The contribution of deep root functioning to whole-plant water relations and xylem transport in *Juniperus ashei*.

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ABSTRACT

Daily and seasonal measurements of whole-plant water relations, morphology, and xylem transport in *Juniperus ashei* were taken on the surface and 7 m underground in a cave system in central Texas, USA. Sap flux data indicated that over the growing season 24% of total daily transpiration on average came from below 7 m. During periods of high water availability sap flux density was similar in deep roots and the main stem, but during an extended drought sap flux density in the deep root was less than in the main stem. Hygrometer measurements of deep root water potential, compared with sap flux density, indicated that hydraulic conductance of deep roots declined during the onset of the drought and increased again following precipitation more than two months later. Consistent with this decrease, deep roots were most vulnerable to xylem cavitation while branches were least vulnerable and shallow roots were intermediate. These differences in vulnerability to cavitation were accompanied by parallel differences in xylem tracheid diameter distribution. Mean tracheid diameter and hydraulically-weighted tracheid diameter were largest in deep roots, intermediate in shallow roots, and smallest in branches. These data demonstrate that deep roots can contribute significantly to total plant water use and suggest that an integrated understanding of whole root system function should incorporate differences in the physiological limits of its component parts.

INTRODUCTION

Total daily transpiration for trees typically ranges from 10 to 200 kg d^{-1} (Wullschleger et al. 1998). Water stored in plant tissues contributes a significant portion of this amount in some species (Holbrook 1995) but transpirational losses must ultimately be replaced by water uptake from the soil. Many empirical and modeling studies have shown that hydraulic conductance (k) and cavitation resistance of stem xylem can limit transpiration (Meinzer and Grantz 1990; Tyree and Sperry 1988). Recent studies have shown that roots often impose an even greater limitation than do shoots (Alder et al. 1996; Sperry et al. 1998). These results emphasize the potential importance of root hydraulic architecture across a range of depths for whole plant gas exchange and drought tolerance. In this paper, we describe direct measurements of the structure and function of *Juniperus ashei* roots from 7 m belowground through the shoot, integrating the contribution of deep roots to whole plant water relations and xylem transport.

The soil depths where plants extract water are determined by biotic and abiotic variables that include system hydrology, water availability at depth, and the distribution and functioning of plant roots. Although the majority of roots are relatively shallow, root distribution varies widely among species and functional types (Jackson et al. 1996); roots of many woody species occur below 5 m while some exceed 60 m (Canadell et al. 1996; Nepstad et al. 1994; Stone and Kalisz 1991). Deep root functioning is difficult to measure because reaching these roots usually requires disrupting the root system and its environment. Non-invasive stable isotope studies have documented patterns of water use in woody plants that range from total dependence upon deep water to substantial water uptake from shallow soil (Dawson 1993; Jackson et al. 1999a; Jackson et al. 1999b; Williams and Ehleringer 2000). Furthermore, studies of hydraulic lift (Caldwell et al. 1998 and references therein) and inverse hydraulic lift (e.g. Sakuratani et al. 1999; Schulze et al. 1998) have demonstrated that deep roots can facilitate water transfer between deep and shallow soil layers. Such data highlight the importance of studying the root system in total, and many questions remain about the functional characteristics of deep and shallow roots. For

differences appeared to be characteristic of deep vs. shallow roots rather than a function of the distance from the root crown.

In addition to the important effects of xylem conduit diameter, k also depends on xylem pressure (Ψ_x) through cavitation (Tyree and Sperry 1989). Cavitation refers to the nucleation of water vaporization under negative pressure resulting in an air filled or "embolized" conduit. Cavitation occurs when Ψ_x is sufficiently negative to overcome the capillary forces of water in the pores of the cell wall (Crombie et al. 1985; Pockman et al. 1995; Sperry and Tyree 1988). The critical Ψ_x required to induce cavitation is determined by the diameter of the largest cell wall pore, typically in inter-conduit pit membranes (Jarbeau et al. 1995). As this diameter decreases, the minimum sustainable Ψ_x without cavitation also decreases. Because flow cannot occur through gas-filled conduits, extensive cavitation reduces or eliminates water transport. The effect of cavitation is described by the relationship between decreasing k and decreasing Ψ_x referred to as a vulnerability curve (Tyree and Sperry 1989).

Available data suggest that shallow roots are often more vulnerable to cavitation than aboveground organs (Hacke and Sauter 1996; Kavanagh et al. 1999; Linton et al. 1998; Sperry and Ikeda 1997). For example, in *Acer grandidentatum* the safety margin from the critical Ψ_x was smaller in roots than in shoots (Alder et al. 1996). Individuals at wet and dry sites showed no difference in stems but greater resistance to cavitation in roots at the dry site, suggesting greater developmental plasticity in the cavitation response of root vs. stem xylem. Thus, the water transport characteristics of roots may be more important in limiting transpiration than the more frequently studied stem xylem. To our knowledge changes in vulnerability to cavitation have not previously been evaluated in roots as a function of depth.

The purpose of this study was to integrate the role of deep roots into whole-plant functioning, including plant water relations and xylem transport. Sap flux measurements seven meters underground and through the main stem were compared for eighteen months in *Juniperus*

Tree identification

Roots were initially identified to species using DNA sequence variation from the ITS region of the genome (Jackson et al. 1999b). The connection between the root in the cave and the corresponding tree on the surface was determined by injecting a 1:1 mix of deuterium oxide (Sigma, St. Louis USA) and distilled water into a small lateral root that branched from the main root in the cave. The lateral root was cut under water 30-40 mm from the point where it joined the main root to allow injection of the label without introducing air emboli. A length of plastic tubing was clamped to the lateral root and filled with the labeled mixture. To force the labeled water into the uninterrupted xylem stream, a pressure of approximately 100 kPa was applied above the solution using compressed nitrogen. Pressure was maintained during daylight hours for three days while twigs and increment cores were collected at intervals from 16 trees on the surface. Stem water was extracted at the Cornell Laboratory for Stable Isotope Analysis (Cornell University, Ithaca USA) using vacuum distillation. The deuterium composition of these samples was then determined using a Europa GEO 20-20 mass spectrometer (PDZ-Europa, Cheshire, UK). The δD of stem water in the measured tree rose 1686‰ between the second and fourth day after labeling began while the δD of stem water for the other trees remained at pre-labeling values (Fig. 1B).

Sap flux

Sap flux was measured from the late summer of 1998 through 1999 in the deep root and the main stem of the tree using 30 mm constant heating probes (Granier 1987). This method measures the temperature difference between an upper heated probe and an unheated reference probe installed 5 cm directly below. Under conditions of no flow, each installation has a characteristic maximum temperature difference (dTM). Sap flux density (J_s) is calculated from continuous measurements of the temperature difference (dT) using an empirical calibration

incorporating dTM (Granier 1987). Prior to the start of our study, we confirmed the empirical calibration by generating known flow rates through excised stem segments of *J. ashei* (data not shown) using methods similar to those used in previous studies (Clearwater et al. 1999; Goulden and Field 1994).

Three probes (Dynamax, Houston USA) were installed in both the main root and the stem. A datalogger (CR10, Campbell Scientific, Logan USA) was used to measure the temperature difference between the probes was measured every minute and averaged values were stored at 15-minute intervals. The daily maximum temperature difference was determined from the stored data for each probe and used to calculate sap flux density. Sap flux density from each probe was multiplied by the cross sectional sapwood area of the root (58 cm^2) or the shoot (200 cm^2) which was determined independently with an increment coring tool. These cores were also used to determine the radial thickness of the sapwood. Where the increment cores indicated that probe length exceeded the thickness of the sapwood, temperature differences from the probe were corrected using the methods of Clearwater et al. (1999) before sap flux density was calculated. The average proportion of the probe in sapwood was 0.75. To compare sap flux density measurements of the deep root and the main stem, we used t-tests for each 15 minute sampling interval (Phillips et al. 1996).

Climate data

For comparison with sap flux and water relations data, photosynthetically active radiation with a quantum sensor (LI-190, LI-Cor, Lincoln USA) and precipitation with a tipping bucket rain gauge (Texas Electronics TR525I, Dallas USA) attached to the same datalogger used for sap flux measurements. Minimum, maximum and average temperature (T) and minimum and maximum relative humidity (RH) were obtained for Camp Mabry, TX (4 km south of the site) from the National Climate Data Center, Asheville NC. Air T in the cave chamber and 1.5 m

above the soil surface aboveground were measured with copper-constantan thermocouples from July to October, 1999.

Water relations

For comparison with sap flux measurements, xylem pressure and water potential measurements were made at several points along the plant flow path. Leaf Ψ was measured at predawn (Ψ_{pd}) and midday (Ψ_{md}) by collecting small twigs and sealing them in a plastic bag with a moist paper towel before determining the balancing pressure with a pressure chamber (PMS, Corvallis USA) within several minutes of collection. Xylem pressure (Ψ_x) of the main stem within 1.5 m of the root crown was determined by wrapping small twigs that branched directly from the main stem in aluminum foil. This treatment, applied the evening before measurement, eliminates flow through each twig, allowing it to remain in equilibrium with the main stem xylem to which it is attached. A small number of twigs were available for this method so these data were used to relate stem Ψ_x as a proportion of the total pressure difference between Ψ_{md} and Ψ_{pd}. Subsequently, this proportion was used to estimate Ψ_x at the root-shoot interface based on Ψ_{pd} and Ψ_{md} when appropriately placed twigs were not available.

To measure Ψ of the deep root, stem hygrometers (Dixon and Tyree 1984, Plant Water Status Instruments, Guelph Canada) were sealed to the xylem of the root with silicon grease and held in place with electrical tape and hose clamps. The installation site was prepared by exposing the xylem using a razor blade and rinsing the area with distilled water to remove the contents of ruptured living cells. Each instrument was measured psychrometrically at 15 or 30 min intervals with a datalogger (Campbell Scientific CR-7, Logan USA) to obtain detailed diel measurements of xylem Ψ. The chamber thermocouple junction was cooled for 30 s after which thermocouple output was measured 28 times over approximately two minutes. All values were stored to check the data but the eighth point generally fell in the psychrometric plateau and was taken as the

endpoint. Plateau values from these instruments were standardized to 25 °C, corrected for temperature gradients between the sample and the instrument, and converted to Ψ using the most recent calibration. Each hygrometer was initially calibrated using salt solutions and recalibrated periodically during field use. Hygrometers were removed, cleaned and reinstalled at 6-8 week intervals to prevent potential problems caused by wound responses at the installation site. Although psychrometric measurements may be subject to errors resulting from temperature fluctuations (Jackson et al. 2000), these problems were minimized here because the cave air temperature changed <1°C.

Vulnerability to cavitation

Vulnerability to cavitation of stems, shallow roots and deep roots 5-8 mm in diameter was measured during June and July, 1999. Shallow roots were excavated using hand tools from approximately 10 cm below the soil surface. Deep roots of the appropriate diameter and species were collected from cave soil. All samples were stored in plastic bags with moist paper towels, taken to Duke University, and analyzed within four days. Vulnerability to cavitation was measured on each segment using the centrifugal force method (Alder et al. 1997; Pockman et al. 1995). Prior to measurement, each sample was cut to length (140 mm) underwater prevent introduction of air emboli. Segments were then placed in a custom centrifuge rotor to generate negative pressure in the xylem. The rotor generates specific negative pressures at the center of a segment while the ends are held in water to allow repeated measurements.

Prior to centrifuging, the initial hydraulic conductance (k) of the segment was determined by measuring the mass flow rate through the segment onto an electronic balance. A known pressure difference was applied by elevating a reservoir of pure water (filtered to 0.22 μm). The segment was then placed in the centrifuge rotor and spun to generate −0.5 MPa for 3-5 minutes with its ends held in water. The effect of the negative pressure was determined by making a

second measurement of k and calculating the percent decrease in k (embolism). This procedure was repeated using increasingly negative pressures generated in the centrifuge in 1.5 MPa increments until embolism was complete or the maximum speed of the centrifuge was reached (21,000 rpm or approximately -9.5 MPa).

Xylem anatomy

Representative samples of branches, shallow roots and deep roots were examined with a Phillips XL30 Scanning Electron Microscope (at 352x) and photographed with a Polaroid CU-5 Land Camera. The negatives were scanned and stored in TIFF format. To quantify the differences observed in the SEM images, tracheid diameter distributions were determined for the same samples used to determine vulnerability to cavitation. We cut transverse sections (*ca.* 50 µm thick) from each segment using a rotary microtome (Spencer 820 Microtome; American Optical Co., Buffalo, New York, USA). The sections were stained with toluidine blue (0.5%) to improve contrast and mounted in glycerol. The slides were viewed with a compound microscope (ZEISS Axioscop; Carl ZEISS, Germany) attached to a black and white video camera (MTI CCD-72; Dage MTI, Michigan City, USA) and images were captured with a computer. The magnification was 100x for roots, and 200x for stems. We used an image analysis program (Image 1 v. 4; Imaging Corporation, West Chester, Pennsylvania, USA) to analyze the sections. For each sample two representative sectors of the same section about 90° apart were measured. For stems, vascular rays were used to establish the boundaries of each sector. Unstained tracheid lumens were easily distinguished from the rest of the tissue (blue stained) by means of their different color (gray level). All open tracheids within each sector were sampled. To eliminate artifacts, such as partially occluded tracheids or gaps in the wood, a filter was applied such that all the "conduits" with an area smaller than 5 µm (stems), 25 µm (roots), or with a shape (sphericity) factor less than 0.4 were excluded. These values were selected in each case to maximize the agreement

between the visually identified tracheids and the ones selected by the computer. Because of their small diameter, the few functional tracheids below the selected sizes would have a negligible effect on the conductive capacity of the xylem. For each selected conduit, the program determined the total cross sectional area and perimeter. At least 100 conduits were measured from each section.

We calculated, for each section, the proportion of tracheids in 2-μm size classes and the proportion of the total calculated hydraulic conductance that was contributed by the tracheids in each of these diameter classes. To calculate the latter, we assumed that hydraulic conductance was proportional to the fourth power of the radius as described by the Hagen-Poiseuille equation (Tyree and Ewers 1991). For each size class, the summed fourth powers of every tracheid diameter in the class was divided by the sum of the fourth powers of the diameter of all tracheids measured from the section. For each section we also calculated the mean tracheid diameter and the "hydraulic mean" equal to the sum of all tracheids to the fifth power divided by that sum to the fourth power.

RESULTS

Although daily sap flux density (Fig. 2A) varied with climate conditions during the 1999 growing season (Fig. 2B), the mean proportion of total daily flow supplied by the deep root was 0.24 (SE = 0.006, n = 279 days). Daily sap flow was calculated from daily J_s by multiplying by the cross-sectional area of the sapwood in the deep root and main stem. Total flow through the main stem was substantially greater than through the deep root because of its larger cross sectional sapwood area.

Between late July and late October 1999, the site was subjected to a severe drought with maximum daily temperatures frequently reaching 35-40 °C (Fig. 2B). Although total daily flow through the main stem and deep root generally increased with increasing evaporative demand,

during this period the total daily flow through the main stem and deep root was lower than when water availability was higher (Fig. 3A). Surprisingly, the ratio between daily root and shoot sap flux density and flow (which are inter-convertible using the ratio of their sapwood areas) decreased with increasing vapor pressure deficit (Fig. 3B). The mean proportion of total daily flow supplied by the deep root during the drought was 0.17 (SE = 0.005, n = 79 days). This decrease in deep root contribution with increased evaporative demand and decreased water availability suggests that deep root hydraulic conductance (k) decreased during the drought.

The decrease in deep root k was also evident from daily J_s compared over the same period. Comparison of the main stem and deep root sap-flow probes indicated that before the drought there were few days with significant differences between J_s at each position (Fig. 2A). This pattern changed with the onset of the drought when daily J_s decreased in both root and main stem and deep root J_s was lower than in the main stem.

Changes in k can result from cavitation associated with decreasing Ψ during drought. Until August 1999, Ψ_{pd} and Ψ_{md} changed only gradually and there were no differences in Ψ between the tree measured for sap flow and neighboring plants (Fig. 2C). During the drought, however, differences emerged in Ψ_{pd} and Ψ_{md} between the plant rooted in the cave and the surrounding plants. Mean Ψ_{pd} and Ψ_{md} of the neighboring plants decreased more than 3 MPa (Fig. 2C) while Ψ_{pd} of the target tree decreased less than 0.5 MPa and Ψ_{md} decreased less than 1 MPa . The group of neighboring plants included one individual that closely tracked the target tree while the remaining 5 individuals accounted for the decreases in Ψ_{pd} and Ψ_{md}. A broader survey of Ψ_{md} for other Juniper trees within 30 m of the target tree (n = 55) showed the same pattern (Fig. 2C inset) observed in longer term measurements of the smaller group (Fig. 2C). Some trees, including the measured tree (Fig. 2C inset, arrow), maintained high Ψ during the drought while a larger number of plants exhibited substantially decreased Ψ_{md}. The variation in minimum Ψ_{md} was not correlated with basal cross sectional stem area, canopy ground area or estimated canopy

volume (data not shown) and access to deep water could help explain the higher Ψ_{md} in the subset of trees with higher water potentials.

For comparison with sap flux data, we measured Ψ at three points along the flow path from deep roots to distal twigs (Fig. 4). As expected, Ψ_{pd} and deep root Ψ were highest while stem Ψ was intermediate and Ψ_{md} was most negative. Comparing J_s and Ψ showed that at all positions Ψ was highest when J_s was zero and most negative during the periods of peak J_s.

Were the relatively small decreases in Ψ of the target tree associated with the decrease in deep root k? Dividing sap flux density by the pressure difference driving xylem flow (ΔP) provides an estimate of sapwood area specific hydraulic conductance (k_s) of the portion of the plant represented by ΔP. We used measurements of J_s and Ψ throughout the drought to calculate k_s. These data indicated substantial changes in deep root k_s during the onset of the drought (Fig. 5A) and some recovery following precipitation at the end of the drought (Fig. 5B). Over the two week onset of the drought, the apparent k_s declined 85% and remained relatively constant throughout the remainder of the drought (Fig. 5A, B). Following the decrease in vapor pressure deficit and the first substantial precipitation in late October k_s increased threefold (Fig. 5B, inset). Over the same period whole plant k_s, calculated using predawn and midday leaf water potentials and Js in the main stem, showed a similar pattern (Fig. 6) decreasing during the onset of the drought. The increase in deep root k_s following precipitation in October (Fig. 5B) was not detectable in the whole plant measurements where the drought and post-drought k_s was not different (Fig. 6).

To evaluate one possible cause of the observed change in hydraulic conductance, we compared vulnerability to xylem cavitation among branches, shallow roots and deep roots (Fig. 7). Branches exhibited no cavitation until pressures below –8 MPa. A slight increase in stem hydraulic conductance following the initial spinning treatment in stems accounts for the slight initial decrease in embolism. Although the reason is unknown, this is sometimes observed in

conifer stems (Pockman, unpublished). Shallow and deep roots were more vulnerable to cavitation than stems. Percent embolism increased gradually in shallow roots reaching approximately 60% by –9.5 MPa; embolism in deep roots increased much more rapidly, approximately one-third by -2 MPa and approaching 100% at –9.5 MPa. Our measurements of deep root water potential (Fig 4 & 5) suggest that these roots are unlikely to experience such negative xylem pressures under all but the most extreme circumstances. However, the increase in xylem embolism as pressures fell to –2 MPa could reduce k in deep roots of the diameter measured.

Scanning electron micrographs of branch, shallow root, and deep root cross sections revealed clear differences in xylem conduit diameter (Fig 8). Mean conduit diameter was 40% larger in deep roots than in shallow roots and four times larger than in shoots ($P<0.01$; Fig 8B-D). Mean conduit diameters were 5.2 μm, 15.8 μm, and 21.8 μm for stems, shallow roots, and deep roots, respectively. Similarly, the hydraulic mean (light arrows) also increased from branches (6.7 μm) to shallow roots (20.3 μm) to deep roots (27.3 μm), reflecting the greater predicted hydraulic conductance (k) of the larger conduits in shallow and deep roots (Fig 8 B - D). The mean initial k_s (mg m^2 MPa^{-1} s^{-1}) of the samples measured for vulnerability to cavitation was 0.35 (SD = 0.12, n = 3) for branches, 2.31 (SD = 0.88, n = 5) for shallow roots and 2.93 (SD = 2.35, n = 3) for deep roots.

DISCUSSION

To our knowledge the present study is the first to directly measure the contribution of deep water uptake. The contribution of deep roots to plant water uptake has been demonstrated previously using stable isotope measurements (Ehleringer and Dawson 1992). However, such studies do not address the mechanistic basis for observed shifts between uptake of shallow and deep water (e.g. Williams and Ehleringer 2000). Our study is also unique in incorporating

measurements of plant water relations along the entire plant flow path diurnally and seasonally. Results show that water absorbed from below 7 m depth contributed 24% of the total daily transpiration of the plant during the growing season, a value that represents a lower bound since other deep roots may have been present. Our data also suggest that changes in hydraulic conductance caused by cavitation may be responsible for shifts in water uptake among different portions of the root system.

The decrease in hydraulic conductance (k) of the deep root (Fig. 5A), and particularly the decline in the proportion of daily transpiration supplied by the deep root during the drought (Fig. 2A), were unexpected. Deep roots are often regarded as buffered from fluctuations in surface water availability. During the first part of the study, Ψ_{pd} and Ψ_{md} (Fig. 2C) changed in concert among all measured individuals suggesting that these plants had relatively uniform access to a reliable water supply. The separation that occurred during the drought revealed considerable variation in water availability (Figs. 2C and 2C-inset) and may reflect differences in the number and extent of deep roots and the size of the deep water pool they use. The range of Ψ_{md} during the drought may reflect variation in rooting depth among the measured plants. Alternatively, the observed pattern could also develop among consistently deep rooted plants where the volume and recharge of deep water varies spatially with substrate structure and the soil volume available to hold water.

Xylem cavitation is the most likely cause of the decreased deep root k_r we observed. Shallow roots have been shown to be more vulnerable to cavitation than stems (e.g. Hacke and Sauter 1996) suggesting they are the most limiting portion of the flow path during drought (Alder et al. 1996; Sperry et al. 1998). The vulnerability curves for *J. ashei* were consistent with this pattern but showed that deep roots were even more vulnerable to cavitation than shallow roots (Fig. 7). Based on the vulnerability curve for deep roots (Fig. 7), the decrease in deep root Ψ that we observed (Figs. 4C & D, 5) could result in a decrease in k of up to 20%. Vulnerability to

cavitation has been shown to increase in more distal, smaller diameter portions of the root system (Sperry and Ikeda 1997; Tyree and Ewers 1991). If this pattern holds in roots of *J. ashei* it could account for the larger decrease in deep root k_s than the 20% predicted by our measurements of a single size class of roots. Likewise, changes in radial hydraulic conductance could also contribute to reducing deep root k_s.

The shape of the relationship between deep root J_s and Ψ during the onset of drought (Fig. 5A) suggests that hydraulic conductance might have decreased and recovered during the course of a day. At the beginning and end of each day the instantaneous slope of the relationship between J_s and ΔP was steeper than at midday (Fig. 5A) suggesting that k declined during the day and recovered at the end of the day. Similarly, the increase in deep root k following precipitation suggests that the deep roots could recover from reduced k during drought (Green and Clothier 1995). Although refilling has been thought to occur by seasonal or diurnal positive root pressures that dissolve air emboli in cavitated conduits (Ewers et al. 1991; Tyree and Yang 1992), recent work suggests that refilling may occur on a daily basis (Zwieniecki and Holbrook 1998) and even while transpiration is occurring (Canny 1997; Holbrook and Zwieniecki 1999). Although several studies have suggested mechanisms that may refill conduits under negative pressure in conifers (Borghetti et al. 1991; Edwards et al. 1994) and in a Mediterranean shrub (Salleo et al. 1996), the actual mechanism remains unclear.

The anatomical differences among xylem of branches, shallow roots and deep roots (Fig. 6) are consistent with those in other species (Pate et al. 1995). The trend in our measurements of xylem function generally supported the expectation that k_s should increase with increasing conduit diameter (Zimmermann 1983). The relationship between increasing conduit diameter and vulnerability to cavitation has been observed in several species (Salleo and LoGullo 1986; Sperry and Saliendra 1994). Thus the increased k associated with larger diameter conduits is accompanied by more severe limits on the range of Ψ_x over which xylem function is maintained.

Our results suggest that deep root function during drought depends on water availability at depth. The depletion of deep water reserves during drought in this study was associated with a reduction in deep root k. In systems where deep water abundance is independent of surface conditions (i.e. in the presence of groundwater), deep roots are unlikely to experience such a decrease. The decreased deep root contribution during drought implies that other portions of the root system increased their relative contribution to whole plant water use. Given the prevailing environmental conditions during this period (Fig. 3B), surface roots in shallow dry soil are unlikely to increase their water uptake. The only roots likely to avoid a similar loss of hydraulic conductance to that observed in the measured deep root are other deep roots with access to a more abundant and long-lasting supply of water. Cave systems like the one utilized for this study provide an opportunity to test whether such shifts occur within the root system of a single individuals where multiple roots differ in their access to water.

Although stored water can contribute significantly to total daily transpiration (Holbrook and Sinclair 1992b; Tyree et al. 1991), it was probably not an important factor in this study. The availability of stored water depends upon tissue structure and plant water status (Holbrook and Sinclair 1992a). Water storage can occur as 1) capillary storage in fibers, apoplastic spaces and cavitated conduits, 2) as elastic storage and 3) as water released by xylem cavitation (Holbrook 1995). At high Ψ capillary water storage in stems can be substantial (Waring and Running 1978) but is typically depleted by approximately –0.6 MPa (Tyree and Yang 1990), which approximates the highest deep root Ψ observed during the drought. At high Ψ large woody roots could contribute water to the stem during the day and refill gradually at night. However, this is unlikely during the drought because observed xylem pressures would prevent refilling of these capillary spaces.

Recent empirical and modeling studies have shown that considering structural and physiological limits at all points in the flow path helps improve our understanding of limitations

to plant performance (Alder et al. 1996; Sperry et al. 1998). The difficulty of integrating measurements across all portions of the root system and shoots represents one such limitation. Access to deep roots in caves provides a unique opportunity to study plant functioning and the extent of deep water use in situ over a range of time scales and climatic conditions. By combining deep root measurements with isotopic methods and other approaches, including sap flux measurements of smaller and lateral roots (e.g. Burgess et al. 1998; Lott et al. 1996; Senock and Leuschner 1999; Smith et al. 1999), whole-plant measurements and mechanistic responses to the environment are now possible in this and other systems.

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REFERENCES

Alder NN, Pockman WT, Sperry JS, and Nuismer S (1997) Use of centrifugal force in the study of xylem cavitation. J Exp Bot 48:665-674.

Alder NN, Sperry JS, and Pockman WT (1996) Root and stem xylem embolism, stomatal conductance and leaf turgor in *Acer grandidentatum* populations along a soil moisture gradient. Oecologia 105:293-301.

Borghetti M, Edwards WRN, Grace J, Jarvis PG, and Raschi A (1991) The refilling of embolized xylem in *Pinus sylvestris* L. Plant Cell Environ14:357-369.

Burgess SSO, Adams MA, Turner N, and Ong CK (1998) The redistribution of soil water by tree root systems. Oecologia. 115:306-311.

Caldwell MM, Dawson TE, and Richards JH (1998) Hydraulic lift: consequences of water efflux from the roots of plants. Oecologia 113:151-161.

Canadell J, Jackson RB, Ehleringer JR, Mooney HA, Sala OE, and Schulze E-D (1996) Maximum rooting depth of vegetation types at the global scale. Oecologia 108:583-595.

Canny MJ (1997) Vessel contents during transpiration--embolisms and refilling. Am J Bot 84:1223-1230.

Carlquist S (1988) Comparative Wood Anatomy. Springer-Verlag, Berlin.

Clearwater MJ, Meinzer FC, Andrade JL, Goldstein G, and Holbrook NM (1999) Potential errors in measurement of nonuniform sap flow using heat dissipation probes. Tree Physiol 19:681-687.

Crombie DS, Hipkins HF, and Milburn JA (1985) Gas penetration of pit membranes in the xylem of *Rhododendron* as the cause of acoustically detectable sap cavitation. Australian Journal of Plant Physiol 12:445-453.

Dawson TE (1993) Water sources of plants as determined by xylem-water isotopic composition: perspectives on plant competition, distribution, and water relations. Pages 465-496 *in* Ehleringer JR, Hall AE, and Farquhar GD Editors, Stable isotopes and plant carbon-water relations, Academic Press, San Diego.

Dixon MA, and Tyree MT (1984) A new stem hygrometer, corrected for temperature gradients and calibrated against the pressure bomb. Plant Cell Environ 7:693-697.

Edwards WRN, Jarvis PG, Grace J, and Moncrieff JB (1994) Reversing cavitation in tracheids of *Pinus sylvestris* L. under negative water potentials. Plant Cell Environ 17:389-397.

Ehleringer JR, and Dawson TE (1992) Water uptake by plants: Perspectives from stable isotope composition. Plant Cell Environ 15:1073-1082.

Elliot WR, and Veni G, Editors (1994) The Caves and Karst of Texas. The National Speleological Society (U.S.), Huntsville, Alabama.

Ewers FW, Carlton MR, Fisher JB, Kolb KJ, and Tyree MT (1997) Vessel Diameters in Roots Versus Stems of Tropical Lianas and Other Growth Forms. IAWA J 18:261-279.

Ewers FW, Fisher JB, and Fichtner K (1991) Water flux and xylem structure in vines. Pages 127-160 *in* Putz FE, and Mooney HA Editors, The biology of vines, Cambridge University Press, Cambridge.

Goulden ML, and Field CB (1994) Three methods for monitoring the gas exchange of individual tree canopies: Ventilated-chamber, sap-flow and Penman-Monteith measurements on evergreen oaks. Functional Ecol 8:125-135.

Granier A (1987) Evaluation Of Transpiration In a Douglas-Fir Stand By Means Of Sap Flow Measurements. Tree Physiol 3:309-320.

Green SR, and Clothier BE (1995) Root water uptake by kiwi fruit vines following partial wetting of the root zone. Plant Soil 173:317-328.

Hacke U, and Sauter JJ (1996) Drought-induced xylem dysfunction in petioles, branches, and roots of *Populus balsamifera* and *Alnus glutinosa* (L.) Gaertn. Plant Physiol 111:413-417.

Holbrook NM (1995) Stem water storage. Pages 151-174 *in* Gartner BL Editor, Plant Stems: Physiology and Functional Morphology, Academic Press, San Diego.

Holbrook NM, and Sinclair TR (1992a) Water balance in the arborescent palm, *Sabal palmetto*. I. Stem structure, tissue water release properties and leaf epidermal conductance. Plant Cell Environ 15:393-399.

Holbrook NM, and Sinclair TR (1992b) Water balance in the arborescent palm, *Sabal palmetto*. II. Transpiration and stem water storage. Plant Cell Environ 15:401-409.

Holbrook NM, and Zwieniecki MA (1999) Embolism repair and xylem tension: Do we need a miracle? Plant Physiol 120:7-10.

Jackson PC, Meinzer FC, Bustamante M, Goldstein G, Franco A, Rundel PW, Caldas L, Igler E, and Causin F (1999a) Partitioning of soil water among tree species in a Brazilian Cerrado ecosystem. Tree Physiol 19:717-724.

Jackson RB, Anderson LJ, and Pockman WT (2000) Measuring water availability and uptake in ecosystem studies. *in* Sala OE, Jackson RB, Mooney HA, and Howarth RW Editors. Methods in Ecosystem Science, Springer Verlag, New York.

Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, and Schulze ED (1996) A global analysis of root distributions for terrestrial biomes. Oecologia 108:389-411.

Jackson RB, Moore LA, Hoffmann WA, Pockman WT, and Linder CR (1999b) Ecosystem rooting depth determined with caves and DNA. Proc Nat Acad Sci USA 96:11387-11392.

Jarbeau JA, Ewers FW, and Davis SD (1995) The mechanism of water stress-induced embolism in two species of chaparral shrubs. Plant Cell Environ 18:189-196.

Kavanagh KL, Bond BJ, and Knowe S (1999) Shoot and root vulnerability to xylem cavitation in four populations of Douglas-fir seedlings. Tree Physiol. 19:31-37.

Kramer PJ, and Boyer JS (1995) Water relations of plants and soils. Academic Press. San Diego.

Landsberg J, and Fowkes N (1978) Water movement through plant roots. Ann Bot 42:493-508.

Linton MJ, Sperry JS, and Williams DG (1998) Limits to water transport in *Juniperus osteosperma* and *Pinus edulis*: implications for drought tolerance and regulation of transpiration. Functional Ecol 12:906-911.

Lott JE, Khan AAH, Ong CK, and Black CR (1996) Sap flow measurements of lateral tree roots in agroforestry systems. Tree Physiol 16:995-1001.

Meinzer FC, and Grantz DA (1990) Stomatal and hydraulic conductance in growing sugarcane: stomatal adjustment to water transport capacity. Plant Cell Environ 13:383-388.

Nepstad DC, de Carvalho CR, Davidson EA, Jipp PH, Lefebvre PA, Negreiros GH, da Silva ED, Stone TA, Trumbore SE, and Vieira S (1994) The role of deep roots in the hydrological and carbon cycles of Amazonian forests and pastures. Nature 372:666-669.

Oren R, and Sheriff DW (1995) Water and nutrient acquisition by roots and canopies. Pages 39-74 *in* Smith WK, and Hinckley TM Editors, Resource Physiology of Conifers, Academic Press, San Diego.

Passioura JB (1988) Water transport in and to roots. Annual Review of Plant Physiology and Molec Biol 39:245-265.

Pate JS, Jeschke WD, and Aylward MJ (1995) Hydraulic architecture and xylem structure of the dimorphic root systems of SouthWest Australian species of the Proteaceae. J Exp Bot 46:907-915.

Pate JS, Jeschke WD, Dawson TE, Raphael C, Hartung W, and Bowen BJ (1998) Growth and seasonal utilisation of water and nutrients by *Banksia prionotes*. Aust J Bot 46:511-532.

Phillips N, Oren R, and Zimmermann R (1996) Radial patterns of xylem sap flow in non-, diffuse- and ring-porous tree species. Plant Cell Environ 19:983-990.

Pockman WT, Sperry JS, and O'Leary JW (1995) Sustained and significant negative water pressure in xylem. Nature 378:715-716.

Rieger M, and Latvin P (1999) Root system hydraulic conductivity in species with contrasting root anatomy. J Exp Bot 50:201-209.

Sakuratani T, Aoe T, and Higuchi H (1999) Reverse flow in roots in *Sesbania rostrata* measured using the constant power heat balance method. Plant Cell Environ 22:1153-1160.

Salleo S, Lo Gullo MA, De Paoli D, and Zippo M (1996) Xylem recovery from cavitation-induced embolism in young plants of *Laurus nobilis*: a possible mechanism. New Phytol 132:47-56.

Salleo S, and LoGullo MA (1986) Xylem cavitation in nodes and internodes of whole *Chorisa insignis* plants subjected to water stress: Relations between xylem conduit size and cavitation. Ann Bot 58:431-441.

Schulze ED, Caldwell MM, Canadell J, Mooney HA, Jackson RB, Parson D, Scholes R, Sala OE, and Trimborn P (1998) Downward flux of water through roots (i.e. inverse hydraulic lift) in dry Kalahari sands. Oecologia 115:460-462.

Senock R, and Leuschner C (1999) Axial water flux dynamics in small diameter roots of a fast growing tropical tree. Plant Soil 208:57-71.

Smith DN, Jackson A, Roberts JM, and Ong CK (1999) Reverse flow of sap in tree roots and downward siphoning of water by *Grevillea robusta*. Functional Ecol 13:256-264.

Sperry JS, Adler FR, Campbell G, and Comstock JP (1998) Limitation of plant water use by rhizosphere and xylem conductance: results from a model. Plant Cell Environ 21:347-359.

Sperry JS, and Ikeda T (1997) Xylem cavitation in roots and stems of Douglas fir and white fir. Tree Physiol 17:275-280.

Sperry JS, and Saliendra NZ (1994) Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. Plant Cell Environ 17:1233-1241.

Sperry JS, and Tyree MT (1988) Mechanism of water stress-induced xylem embolism. Plant Physiol 88:581-587.

Steudle E, and Peterson CA (1998) How does water get through roots? J Exp Bot 49:775-788.

Stone E, and Kalisz PJ (1991) On the maximum extent of tree roots. For Ecol Manage 46:59-102.

Tyree MT, and Ewers FW (1991) Tansley Review No. 34. The hydraulic architecture of trees and other woody plants. New Phytol 119:345-360.

Tyree MT, Snyderman DA, Wilmot TR, and Machado J-L (1991) Water relations and hydraulic architecture of a tropical tree (*Schefflera morototoni*). Plant Physiol 96:1105-1113.

Tyree MT, and Sperry JS (1988) Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Answers from a model. Plant Physiol 88:574-580.

Tyree MT, and Sperry JS (1989) Vulnerability of xylem to cavitation and embolism. Annual Rev Plant Physiol Mol Biol 40:19-38.