scale, with the potential to affect stomatal behaviour and impact upon the dynamics of daily assimilation (Brodribb, 2009; Scoffoni *et al.*, 2016). Roots also possess significant outside-xylem components, including well defined symplastic barriers in their hydraulic pathway, and are known to be sensitive to changes in cell membrane permeability produced by proteins such as aquaporins (Steudle & Peterson, 1998; Maurel *et al.*, 2010). Thus, it is important to establish whether root K is stable or dynamic during water stress, prior to xylem cavitation.

Despite the likelihood that roots are major resistors in the hydraulic system, their water transport physiology remains poorly understood. Studies of root tips, behind which water uptake preferentially occurs (Gambetta *et al.*, 2013; Zarebanadkouki *et al.*, 2013), suggest that root hydraulic conductance may be dynamic and dependent upon the hydraulic flux (Frensch & Steudle, 1989). Seminal works have also reported that roots, preferentially the cortex of the root, may shrink as soil dries (Passioura, 1988) developing a loss of hydraulic continuity within the soil-root interface even during diurnal drops of plant water potential (Huck *et al.*, 1970; Faiz & Weatherley, 1982). More recently, Cuneo *et al.* (2016) showed evidence that root cortical tissue may be highly sensitive to damage even under very mild soil water deficit. This raises the possibility that dynamic changes in root conductivity may

impact whole plant K sufficiently to impact stomatal regulation of water use and forest productivity (McCormack *et al.*, 2015).

A knowledge gap in understanding the whole-root hydraulic resistance and its integration within the soil-root system is clearly recognized (Lobet *et al.*, 2014; Poyatos *et al.*, 2018; Passot *et al.*, 2019), yet techniques for measuring the hydraulic resistance of the root-soil pathway are limited. Here we introduce a new method for measuring the components of root hydraulic resistance using rehydration kinetics to partition resistances in the shoot and root of olive plants subjected to water stress. Olive is recognized as a drought-resistant species, with highly cavitation-resistant roots (Rodriguez-Dominguez *et al.*, 2018). Thus, our aim was to determine whether the hydraulic conductance of shoots and roots changes when exposed to water deficits sufficient to close stomata (Diaz-Espejo *et al.*, 2018), but not enough to cause cavitation in the xylem of both stem and root tissues (Torres-Ruiz *et al.*, 2017; Rodriguez-Dominguez *et al.*, 2018).

Materials and Methods

Overview

We present here a brief overview of the experiment conducted (see Fig. S1 for details). We monitored daily plant water status, as stem water potential (ψ_{stem}), and canopy conductance (g_c) in four potted two-year-old olive plants subjected to three levels of moderate water stress, defined here as ψ_{stem} values prior to cavitation thresholds for olive (Torres-Ruiz *et al.*, 2017; Rodriguez-Dominguez *et al.*, 2018). Rehydration techniques were used to measure whole root hydraulic conductance by either hydrating plants externally from the soil (K_{root+i}), or by hydrating roots internally via the xylem by cutting the root system off and attaching it to tubing (see below). This allowed differentiation of internal hydraulic pathways that included cortical pathways (K_{root}) from those external including the soil-root interface (K_i). On the same plants we measured shoot hydraulic conductance (K_{shoot}), that included stem and leaf hydraulic pathways. From these measurements, total hydraulic conductance (K), or total hydraulic resistance (R), of the plants were calculated as:

$$(1) \qquad \frac{1}{K} = \frac{1}{K_{\mathrm{root+i}}} + \frac{1}{K_{\mathrm{shoot}}} = \frac{1}{K_{\mathrm{root}}} + \frac{1}{K_{\mathrm{i}}} + \frac{1}{K_{\mathrm{shoot}}},$$

or, as resistances,

$$(2) \qquad R = R_{\mathrm{root+i}} + R_{\mathrm{shoot}} = R_{\mathrm{root}} + R_{\mathrm{i}} + R_{\mathrm{shoot}}.$$

We also calculated total root K (internal + interface) from pressure drops in the plant during steady-state transpiration at midday (averages from 1100 to 1300 local time) as:

$$(3) \qquad K_{\mathrm{root+i}} = \frac{E}{\psi_{\mathrm{soil}} - \psi_{\mathrm{stem-md}}},$$

where E is midday canopy transpiration rate, $\psi_{\mathrm{stem-md}}$ is midday ψ_{stem}, and ψ_{soil} is the average between predawn ψ_{stem} (averaged from 0100 to 0400 local time) the day before and the day after the one under consideration. This calculated $K_{\mathrm{root+i}}$ did not contain leaf water potential but ψ_{stem} monitored by a stem psychrometer placed at ca. 30 cm from the base of the plant, so plant K could not be derived from Eqn 3.

In addition, we derived maximum diffusive conductance (g_{max}) using a hydraulic model and assessed the effects of observed variation in $K_{\mathrm{root+i}}$ on g_{max} over the water stress levels to which the olive plants were subjected (see derivation of the models in Methods S1 of the Supporting Information File).

Plant material and glasshouse conditions

Ungrafted one-year-old olive seedlings (*Olea europaea* L. var. *arbequina*) were grown in glasshouse facilities at the University of Tasmania from March 2017 to January 2018. Following acquisition from a local nursery, seedlings were replanted to 2-L pots using a 3 : 1 mixture of coarse river sand and potting mix. Roots were carefully washed off prior to replanting. The experiment was performed from February to August 2018. Glasshouse conditions were 25°C : 15°C (day : night temperatures), photoperiod of 14 h with sodium vapour lamps continuously illuminating the plants from 0600 to 2000 local time and relative humidity matched the ambient (~ 40%). Pots were irrigated daily until the beginning of the experiment. Plants ranged from 120 to 150 cm in height.

Daily plant water status and canopy conductance

Daily plant water status (as ψ_{stem}) was monitored every 20 min using a stem psychrometer (PSY1, ICT International, Armidale, NSW, Australia) installed on the main stem of the plant, according to Rodriguez-Dominguez *et al.* (2018), at ca. 30 cm from its base and insulated to minimize the effect of temperature changes on the psychrometric reading. Even so, data from periods of the day with rapid changes in temperature (e.g. day-night transitions) were not considered (Fig. S1 Step 1).

Canopy transpiration (E, mmol m^{-2} s^{-1}) was monitored every 30 min with a balance (XS6002S, 6100 g × 0.01 g, Mettler-Toledo GmbH, Greifensee, Switzerland), normalizing by the corresponding leaf area (LA) measured with a scanner and ImageJ (Schindelin *et al.*, 2012) at the end of the measurements. The pot was previously double-bagged and sealed to prevent evaporation from the soil. A temperature and humidity probe (HMP45AC, Vaisala Inc., Helsinki, Finland) connected to a datalogger (CR850, Campbell Scientific, Logan, UT, USA) monitored the atmospheric vapour pressure deficit (D) (Buck, 1981) close to the targeted plant. Fans were continuously operating in the glasshouse, producing conditions of high aerodynamic conductance which, together with the small size of olive leaves, allowed calculation of g_c as $g_c = E/D$ (Jarvis & McNaughton, 1986). Moreover, glasshouse conditions were designed to produce midday D values > 1.5 kPa (Fig. S1 Step 1), ensuring that maximum stomatal opening was always constrained by hydraulics (Diaz-Espejo *et al.*, 2018), i.e. conditions that produced enough transpiration to drive leaf water potential into ranges where stomata are responsive to leaf water potential. We derived midday ψ_{stem} and midday g_c by averaging diurnal values from 1100 and 1300 local time. After plant rehydration (see below), g_c that did not reach predrought values were not considered.

Water stress levels and plant rehydration through the soil

Plants were dehydrated by withholding water under glasshouse conditions to three water stress levels (ψ_{stem} ca. -1, -2 and -4 MPa) and rehydrated through the soil to determine K_{root+i}. When each plant reached the initial targeted ψ_{stem} (-1 MPa) it was rehydrated rapidly by flooding the soil and bagging the leaves until its ψ_{stem} was completely recovered (Fig. S1 Step 2), taking on average 100 min (Fig. S2). After complete rehydration, plastic bags were removed, and the plant was placed again on the balance and remained unwatered until it

reached the second targeted ψ_{stem} (-2 MPa) at which point it was rehydrated again, and the procedure repeated finally at -4 MPa. This enabled the determination of K_{root+i} by rehydration at the three target water potentials.

Rehydration kinetics of ψ_{stem} and K_{root+i}

Hydraulic conductance was measured using rehydration kinetics, whereby the dynamic changes in the water potential or flow of water into hydrating samples is used to calculate the hydraulic conductance of the sample. The rate of change in the rehydration flow or water potential is determined by the capacitance of the hydrating tissue and the hydraulic conductance (high capacitance and low conductance causing slow kinetics). The ψ_{stem} relaxation kinetics were assessed using the psychrometer outputs monitored every 10 min during rapid rehydration of plants through the soil. Rapid rehydration required water to be immediately in contact with roots, so pots were submerged and flooded such that the porous potting mix became immediately flooded with water (Fig. S1 Step 2). The ability of the psychrometer to capture rapid increases in water potential has been reported (Milliron *et al.*, 2018), but notwithstanding, equilibration of the psychrometer and ψ_{stem} was validated using simultaneous ψ_{stem} and flow recordings during hydrating stem sections internally via the xylem (Fig. S3). An exponential decay function (SigmaPlot, Systat Software Inc., California, USA) was fitted to the first 40 minutes of ψ_{stem} data from whole plants (Fig. S2):

(4) $\psi_{stem} = \mathrm{a}e^{(-bt)}$,

where a and b are the fitted parameters and *t* is time since rehydration (min). $\mathrm{b} = \dfrac{K}{C}$ (Brodribb & Holbrook, 2003), where K is K_{root+i} and C is the plant hydraulic capacitance. By analysing the first 40 minutes of rehydration we captured the fastest phase of the rehydration kinetic, characterizing the resistance that would be most influential in regulating dynamic transpiration and water potential in plants subjected to typical diurnal fluctuations in D, and avoiding uncertainties about what slower compartments represent. This K_{root+i} was normalized by plant LA at the time of rehydration (mmol m^{-2} s^{-1} MPa^{-1}), and included the pathway from the soil-root interface to the main stem.

K_{root} and K_{shoot} determined by the rehydration technique

Following full rehydration, plants were again allowed to dehydrate under glasshouse conditions. After each plant reached a desired water stress level (see below), it was moved to the laboratory and allowed to equilibrate overnight. The rehydration kinetic technique (Brodribb & Cochard, 2009), with some modifications for roots, was used to measure both K_{root} and K_{shoot} (Fig. S1 Step 3 to 5). In both cases, the targeted portion of the plant was attached to tubing connected to a beaker with degassed pure water placed onto an analytical balance (Newclassic MS204S, 220 g × 0.1 mg, Mettler-Toledo GmbH). Briefly, the plant was de-topped under water, and the root-stem portion immediately connected to a logging balance. The branch, stored to prevent transpiration, was later used for K_{shoot} measurements by re-cutting it under water and connecting it to the same balance set-up. In both cases, flow was recorded every 5 s, corrected by LA and water viscosity, and points from the first minute of rehydration were used to fit an exponential curve, which was extrapolated back to the initial point of excision to determine the maximum initial flow (F_{max}). The root-stem portion was rehydrated until flow approximated zero (approximately 85 min) at which point it was disconnected and used for root capacitance (C_{root}) determinations. The shoot portion was connected until flow decreased by half from its maximum (approximately 100 s), at which point it was disconnected and stored for measuring final water potential (ψ_{fin}) to determine shoot capacitance (C_{shoot}). Both K_{root} and K_{shoot} (mmol m^{-2} s^{-1} MPa^{-1}) were calculated as:

(5) $\qquad K_{root} \text{ or } K_{shoot} = \dfrac{F_{max}}{\Delta\psi},$

where $\Delta\psi$ is the water potential difference between source and the water potential of the sample prior to rehydration ($0 - \psi_{ini}$). ψ_{ini} and ψ_{fin} were verified using two randomly sampled leaves measured with a Scholander chamber. Although four plants were used in total for the experiment, K_{root} and K_{shoot} measurements were destructive. Thus, K_{root} and K_{shoot} were measured in one plant at -0.98 ± 0.08 MPa, one plant at -2.49 ± 0.15 MPa and two plants at -3.86 ± 0.11 and -4.07 ± 0.01 MPa, respectively and considering both the value from the psychrometer and ψ_{ini}.

Plant hydraulic capacitance

C for each plant was calculated as $C = C_{root} + C_{shoot}$. C_{root} was determined by simultaneous measurement of water potential and root mass as roots were allowed to dehydrate from 0 to -5MPa (Fig. S1 Step 6). Roots were carefully washed (Pérez-Harguindeguy *et al.*, 2013) and a psychrometer installed and equilibrated with laboratory temperatures for a minimum of 1 h, while maintaining the roots under water. Then, the roots were carefully dried with absorbent paper towel, covered with aluminium foil and the root-stem clamped on a stand and placed onto a balance (PG5002-S, 5100 g × 0.01 g, Mettler-Toledo GmbH). ψ_{stem} and root mass (g) were recorded every 15 min and C_{root}, normalized by LA (mmol m^{-2} MPa^{-1}), was derived from slopes that considered ψ_{stem} vs root mass data for each range of change in ψ_{stem} during the 40-min rehydration performed for K_{root+i} measurements (Fig. S4).

C_{shoot} (Fig. S1 Step 5) was measured directly during K_{shoot} measurements, according to Blackman & Brodribb (2011), as:

(6) $$C_{shoot} = \frac{\Sigma F}{\psi_{fin} - \psi_{ini}},$$

where ΣF is the sum of the flow of water into the shoot during rehydration from ψ_{ini} to ψ_{fin} normalised by LA supported by the shoot and water viscosity (mmol m^{-2}), determining F_{max} as explained before.

Results

During soil dehydration, the sensitivity of g_c to ψ_{stem} could be described by three phases (Fig. 1a): an insensitive phase between 0 and -1.0 MPa (Fig. 1a, b); a steep decline between -1.0 and -2.5 MPa (Fig. 1a), where diurnal values hardly exceeded 0.01 mol m^{-2} s^{-1} (Fig. 1c); and a shallow decline below -2.5 MPa (Fig. 1a), with stomata virtually closed (Fig. 1d). Whole plant hydraulic conductance followed a similar pattern, with an 74% decrease in K from 0.68 ± 0.13 mmol m^{-2} s^{-1} MPa^{-1} at ψ_{stem} = -0.97 ± 0.07 MPa to 0.17 ± 0.06 mmol m^{-2} s^{-1} MPa^{-1} at ψ_{stem} = -2.39 ± 0.07 MPa (striped dark grey box plots in Fig. 2). No changes in root hydraulic conductance (K_{root}), including xylem and cortical pathways, and shoot hydraulic conductance (K_{shoot}) were observed within this range of water potentials (Fig. 2). Instead, whole root

hydraulic conductance plus soil-root interface (K_{root+i}) appeared to drive this change in total K, decreasing from 0.89 ± 0.23 to 0.19 ± 0.06 mmol m^{-2} s^{-1} MPa^{-1}. A smaller decrease in total K was observed between ψ_{stem} of -2.39 and -4.11 ± 0.17 MPa. The mean total K observed at this minimum water potential was 0.08 ± 0.02 mmol m^{-2} s^{-1} MPa^{-1}, which was similarly due to a very reduced K_{root+i} (91% of maximum K_{root+i}), but with minimal reductions in K_{root} and only a slight decrease in K_{shoot} (19% of maximum K_{shoot}).

To further understand changes in the partitioning of hydraulic limitation within the olive plants, hydraulic resistances (R) were considered (Fig. 3). In all cases, whole root hydraulic resistance plus soil-root interface (R_{root+i}) contributed a much higher percentage of the total plant R than shoot hydraulic resistance (R_{shoot}), constituting already 81% in unstressed plants (at ψ_{stem} = -0.97 ± 0.07 MPa) and increasing to 98% in plants at -4.11 ± 0.17 MPa. Root, including xylem and cortical resistances (R_{root}), and resistance of the soil-root interface (R_i) were similar in unstressed plants (50% R_{root} and 31% R_i), but at lower water potentials there was a very large increase in R_{root+i} due to R_i. At the minimum ψ_{stem}, R_i constituted >90% of total plant resistance while both R_{root} and R_{shoot} (which included the leaves) contributed only 7% of the total plant R.

Both K_{root+i} measured with the rehydration technique and K_{root+i} calculated from water potential drops in the plant during midday canopy transpiration (Eqn 3) agreed across the range of water potentials measured (Fig. 4a). These changes in root K including soil-root interface were sufficient to prevent maximum stomatal opening because, under the evaporative demand in the glasshouse (D > 1.5 kPa), the very low K_{root+i} was sufficient to drive the water potential into the stomatal sensitive range described above (Fig. 4b).

Discussion

Our results identify dynamic root hydraulic conductance during water stress as an important driver of early stomatal closure in olive plants. Our novel hydraulic method allowed us to observe sharp declines in whole root K, including the soil-root interface, occurring over the same range of water potentials as stomatal closure, far above those required to trigger xylem cavitation (Torres-Ruiz *et al.*, 2017; Rodriguez-Dominguez *et al.*, 2018). Our findings indicate that the most important changes in the hydraulic system of these olive plants

during moderate water stress did not occur in leaves or stems, and did not involve cavitation. Instead, we find that root hydraulic conductance in olive constitutes a primary regulator of plant water use during early water deficit.

Using the rehydration kinetic principles to determine whole plant K

The rehydration kinetic technique has been long established as a tool for measuring leaf hydraulic conductance (Brodribb & Holbrook, 2003; Brodribb & Cochard, 2009; Blackman & Brodribb, 2011) and has been successfully used recently for measuring root hydraulic conductance (Creek *et al.*, 2018). Here, we used a novel combination of rehydration techniques to determine the conductance of hydraulic pathways in shoots and roots, while partitioning the root resistance into internal, including xylem and non-xylem components, and external, including soil-root interface, pathways. Other methods, e.g. the HPFM method (Tyree *et al.*, 1995; Nardini & Tyree, 1999), have been used to measure root and shoot hydraulic conductance. However, the ability to measure whole root K under tension, and to capture the hydraulic component of the soil-root interface, gives our methodology new insight. This allowed us to determine the contribution of each hydraulic component to the whole plant resistance, identifying the important and dynamic role of the hydraulic pathways from the soil to the root as a primary factor reducing whole plant K within the sensitive water potential range of stomatal function. Furthermore, the finding that K_{root} did not change within a range of ψ_{stem} down to <-4 MPa also confirmed previous results indicating a high resistance to cavitation in the coarse roots of olive (Rodriguez-Dominguez *et al.*, 2018).

Regarding the above-ground part of the plant, reductions in outside-xylem hydraulic conductance in leaves have been suggested as contributing to dynamic leaf K and, hence, as a potential cause of stomatal closure (Scoffoni *et al.*, 2017; Scoffoni & Sack, 2017). This has been proposed as playing an important role to decouple and protect the leaf xylem under high atmospheric demands. Although we did not find evidence of changes in shoot (leaf and stem) hydraulic conductance over the water potential range of stomatal closure in olive, we did find evidence of large changes in K_{root+i}. The magnitude of these changes was clearly sufficient to prevent stomatal opening at a moderate vapour pressure deficit of 1.5 kPa due to the low leaf water potential produced by transpiration when plant K was very low.

Furthermore, these changes in K_{root+i} were measured by a rehydration technique that was independent of evaporative flux, thereby avoiding the co-dependence that is innate when reporting parallel responses of K, measured by the evaporative flux, and g_s (measured from evaporation) to leaf water potential. We believe that demonstrating this important connection by using a method independent of transpiration is a major step forward, as well as providing a new perspective on root function.

An explanation for the discrepancies between our findings regarding unchanged K_{shoot} and those that report a decline in leaf hydraulic conductance at low levels of water stress in olive under field conditions (Torres-Ruiz *et al.*, 2015; Hernandez-Santana *et al.*, 2016) still needs to be elucidated. For instance, combinations of different hydraulic approaches, including leaf anatomical analyses, to quantify the outside-xylem hydraulic conductance of the leaves and to localize where the main constrains to water flow occur, may help to make more progress on this question.

Significance of root K in terms of plant water stress performance

Midday stomatal opening in unstressed olive plants was already quite limited by the dominant resistance imposed by their roots (81% of total R). Being potted plants may have contributed to the unexpectedly high resistance component found in the roots, a point that deserves more attention since plants growing in soils where their roots can explore a greater volume may have less constrained K_{root+i}. However, the pot limitation is unlikely to have modified the dynamic responses observed. Glasshouse conditions were controlled to maintain midday $D > 1.5$ kPa contributing to the low values of g_c recorded for our potted olive plants. Nevertheless, plants were able to sustain low g_c after water was withheld delaying the moment in which ψ_{stem} started to steeply decrease by up to ca. 10 days (e.g. Fig. S1 Step 1). The importance of this low stomatal conductance on plant water use has been previously pointed out as an advantageous strategy for droughted plants to conserve water (Duursma *et al.*, 2018). In mildly stressed olives ($\psi_{stem} \approx -2.5$ MPa) we found K_{root+i} fell to very low levels and, due to its high contribution to whole-plant hydraulic resistance, it was insufficient to support midday $g_c > 0.01$ mol m^{-2} s^{-1} (according to a hydraulic model) thereby constraining the maximum rate of water loss from leaves. The dynamism of root K observed here agrees with the dynamic nature of below-ground hydraulics reported recently in

droughted pine forests (Poyatos *et al.*, 2018) where sensitivity of K_{root+i} was considered as an adaptive strategy to conserve water.

Our results may point to changes in the function of fine roots that could drive the decline in whole plant hydraulic system during water stress (Cuneo *et al.*, 2016). Indeed, we found that shrinkage of coarse olive roots occurred at similar ranges of water potential found to cause declining whole root *K* (Fig. S5). This is likely to cause physical damage to fine roots and root hairs by moving the root away from the soil (Passioura, 1988; Lo Gullo *et al.*, 1998). Together with this damage, deposition of suberin in exodermis and endodermis cells of roots has also been reported in wild olive seedlings under severe water stress (Lo Gullo *et al.*, 1998). Whether these changes we observed in root conductance provide a selective advantage in terms of accelerating water conservation by forcing stomatal closure, or by isolating plants from drying soil, will require further work. Investigating these questions applying the new techniques described here will provide opportunities to explore variation in root behaviour in diverse plant species growing in a variety of soils.

Author contributions

C.M.R-D. and T.J.B. conceived and designed the experiment. C.M.R-D. collected and analysed the data with help from T.J.B., and both C.M.R-D. and T.J.B. wrote the manuscript.

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Supporting information

Methods S1. Hydraulic model of maximum diffusive conductance, g_{max}

Fig. S1. Step-by-step representation of the experiment conducted, and supplementary methodology applied.

Fig. S2. Stem water potential (ψ_{stem}) relaxation kinetics obtained from stem psychrometer outputs while rehydrating each olive plant through the soil at different water stress levels.

Fig. S3. Simultaneous recordings of stem water potential (ψ_{stem}) measured with a stem psychrometer and flow of water (measured with a balance) entering through the stem (via xylem), and comparison between ψ_{stem} relaxation kinetics rehydrating the root-stem portion of the plant internally (via xylem) or externally (through soil).

Fig. S4. Simultaneous measurements of root mass and stem water potential (ψ_{stem}) of the root-stem portion of each olive plant to calculate the root hydraulic capacitance (C_{root}).

Fig. S5. Shrinkage observed in olive coarse roots derived by analysing the width of the roots over a range of stem water potential (ψ_{stem}) down to ca. -4.5 MPa from collections of images used to determine root optical vulnerability curves (Rodriguez-Dominguez *et al.*, 2018) and root hydraulic conductance plus soil-root interface (K_{root+i}) measured in this study.

Figure legends

Figure 1. Midday canopy conductance (g_c) and midday stem water potential (ψ_{stem}) derived from daily monitored olive plants. (a) Each blue circle corresponds to midday averages (1100 to 1300 local time) of g_c and ψ_{stem} from four olive plants subjected to drought cycles. g_c values were calculated from canopy transpiration measured with a balance and vapor pressure deficit data monitored with a meteorological probe. ψ_{stem} was measured with a stem psychrometer installed on the main stem of the plant. Black points with the letters (b), (c) and (d) are three examples for which diurnal variations are presented at the lower panels. Thus, panels (b), (c) and (d) show diurnal values (every 30 min) of g_c (blue dots) and ψ_{stem} (orange dots) at three levels of water stress for which midday average values are presented above each panel. Blue and orange lines are smoothed data using a 2-point FFT Filter routine for g_c and ψ_{stem} values, respectively. Grey bands indicate night time.

Figure 2. Hydraulic conductance (K) measured using rehydration techniques on different components of four olive plants dehydrated to three target stem water potentials (ψ_{stem}). K including soil-root interface, cortex and root xylem (K_{root+i}) was determined from the ψ_{stem} relaxation kinetics resulted from rehydrating the plants through the soil. K from the root (K_{root}), including xylem and cortical pathways, and K from the shoots (K_{shoot}), including stem and leaf, were measured rehydrating internally via xylem. Triangles up and down correspond to measurements done in two individuals, respectively, and diamonds are average values from more than one individual. Error bars indicate standard deviations. Striped dark grey box plots show the 10th, 25th, 75th, 90th percentiles, median (grey line) and mean (black line) of whole-plant K determined from Eqn 1 at three levels of ψ_{stem}. Note the different y-axis used for K from each component or for whole-plant K.

Figure 3. Hydraulic resistance (R) at the three levels of stem water potential (ψ_{stem}) that olive plants were measured. (a) R for each component and whole-plant R (striped dark grey bars) determined from Eqn 2. Error bars denote standard errors. (b) Percent contribution of each component to total R for the three levels of water stress using the same code of

colours for each component than in (a). Percentages values for R_{root+i} and R_{shoot} are presented.

Figure 4. (a) Relationship between calculated or measured hydraulic conductance (K) at the root level, including the soil-root interface (K_{root+i}) or not (K_{root}), and stem water potential (ψ_{stem}) measured on four olive plants subjected to multiple stages of water stress. Each grey point represents the midday average of calculated K_{root+i} (from 1100 to 1300 local time) derived from evaporation (Eqn 3). Black circles with standard errors are pooled averages of calculated K_{root+i} and ψ_{stem} values every 0.3 MPa until ψ_{stem} = -1 MPa, and every 0.5 MPa from -1 to -4.5 MPa. Values for measured K_{root+i} and K_{root} are the same than those in Fig. 2, but in this case error bars are standard errors. (b) Modelled maximum canopy diffusive conductance (g_{max}) compared to measured midday canopy conductance (g_c). Measured g_c (blue dots) are the same values as the ones presented in Fig. 1a. The first hydraulic model (black circles and dashed black lines) shows changes in g_{max} due solely to variation in K_{root+i} (white diamonds in panel a). The second hydraulic model (blue circles and blue dashed lines) shows the same limitation of g_{max}, but with the combined effect of declining soil water potential (ψ_{soil}) on g_{max}. Details on the derivation of both models are presented in Supporting Information Methods S1.