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Original Article

Bark water uptake promotes localized hydraulic recovery in coastal redwood crown

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ABSTRACT

Coastal redwood (*Sequoia sempervirens*), the world's tallest tree species, rehydrates leaves via foliar water uptake during fog/rain events. Here we examine if bark also permits water uptake in redwood branches, exploring potential flow mechanisms and biological significance. Using isotopic labelling and microCT imaging, we observed that water entered the xylem via bark and reduced tracheid embolization. Moreover, prolonged bark wetting (16 h) partially restored xylem hydraulic conductivity in isolated branch segments and whole branches. Partial hydraulic recovery coincided with an increase in branch water potential from about -5.5 ± 0.4 to -4.2 ± 0.3 MPa, suggesting localized recovery and possibly hydraulic isolation. As bark water uptake rate correlated with xylem osmotic potential ($R^2 = 0.88$), we suspect a symplastic role in transferring water from bark to xylem. Using historical weather data from typical redwood habitat, we estimated that bark and leaves are wet more than 1000 h per year on average, with over 30 events being sufficiently long (>24 h) to allow for bark-assisted hydraulic recovery. The capacity to uptake biologically meaningful volumes of water via bark and leaves for localized hydraulic recovery throughout the crown during rain/fog events might be physiologically advantageous, allowing for relatively constant transpiration.

Key-words: embolism; fog; foliar uptake; phellem; sequoia sempervirens.

INTRODUCTION

Xylem embolism threatens plant vitality by limiting hydraulic conductivity, transport of water and nutrients, leaf gas exchange and, ultimately, plant growth and survival. Embolism forms diurnally and seasonally in response to low water potential caused, for example, by drought and freezing stress (Tyree & Sperry 1989; Hacke *et al.* 2001; Brodersen *et al.* 2010). Following embolism, plants can recover hydraulic conductivity by forming new xylem or refilling previously embolized conduits (Tyree & Sperry 1989). Both actions require partial or complete relief from water stress. At a minimum, refilling requires a water source and a free energy gradient (Zwieniecki & Holbrook 2009). In many herbaceous and smaller woody plants, when the soil is fully saturated, root pressure can provide water for restoring hydraulic conductivity in embolized stems and branches (Sperry *et al.* 1994). However, root pressure is unlikely to restore hydraulic conductivity in tall trees, as it rarely exceeds tens of kilopascals even under the most suitable (i.e. saturated) conditions (Sperry *et al.* 1994; De Swaef *et al.* 2013). In fact, the effect of root pressure in restoring embolized xylem has not been observed in conifers. It is unclear, if and how tall coniferous trees can restore hydraulic conductivity in distal regions (i.e. branches and leaves) where the most negative xylem pressures occur.

When xylem tension is close to zero, capillary action could drive the refilling process (Yang & Tyree 1992). Because typical tracheid diameters are *ca.* 25 μm, refilling cannot occur solely by capillary action at tensions greater than a few kilopascals (Yang & Tyree 1992). Instead, refilling would require additional energy in the form of a water potential gradient between xylem and parenchyma cells, a process proposed to exist in some woody angiosperms (Secchi & Zwieniecki 2012) and/or under hydraulic isolation (Zwieniecki & Holbrook 2009). Even then, the amount of solutes measured in xylem sap of non-functional conduits barely accounts for 0.2 MPa of osmotic gradient. In tall trees (>40 m), the bulk of stem water remains under significant tension (>0.4 MPa) because of gravity, even when soil is wet and no transpiration occurs. Thus, any refilling activity should require localized relief from tension, independent of bulk water in the stem. Previous studies demonstrated that foliar water uptake locally rehydrates leaves in numerous tree species, across rainforest, temperate, boreal and desert biomes (Boucher *et al.* 1995; Gouvra & Grammatikopoulos 2003; Breshears *et al.* 2008; Limm *et al.* 2009; Eller *et al.* 2013; Laur & Hacke 2014; Mayr *et al.* 2014). How water enters the leaf, despite a highly effective barrier to evaporative water loss (i.e. cuticle), is not exactly clear, but some cuticular membranes were shown to be permeable to water (Schreiber *et al.* 2001). If a cutinized organ like a leaf permits water uptake for refilling, perhaps other aerial organs also function as water absorption surfaces that promote hydraulic recovery.

Tree stems and branches are covered with suberized tissue, that is, cork (phellem). This surface provides protective

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functions, including fire resistance and avoidance of mechanical, insect and pathogen damage (Rosell *et al.* 2013). The bark also provides an effective barrier to evaporation. Similar to leaves, however, a prior study showed that water could permeate bark when wet. *Picea abies* branches soaked for 200 min increased their mass up to 8% and decreased water potential from −1.4 to −0.2 MPa (Katz *et al.* 1989). Hydrologists also recognize the significant role of bark in rainfall interception and storage, noting that some water might be absorbed by the xylem (Liu 1998). Moreover, the forest products industry must consider bark moisture content during processing, as some species have moisture contents over 100% of dry weight (Miles & Smith 2009). These observations suggest that bark might not only protect from water loss, but under specific circumstances (e.g. rainfall or fog), could locally relieve tension in branches by providing a prolonged source of water. If so, could bark water uptake represent a biologically meaningful mechanism for xylem hydraulic recovery? The answer to this question would clarify the role of fog and rainfall in sustaining tall trees through reliance on bark and foliar water uptake to restore hydraulic conductivity, as well as help predict the effect of sudden exposure to low humidity and high evaporative demand on tree growth and survival.

In this study, we examine bark water uptake of coastal redwood (*Sequoia sempervirens*), a gymnosperm restricted to foggy coastal environments, that is the world's tallest tree species. Burgess & Dawson (2004) provide evidence that coastal redwood trees absorb water through leaves, a strategy thought to have evolved in response to regularly available fog. However, given the capacity of bark to absorb water, we hypothesize that coastal redwood may utilize surfaces throughout the entire crown, both leaves and bark, to restore hydraulic conductivity. Foliar uptake has only been observed in the most distal parts of the crown and does not account for large internal fluxes (Burgess & Dawson 2004). Consequently, restoration of hydraulic conductivity in branches might rely primarily on bark water uptake, suggesting localized hydraulic recovery, in which water for refilling is sourced from nearby plant surfaces. If localized hydraulic recovery indeed exists, it raises novel questions about the biophysics, ecology and evolution of plant surfaces, such as bark and leaves.

MATERIALS AND METHODS

Plant materials

Plant materials used in this study were collected from 10 mature coastal redwood (*S. sempervirens* (Lamb. ex D. Don) Endl.) trees (~25 m tall and ~0.3 m diameter) openly grown at the University of California Davis campus arboretum. Small branches (8–12 mm in diameter) were randomly selected from low-hanging limbs (~2.5 m above ground). The selected branches had over 20 cm long segments with no leaves, which ensured full coverage with bark. Branches were collected from limbs facing different aspects around the trees. Branch segments utilized in the experiment were located at a minimum distance of 20 cm from the initial cut separating the branch from the tree.

Determination of water uptake via bark

Change in branch segment mass and volume via soaking

From the collected branches, we cut segments (20–30 mm length) and quickly sealed both ends by covering them with a siliconized acrylic caulk (Ace ®) (ACE, Oakbrook, IL, USA) and wax tape (Parafilm M ®). The use of caulk was necessary as the seal provided by Parafilm only was leaky on rough bark surface. Siliconized acrylic caulk has excellent sticking properties to wax type surfaces and, because of its flexible nature, effectively sealed small surface bark cracks. Based on dye infiltration tests, the segments' ends were confirmed to be well-sealed even during prolonged (>20 h) water submergence. We placed sealed-end branch segments in a deionized water bath (20 °C). At specified time increments (10 min, 30 min, … to 90 h), we removed each submerged branch segment, patted it with a paper towel to remove surface water, weighed it and returned the branch segment to the water bath. We also tested the relative change in volume and mass of bark and xylem separately by excising bark from the xylem. Again, at specified time increments (0 min, 15 min, 60 min and 16 h), we removed each submerged sample, patted it with a paper towel to remove surface water, weighed it and returned it to the water bath.

Isotopic study of water transport into xylem

We tested whether water outside the branch entered the xylem via the bark using $\delta^{18}O$ enriched water. We excised four branches and cut each branch into two segments: initial and treated (i.e. final) segments. For initial segments, we removed the bark and immediately spun segments at 12 000 r.p.m. in a centrifuge to remove xylem water and then cut ~3 mm off the segment, spun again and repeated this procedure until the entire segment was cut and spun. The extracted water was refrigerated at 2 °C before analysis. We sealed the ends of the treated segments as described before. Then, we placed treated segments in $\delta^{18}O$ enriched water (1 atom%). After 16 h, branch segments were removed, stripped of bark, patted dry with a paper towel, allowed to dry for 15 min and then spun 5 min at 12 000 r.p.m. in a centrifuge to extract xylem water (following the same procedure as for control-initial segments). We used a micro-distillation system to obtain water aliquots free of organic compounds, which were then sent to the University of California, Davis Stable Isotope Facility for $\delta^{18}O$ determination. Isotopic analysis was performed using a Laser Water Isotope Analyzer V2 (Los Gatos Research, Inc., Mountain View, CA, USA) using 10 injections per aliquot for oxygen isotope ratio calculations. Isotope ratios were standardized using a range of working standards that have been calibrated against International Atomic Energy Agency standard reference materials. Final isotope values were reported relative to Vienna Standard Mean Ocean Water (VSMOW) as follows:

$$\delta^{18}O\left({}^{0}/_{00}\right) = \left({}^{16}O/{}^{18}O_{\text{Sample}} / {}^{16}O/{}^{18}O_{\text{VSMOW}}\right) - 1 \times 1000 \quad (1)$$

Based on the evaluation of standards, the final $\delta^{18}O$ results were reported with analytical precision of <2‰. Statistical

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differences between initial (unenriched) water and the enrichment treatment were determined using pairwise student's *t*-tests.

Hydraulic conductivity and branch water potential

We measured the change in hydraulic conductivity due to fog/water exposure in isolated branch segments and whole branches with leaves covered, bark covered and bark/leaves uncovered. We also measured the change in water potential associated with the whole branch treatments (Fig. 1).

Isolated branch segments

We removed 12 branches from different trees across campus and cut three 50 mm segments from each branch, resulting in 36 segments total. Segments from each branch were randomly assigned to one of the three treatments: control-initial, control-final (16 h, entire branch segment surface covered with Parafilm on bench) and soaking treatment (16 h of soaking with ends only covered in Parafilm). For the control-initial, we recut a subset of 12 branch segments under water to ~25 mm length and allowed several minutes for tension relaxation (Wheeler *et al.* 2013). We then allowed filtered and degassed water at a 1.96 kPa pressure head to pass through the branch segment from a mass balance to a water bath. We measured the change in mass over time of the water as it left the mass balance. For the control-final, we sealed the ends of the second set of 12 branch segments and also covered the bark with Parafilm. We weighed the branch segments. Then, we placed them on the bench for 16 h, after which time, we weighed a branch segment again and measured mass flow of water through it. For the soaking treatment, we sealed the ends of a third set of 12 branch segments as described earlier and weighed the segments. Then, we soaked the branch segments in a deionized water bath for 16 h, after which time, we weighed the branch segment and measured mass flow of water through it.

We calculated sapwood specific hydraulic conductivity (K_s) using the length and diameter of the branch segment, the pressure head and the mass flow of water. We tested for differences in K_s among branch segments from each treatment using a nested repeated-measures ANOVA, with branch segment as a random factor. We then used a student's t to test for differences among groups.

Whole branches

We tested for the relative contribution of bark and leaves to hydraulic recovery and hydrostatic tension release in whole branches before and after sitting in a fog chamber for 16 h for three treatments: leaves covered (only basal part of the branch not covered with leaves exposed to fog), bark covered (only distal part of the branch covered with leaves exposed to fog) and bark/leaves uncovered (entire branch exposed to fog). We selected 21 branches ($n = 7$ for each treatment), with similar length of branch covered with bark and leaves (based on visual inspection) from different trees across campus. Before excision, we measured branch water potential (Ψ_{br}) by covering a leafy twig in a reflective bag containing a damp paper towel. Following excision, we removed a 50 mm long and ~8 mm

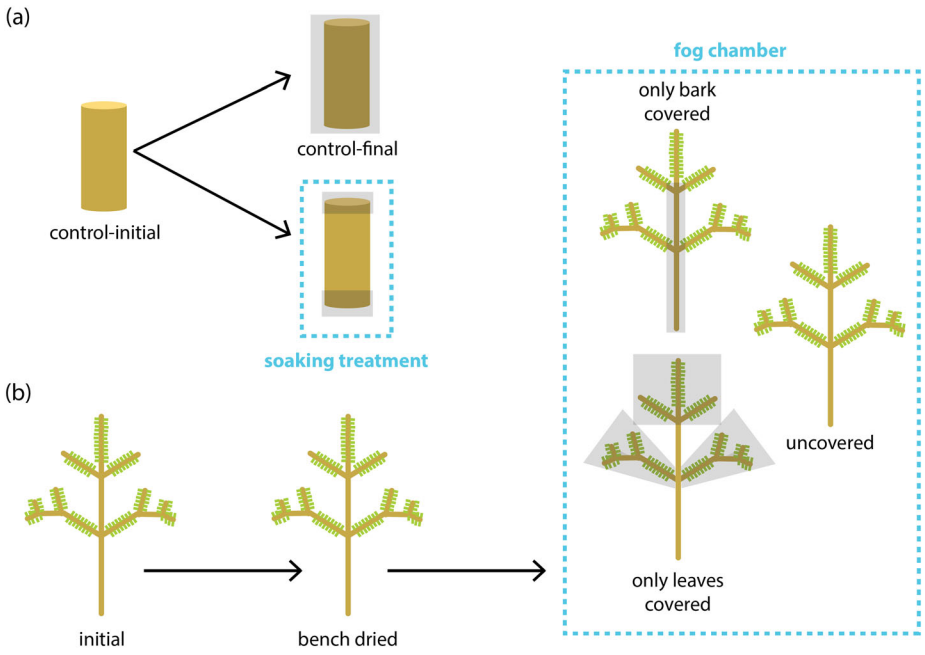

Figure 1. Procedure for investigating hydraulic conductivity and branch water potential of either whole branches or isolated branch segments. Grey boxes represent branch surfaces which are sealed from water entry. Blue-dotted boxes represent soaking/fog treatment. (a) Whole branches: Initial hydraulic recovery measurements were made prior to bench drying and fog treatment. Within fog treatment, we covered only bark or only leaves, or left branches uncovered. (b) Hydraulic recovery was measured on isolated branch segments before soaking treatment (control-initial) or wrapped with Parafilm (i.e. control-final), left on the bench and measured after 16 h when soaking was complete.

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diameter segment for hydraulic conductivity measurements, covered it with Parafilm and stored it at 4 °C. The cut end of the branch was covered with Parafilm and allowed to bench dry for 2 d, after which time, we measured Ψ_{br} and removed a 50 mm segment for hydraulic conductivity measurements. Next, we sealed the cut end of the branch with Parafilm and acrylic caulk. Each time we cut a twig to measure branch water potential, we also sealed the cut with acrylic caulk and Parafilm. At this point, we randomly divided the branches into the three treatment groups: leaves covered, bark covered and bark/leaves uncovered. Leaves were covered using large plastic bags with duct tape along the open end and putty covered by more duct tape where the stem entered the bag. Bark was covered by applying multiple layers of thin plastic wrap and wrapping in Parafilm. Such extensive wrapping procedures were necessary to prevent fog penetration into the covered area. We left the third set of branches uncovered. All three treatment groups were placed in a fog chamber for 16 h. We used foam board to build a box inside of which we placed a commercially available cold air humidifier on a medium setting. After 16 h, we removed the branches, measured Ψ_{br} and cut a 50 mm segment for hydraulic conductivity measurements. For all treatments, we cut distal leafless branch segments and measured hydraulic conductivity no more than 3 h after being stored at 4 °C. Branches were allowed to sit on bench for 20 min prior to measuring to restore segments to room temperature.

We tested for differences in K_s and Ψ_{br} before and after the fog treatment within segments from the same branch using a pairwise *t*-test.

Xylem refilling

We examined if branch segments soaked in water for 16 h had less embolism than those without soaking using synchrotron-based microcomputed tomography (microCT) at the Advanced Light Source (ALS), microtomography beamline 8.3.2, at the Lawrence Berkeley National Laboratories. We selected four branches from different trees across campus and measured Ψ_{br}. As Ψ_{br} was about −1.4 MPa, we bench-dried the branches for 2 d until they reached ca. −4.5 MPa, which corresponds to about ~35–40% loss in conductivity in coastal redwood, according to previous studies (Burgess *et al.* 2006). We then cut two 30 mm long and 5 mm diameter segments from each branch. We covered the cut ends of one segment from each branch with acrylic caulk and Parafilm and placed it in water for 16 h. We covered the cut ends and bark of the other segment from each branch with Parafilm and placed it in a shaded area on the bench for 16 h. After 16 h, we performed a single microCT scan of the soaked and unsoaked segments. We performed a single scan on paired branch segments, as opposed to repeat scans of the same segment, to minimize a noticeable effect of multiple-scan exposure on bark tissue, likely due to high energy x-ray. Specifically, when comparing unscanned control segment (soaked 16 h) with those scanned more than once, microCT images showed a noticeable decrease in voxel intensity in the inner bark. Such an effect appeared to increase with the number of scans.

Segments were scanned at 15 keV in the synchrotron X-ray beam while being rotated through 0–180° in increments of 0.25°. After each incremental rotation, the X-ray image was magnified through a series of lenses and relayed onto a 4 megapixel charge coupled device camera (#PCO 4000, Cooke Corp., Romulus, MI, USA). This yielded ~700, two-dimensional (2D) projections per sample. Raw 2D tomographic projections were reconstructed *c.* 500 sample. Image slices using a custom plugin for Fiji (a Java-based distribution of ImageJ, US National Institutes of Health, Bethesda, MD, USA) built and maintained by ALS staff. The resulting images utilizing the 2× lens produced images with a 4.5 μm voxel resolution.

A radial slice from the longitudinal centre of each branch segment was randomly selected for image analysis. The image was prepared and analyzed in ImageJ software (US National Institutes of Health, Bethesda, MD, USA). First, we removed the bark and background from the image, leaving only xylem. Then, we locally enhanced the contrast to facilitate the distinction between water-filled and embolized xylem. Images were then converted to 8-bit, and the 'threshold' function (available in the standard ImageJ package) was applied. Beginning with the control image for a given branch, we determined the appropriate threshold value by minimizing the presence of artefactual air bubbles in regions of the xylem clearly occupied by water based on visual inspection of the grey scale image. Once a threshold value was determined for the control image, we applied the same value to the treatment. We converted the images to binary and analyzed the area occupied by embolized tracheids via particle analysis. If the difference in average tracheid (i.e. particle) size between the control and treatment was greater than 0.5%, we iteratively changed the threshold size for both images in opposite directions until this criterion was met. In this way, we minimized the potential bias due to initial contrast differences between the control and treatment images. Pairwise *t*-test (two tails) was used to compare percent total image occupied by embolized tracheids. To estimate percent embolized tracheids, we removed the percent total area occupied by cell wall, 69.7% (±3.1% SD, $n = 10$) based on our images, from the percent total area occupied by embolized tracheids. We estimated percent total area occupied by cell wall by analyzing ten randomly selected groups of 5 to 15 embolized tracheids, in which the cell wall was still wet.

Xylem sap soluble sugar content

We soaked branch segments with sealed ends for 90 h, as described previously. After soaking, we centrifuged debarked branch segments at 12 000 r.p.m. to remove xylem water. Segments were placed inside centrifuge tubes with a small plastic ring inside that prevented segments from touching the tube's end, allowing for xylem sap to collect. Next, we analysed xylem water soluble sugar concentration colorimetrically using anthrone (Leyva *et al.* 2008). Specifically, 50 μL of the diluted extracted xylem water (×25) was mixed with 150 μL of sulfuric acid (98%) anthrone (0.1%, w/v) solution in a 96 micro-plate well and placed on ice (<4 °C) for 10 min. Plates were heated at 100 °C for 20 min and then allowed to equilibrate to room temperature for 20 min (ca. 20 °C). The sugar levels were

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determined as glucose equivalent from the colorimetric reading (Thermo Scientific Multiskan) of absorbance at 620 nm (A_{620}) and a predetermined standard curve (0, 0.01, 0.03, 0.1 and 0.3 mg L^{-1} glucose). Equivalent osmotic potential was calculated using the Morse equation, $\Psi_{solsug} = iMRT$, where i is the van't Hoff factor (1 for glucose), M is the molarity, R is the ideal gas constant and T is the absolute temperature.

Crown wetting weather event occurrence and duration

We examined the daily occurrence and duration of crown wetting weather events at Maple Creek, CA, Remote Access Weather Station (RAWS) from 1 January 2000 to 31 December 2005. This station is representative of the weather experienced within redwood habitat along the north coast of California. A crown wetting weather event was defined as occurring when daily average relative humidity equaled 100% and daily precipitation was greater than zero. These criteria likely include some days in which the bark was not wet, for example due to insufficient precipitation, and also likely excluded foggy days in which precipitation was not measured. Despite these shortcomings, we used RAWS data from 2000 to 2005 to calculate the occurrence and duration of weather events that would likely lead to crown wetting, that is, crown wetting weather events.

RESULTS

Water enters xylem through bark

In all tested branches, segment mass increased with soak time over a period of 90 h. Percent increase in branch mass changed most rapidly over the first 2 h. While substantial variability existed in water uptake quantity and rate, the general pattern of saturating uptake was present across all segments examined (Fig. 2a). Over a period of 90 h, segment mass increased by 3.0 and 15.3% of its initial weight. Notably, percent mass increase of bark was approximately equal to the increase in volume change of bark. This was not the case for the xylem, however, where after 16 h of water soaking, mass increased by 7.6% on average but volume change was only 2.9% (Fig. 2b; $n = 4$, $P < 0.05$). This discrepancy between change in xylem mass and volume suggests that water moving into the xylem enters spaces formerly filled with air.

In branch segments, bark and xylem increased mass with soaking time. However, the cambial zone of intact branches might form substantial hydraulic resistance in the path from bark to xylem. To test if water entered the xylem from the bark, we soaked branch segments using ^{18}O-enriched water. After 16 h of soaking with enriched water, the $\delta^{18}O$ values of water collected from xylem increased significantly, from -2.1 ± 3 to 393 ± 50‰ (mean ± SE; Fig. 2c), demonstrating that the hydraulic path linking bark with xylem is present in intact branch segments. No difference between water collected from initial segments and DI-soaked segments was detected (not shown in figure).

Microcomputed tomography analysis provided indirect evidence that water entered the bark and decreased the percent embolized tracheids (Fig. 3). Control branch segments ($\Psi_{br} = -4.5$ MPa), which were not soaked prior to scanning, had 42% embolized tracheids on average. Soaked branch segments had 38% embolized tracheids on average. Pairwise comparison showed that soaked branches had 3.8% ± 1.2% ($n = 4$, $P < 0.05$) fewer embolized tracheids than unsoaked branches.

Soaking restores hydraulic conductivity in isolated branch segments and whole branches

Isolated branch segments

Despite the seemingly small mass of water entering the xylem via bark, it appears to have a biologically meaningful impact. Water-soaked branch segments showed a significant increase of sapwood specific hydraulic conductivity (K_s; Fig. 4a). Initial K_s values ranged from 0.67 to 1.36 kg s^{-1} m^{-1} MPa^{-1}. After 16 h of soaking, K_s values ranged from 0.84 to 1.42 kg s^{-1} m^{-1} MPa^{-1}. These values fall within the range of previously observed values (Burgess *et al.* 2006; Ambrose *et al.* 2009). Final K_s increased by 0.1 ± 0.05 kg s^{-1} m^{-1} MPa^{-1} (Fig. 4a, $\underline{n} = 12$, $P < 0.05$, two-tailed pairwise student's *t*-test), or 13%, with branch segments increasing conductivity up to 47% (Fig. 4a).

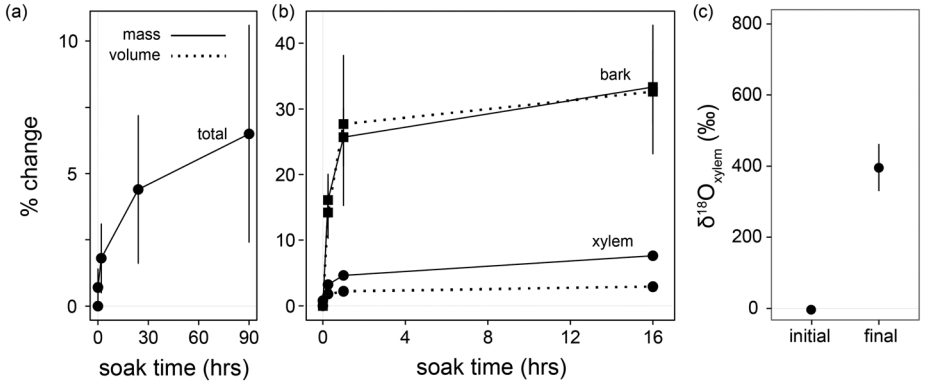

Figure 2. (a) Percent change in total mass of branch segments ($n = 9$) as a function of soak time. Errors bars show ±1 standard deviation. (b) Percent change in mass and volume of bark and xylem ($n = 4$) as a function of soak time. Error bars show ±1 standard deviation (not visible for xylem in graph). (c) Xylem $\delta^{18}O$ values before and after soaking bark in ^{18}O-enriched water ($n = 4$; $P < 0.05$, pairwise *t*-test).

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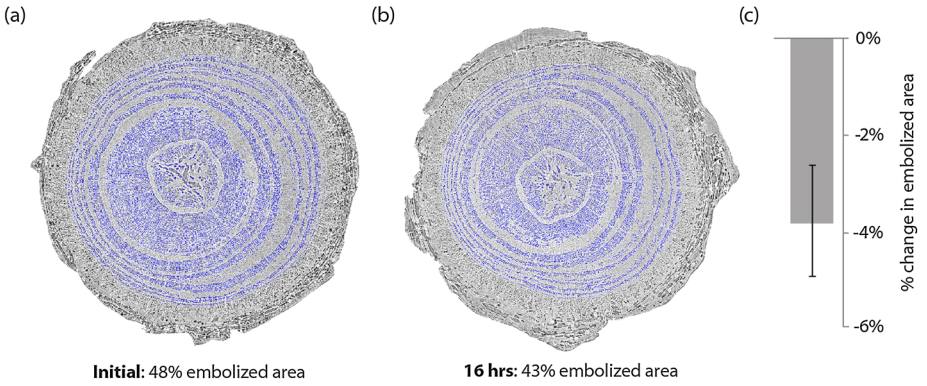

Figure 3. (a) Microcomputed tomography (microCT) image of branch segment prior to soaking in water. Embolized area (shaded blue) = 48%, which corresponds to the sample shown. (b) MicroCT image of branch segment after soaking in water. Embolized area (shaded blue) = 43%, which corresponds to the sample shown. (c) Percent change in embolized area between before and after soaking of adjacent branch segments ($n = 4$; $P < 0.05$, pairwise *t*-test). Error bar shows ±1 standard error.

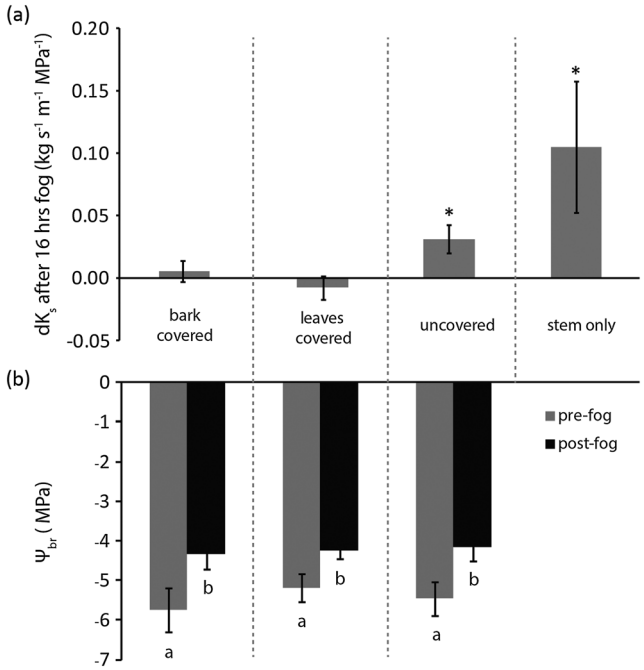

Figure 4. (a) Change in K_s after 16 h of soaking with fog with covered bark, covered leaves, uncovered bark and leaves ($n = 7$; $P < 0.05$, pairwise *t*-test) and stem segment only ($n = 12$, $P < 0.05$, pairwise *t*-test). Error bars show ±1 standard error. (b) Ψ_{br} before and after 16 h of soaking with fog with covered bark, covered leaves and uncovered bark and leaves ($n = 7$; $P < 0.05$, Tukey HSD). Error bars show ±1 standard error.

K_s of branch segments covered with Parafilm, which sat on the bench for 16 h, did not differ significantly from the initial value (Fig. 4a).

Whole branches

Branches from all three treatments, leaves covered, bark covered and bark/leaves uncovered, significantly increased Ψ_{br} from about −5.5 to −4.2 MPa following fog treatment for 16 h (Fig. 4b, $n = 7$, $P < 0.05$, two-tailed pairwise student's *t*-test). However, Ψ_{br} was not different across treatments before or after fog (Tukey's HSD test). Prior to bench drying, K_s values ranged from 0.2 to 0.5 kg s^{-1} m^{-1} MPa^{-1} (not shown). Following bench drying, and prior to soaking, K_s was 0.19 ± 0.12 kg s^{-1} m^{-1} MPa^{-1} (±1 SD). Interestingly, only uncovered branches significantly increased K_s after being soaked in the fog chamber for 16 h (Fig. 4a, $P < 0.05$, two-tailed pairwise student's *t*-test). On average, branch segments from uncovered branches increased K_s by 0.03 ± 0.01 kg s^{-1} m^{-1} MPa^{-1} (Fig. 4a, $P < 0.05$, two-tailed pairwise student's *t*-test), or 56% on average, with branch segments increasing conductivity up to 137% (Fig. 4a). When the leaves or bark were covered, K_s was not significantly different before and after fog treatment.

Xylem osmotic potential is related to long-term bark water uptake rate

An osmotic gradient is the putative driving force for moving water from the bark into the xylem via a symplastic route (Zwieniecki & Holbrook 2009), so we examined soluble sugar concentration of xylem sap. In the six branch segments examined, soluble sugar concentration inside the xylem after 90 h of soaking correlated well ($R^2 = 0.88$) with the percent change in mass of the branch segment (Fig. 5).

Extended periods of crown wetting weather are common in redwood habitat

We examined the duration and occurrence of crown wetting weather at Maple Creek, CA Remote Access Weather Station (RAWS) from 1 January 2000 to 31 December 2005. Crown wetting weather events most frequently lasted for 24 h (Fig. 6; median: 15.5 events per year; range: 14 to 25 events per year). Multi-day events were less common, with 6 to 9 events per year (median: 6.5 events per year) lasting 48 h and 0 to 4 (median: 1.5 events per year) lasting 72 h. Events lasting up to 192 h occurred, but less frequently, during the 6 year period examined. Annually, 696 to 1560 h (average: 1048 h) of crown wetting weather occurred at Maple Creek, CA (Fig. 7a). While crown

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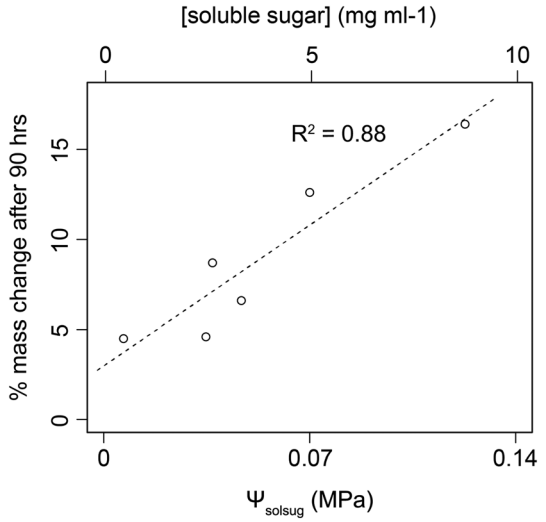

Figure 5. Percent mass change of branch segments ($n = 6$) after 90 h as a function of soluble sugar concentration in xylem liquid extract (upper *x*-axis). Corresponding osmotic potential (MPa) is shown on lower *x*-axis.

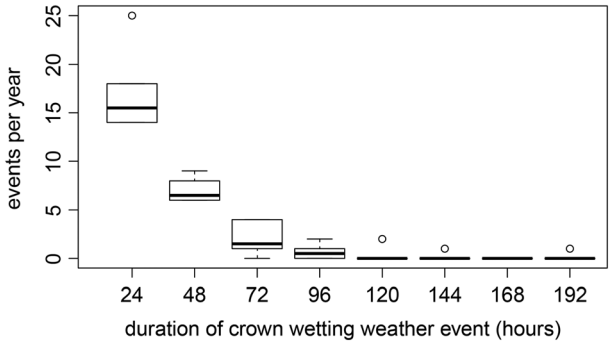

Figure 6. Box plot showing occurrence of crown wetting weather events (events per year) of different durations at Maple Creek, CA, Remote Access Weather Station from 2000 to 2005.

wetting weather events can occur any month, they were most common from November through May (Fig. 7b).

DISCUSSION

Coastal redwood is the tallest tree species in the world, often reaching more than 100 m in height. Such record heights require lifting water to the top of the crown and maintaining water column continuity. Even under fully hydrated conditions, doing so requires up to ~1.0 MPa tension in the tree crown. Leaf water potential can drop significantly during active transpiration, decreasing branch and leaf hydraulic conductivity. Previous studies showed how foliar water uptake from fog, snow and rain facilitates leaf rehydration and hydraulic recovery (Burgess & Dawson 2004; Burgess *et al.* 2006; Ambrose *et al.* 2009; Laur & Hacke 2014; Mayr *et al.* 2014), but did not examine the role of bark in twig and branch rehydration. We identify a novel mechanism for xylem hydraulic recovery of coastal redwood. Soaked bark supplies water over a multi-hour period of time that increases xylem hydraulic conductivity in branches, most likely by refilling embolized tracheids. Our findings suggest that hydraulic recovery occurs locally in the coastal redwood branches, a potentially important yet physically not well-understood process that would facilitate refilling in tall trees.

Previous studies showed that some coniferous species exhibit similar mass/volume uptake curves as observed in coastal redwood (Fig. 2a & b) when branches are soaked in water or when exposed to fully saturated air (Katz *et al.* 1989; Liu 1998; Laur & Hacke 2014). For example, defoliated twigs of *P. abies* increased water content by 6 to 8% (±1% SE) in 200 min (Katz *et al.* 1989). These water uptake rates are at the higher end of what we observed in coastal redwood. In another study, defoliated branches of *Picea glauca* reported a ~4% increase in relative water content after soaking, although this increase was not significant (Laur & Hacke 2014). When studying rainfall interception by tree stems, Liu (1998) observed that not all water absorbed by bark is released, which they presume is because of xylem uptake. Similarly, we find that coastal redwood branch segments increase mass by 4.5% on average in a 24 h period (Fig. 2a). A change in branch mass alone, however, does not prove that water moves beyond the bark/phellem into the xylem. Our observations that $\delta^{18}O$ labelled water permeated bark into the xylem of excised branch segments (Fig. 2c) and that embolized area decreased following soaking of excised branch segments according to microCT images (Fig. 3c) provide novel evidence that coastal redwood branches take water into the xylem via the bark surface. These findings agree with one previous observation in *P. glauca* where dye sprayed on bark was later found in stem parenchyma rays (Katz *et al.* 1989).

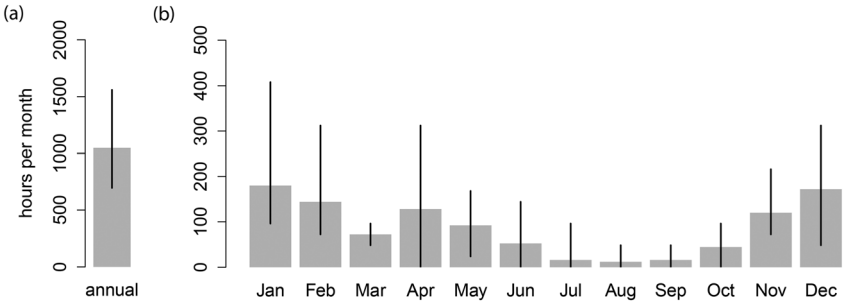

Figure 7. (a) Average hours of crown wetting weather annually at Maple Creek, CA, Remote Access Weather Station from 2000 to 2005. Error bars show minimum and maximum. (b) Average hours of crown wetting weather monthly at Maple Creek, CA, RAWS from 2000 to 2005. Error bars show minimum and maximum.

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Water saturating the entire branch surface (both leaves and bark) at high relative humidity is capable of partially restoring hydraulic conductivity. Hence, it is biologically meaningful, especially over the course of multiple rain/fog events. Our findings shed new light on recent investigations of foliar water uptake. After 5 d of soaking in water (bark + leaves), percent loss in conductivity of *Picea abies* decreased from 86 to 29% (Mayr *et al*. 2014). Similarly, exposing drought-treated *P. glauca* to 100% relative humidity (i.e. fog) reduced percent loss in conductivity from 16 to 3% (Laur & Hacke 2014). Our findings suggest that wetting not only leaves, but simultaneously bark, is necessary for recovering conductivity – particularly in leafless distally located parts of the branch. Recovery of conductivity was not observed when we covered either the leaves or bark of whole branches (Fig. 4a). Moreover, as soaking isolated branch segments restored hydraulic conductivity, we suspect that if either bark or leaves are not exposed to high humidity, they become a sink for water, which prevents localized embolism removal. Thus, only when the entire branch surface, or an excised segment, was wet could localized hydraulic recovery proceed. Such localized recovery requires that soaked bark provides a more energetically favorable path for water movement to nearby xylem conduits than distant leaves.

This study does little to elucidate the cellular pathway along which bark water recovery occurs. However, our finding that the soluble sugar concentration in xylem sap is related to bark water uptake rate suggests that water entry is at least partly facilitated by a symplastic route. Based on this finding, personal observations and prior studies, we propose the following mechanism of water movement from bark into xylem. When a water droplet is placed on bark of coastal redwood, it quickly spreads across the surface, filling hydrophilic capillary spaces of the rhytoderm, that is, the outermost layer composed mostly of dead phloem and cortical cells (personal observation). Thus, a rain or fog event could wet the outer bark surface in minutes to hours, increasing its water potential close to zero. Directly beneath this layer, water flow encounters suberized phellem tissue (i.e. cork cells) whose high hydraulic resistance varies within individual plants and among species depending on phellem thickness, dry mass and density (Schönherr & Ziegler 1980; Groh *et al*. 2002). Based on dye tracing, Schönherr & Ziegler (1980) provide evidence that water moves primarily through the hydrophilic, non-suberized regions of the phellem cell wall. Driven by an osmotic gradient, water likely enters parenchyma cells via aquaporin channels (Laur & Hacke 2014; Mayr *et al*. 2014) and more slowly by diffusion across the cell membrane. In coastal redwood, every tracheid is directly connected to at least one ray parenchyma cell (Choat *et al*. 2014). This ray-tracheid connection could provide an entry point for water from the symplast into the tracheid for refilling, as observed by Katz *et al*. (1989). Moreover, coastal redwood xylem sap contains up to 8.5 mg ml^{-1} soluble sugar (Fig. 5), which could be highly concentrated at the ray-tracheid interface of embolized conduits. Thus, a steep osmotic gradient may exist, which could drive water from the symplast into embolized tracheids similar to that described by Zwieniecki & Holbrook (2009).

We assumed that Ψ_{br} should be at or near 0 MPa before hydraulic recovery could occur, such that capillary and osmotic forces could overcome the hydrostatic forces that prevent air bubble dissolution (Yang & Tyree 1992; Zwieniecki & Holbrook 2009). Unexpectedly, Ψ_{br} only increased from about -5.5 ± 0.4 to -4.2 ± 0.3 MPa following fog treatment for 16 h. Laur & Hacke (2014) observed a similar pattern in which substantial hydraulic recovery occurred, despite stem water potential only rising from -2.9 to -2.1 MPa after 50 h at 100% relative humidity. This repeated observation is intriguing and suggests that localized changes in water potential and hydraulic isolation may be occurring, possibly described by the disjoining pressure theory (Gouin 2015). Without further theoretical and experimental studies on hydraulic isolation, however, we cannot explain how hydraulic recovery could occur without reducing Ψ_{br} close to zero. In addition, it is noteworthy that branch water potential increased to similar levels regardless of the treatment (i.e. bark covered, leaves covered or uncovered). We currently have no good explanation for this observation, but it could be an interesting topic of future research.

Why do redwood saplings not recover embolism when watered from the roots (Choat *et al*. 2014)? Although we do not provide direct evidence of refilling, we provide strong indirect evidence from adjacent branch segments and whole branches, that is, $\delta^{18}O$ values, microCT and hydraulic recovery. Based on this study, we expect recovery to only occur when bark and leaves are wet concurrent to 100% relative humidity, that is, during rain or fog. It is possible that Choat *et al*. (2014) did not see embolism recovery because the leaves and bark were not wet following rewatering. Instead, they possibly created a scenario similar to when we exposed branches with leaves/bark covered to fog, in which we did not observe hydraulic recovery. Future investigations should resolve this discrepancy.

Because extended periods of crown wetting weather are common in redwood habitat, we suspect localized hydraulic recovery is also common. On average, Maple Creek, CA RAWS station experienced 1048 h of crown wetting weather during a single year (Fig. 7a) – mostly as 24 and 48 h events (Fig. 6). Based on our observations that 16 h in the fog chamber increases K_s by ~0.03 kg s^{-1} m^{-1} MPa^{-1}, and assuming that well-watered K_s for these branches is ~0.60 kg s^{-1} m^{-1} MPa^{-1} (average of observed initial values for excised branch segments and whole branches), twenty 24 h crown wetting events (or 480 h total) could be sufficient to fully recover K_s in these branches. This assumes that no additional loss in K_s occurs between surface wetting events and a linear relationship between time and recovery of K_s. Even if substantial loss occurs between wetting events, the total hours of crown wetting weather at Maple Creek suggest that bark and leaves are likely wet for a substantial fraction of the time – especially from November through May when evaporative demand is low. Thus, bark and leaves would likely recover a meaningful fraction of coastal redwood's hydraulic conductivity in its typical habitat.

Our research raises the question: Is bark-facilitated hydraulic recovery specific to high rainfall/fog regions or does bark in all woody species has such transport capacity? For now, we can only suggest that *S. sempervirens* could utilize localized

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hydraulic recovery to cope with temporal variation in water demand and availability. Under climate change conditions, *S. sempervirens* habitat is already experiencing dramatic changes in the length and frequency of rain/fog events (Johnstone & Dawson 2010). Building on our findings, less frequent and shorter periods of rain/fog may reduce the capacity of the crown to recover conductivity following stress and, as a result, endanger further survival of the world's tallest trees.

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