

AN ANNUAL PATTERN OF NATIVE EMBOLISM IN UPPER BRANCHES
OF FOUR TALL CONIFER SPECIES¹

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- *Premise of the study:* The Pacific Northwest of North America experiences relatively mild winters and dry summers. For the tall coniferous trees that grow in this region, we predicted that loss in the hydraulic conductivity of uppermost branches would be avoided because of difficulty reversing accumulated emboli in xylem that is always under negative pressure.
- *Methods:* To test this hypothesis, we measured native percent loss in hydraulic conductivity (PLC; the decrease of in situ hydraulic conductivity relative to the maximum) monthly throughout 2009 in branches at the tops (~50 m) of four species in an old growth forest in southern Washington.
- *Key results:* Contrary to our prediction, freeze–thaw cycles resulted in considerable native PLC. Branches showed hydraulic recovery in the spring and after a moderate increase in native embolism that was observed after an unusually hot period in August. The September recovery occurred despite decreases in the leaf and stem water potentials compared to August values.
- *Conclusions:* Recoveries in branches of these trees could not have occurred by raising the water potential enough to dissolve bubbles simply by transporting water from roots and must have occurred either through water absorption through needles and/or refilling under negative pressure. Excluding the August value, native embolism values correlated strongly with air temperature of the preceding 10 d. For three species, we found that branches with lower wood density had higher specific conductivity, but not greater native PLC than branches with higher wood density, which calls into question whether there is any hydraulic benefit to higher wood density in small branches in those species.

Key words: *Abies grandis*; hydraulic conductivity; *Pseudotsuga menziesii*; *Thuja plicata*; *Tsuga heterophylla*; wood density.

The dominant conifers of the Pacific Northwest of North America are some of the tallest trees in the world. Ranging from northern California to southeastern Alaska, some individuals of these trees routinely achieve 50+ m in height. From a water transport perspective, these heights present a stressful condition, assuming that the water being supplied to the foliage is transported through the plant. The pressure in the xylem water at the top of the tree will be lower than that at the bottom of the tree both due to the force of gravity on the water column (causing a decline of 0.01 MPa·m⁻¹) and due to the friction of pulling water through the microscopic xylem conduits (also measured in MPa·m⁻¹). Thus, all else being equal, the uppermost branches of a taller tree must have a lower xylem pressure to draw water from the soil than those of a shorter tree.

The climate of this region is also stressful to plants. The summers are generally hot and dry with little rainfall. Although the

severity of the summer dry season varies across the region, our study site, the Wind River Canopy Crane Research Facility (WRCCRF), often receives little rain from May to September (20-yr mean ≤ 50 mm of rain per month) with mean July maximum air temperatures of 26.1°C (30-yr means from 1978 to 2008 at the Carson Fish Hatchery ~5 km north of the crane site; <http://cdo.ncdc.noaa.gov/dly/DLY>). During these hot, dry months, the 50–60-m-tall trees at the WRCCRF deplete the soil surrounding their roots of water (Meinzer et al., 2007), and tree growth is limited (Franklin and Waring, 1980). Presumably, this seasonal dry period could cause reduced xylem hydraulic conductivity (volume flow rate per pressure gradient) unless stomatal control of transpiration prevented xylem pressure from falling below thresholds associated with introduction of water-stress-induced emboli to the xylem. The vast majority of the 2223 mm of mean annual precipitation at WRCCRF falls during the winter months as rain, snow or sleet with maximum temperatures in January averaging 3.4°C and minimum temperatures averaging -2.7°C (<http://cdo.ncdc.noaa.gov/dly/DLY>), resulting in many freeze–thaw cycles.

Freeze–thaw cycles are important hydraulically because they can result in embolism formation (Sucoff, 1969; Robson et al., 1988). According to the “thaw–expansion hypothesis”, this process occurs when water in xylem conduits freezes and the air that had been dissolved comes out of solution and forms bubbles. When the water subsequently thaws, the bubbles can either dissolve back into solution, or if there is tension in the water column during the thaw, expand and fill the conduit with air, thereby embolizing the conduit. When a freeze–thaw cycle plus tension creates enough embolisms within a stem, the hydraulic conductivity decreases. Work in both conifers (Sperry and

¹Manuscript received 10 December 2010; revision accepted 21 March 2011.

This project was funded by National Science Foundation grant IBN 09-19871. We are very grateful to L. Bernart, M. Loloum, and S. du Peloux du Saint Romain for assistance with measurements. We are also indebted to the employees at the Wind River Canopy Crane Research Facility, particularly M. Schroeder, M. McCord, K. Anlauf, R. Johnson, and K. Kirchner were helpful with sample collection, and S. Voelker and M. Huso provided very useful discussions on statistics.

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TABLE 1. Morphological and anatomical characteristics (mean $\pm$ SD) of four tall conifer species. Stem diameters were measured after bark was removed. For variables that showed differences between the species, different letters indicate values that differed in post hoc LSD tests in 1-way ANOVAs ($P < 0.05$). d = diameter.

Species	Height (m)	Stem diameter (mm)	Conduit d (μ m)	Hydraulic d (μ m)	Conduit frequency (mm^{-2})
<i>Abies grandis</i> (grand fir)	49.8 $\pm$ 3.3 a	3.4 $\pm$ 1.1	9.5 $\pm$ 0.7 a	12.7 $\pm$ 0.8 a	5191 $\pm$ 136 a
<i>Pseudotsuga menziesii</i> (Douglas-fir)	60.1 $\pm$ 3.2 b	3.6 $\pm$ 0.8	11.3 $\pm$ 0.3 b	13.4 $\pm$ 0.5 ab	4214 $\pm$ 138 ab
<i>Thuja plicata</i> (western redcedar)	50 $\pm$ 3.9 a	3.8 $\pm$ 1.0	10.1 $\pm$ 0.6 ab	13.1 $\pm$ 1.4 a	4435 $\pm$ 688 ab
<i>Tsuga heterophylla</i> (western hemlock)	54.3 $\pm$ 1.0 a	3.6 $\pm$ 0.8	11.4 $\pm$ 0.5 b	15.8 $\pm$ 0.9 b	4044 $\pm$ 52 b

Robson, 2001; Pittermann and Sperry, 2003) and angiosperms (Davis et al., 1999) has shown that the likelihood of a single freeze–thaw cycle at a given, relatively mild tension causing a decline in hydraulic conductivity significantly increases when the mean tracheid or vessel diameter is greater than 44 μ m. However, plant organs with narrower average conduit diameters are not always safe from the damaging effects of freeze–thaw cycles. A number of studies have observed loss of hydraulic conductivity in species with narrow conduits ($<44 \mu\text{m}$) exposed to repeated freeze–thaw cycles (Sperry and Sullivan, 1992; Sperry et al., 1994; Mayr et al., 2003a, b, 2006) or when the thaw was accompanied by severe water stress (Langan et al., 1997; Pittermann and Sperry, 2006).

Regardless of whether embolism is drought-induced or formed because of a freeze–thaw cycle, it is well established that plants can refill these nonfunctional conduits when the xylem pressure is negative but close to 0 MPa (e.g., Sobrado et al., 1992; Edwards et al., 1994). To shrink a bubble and refill a conduit, the pressure in the xylem (P_x) surrounding the bubble must be $> -2T/r_b$, as defined by La Place's law (Yang and Tyree, 1992), where T is the surface tension of water (0.0728 Pa m) and r_b is the radius of the air bubble, which in an embolized tracheid is approximately the radius of the tracheid (Tyree and Zimmermann, 2002). Thus, to refill embolisms at the tops of tall trees, at least one of three conditions must be met. First, the conduit diameter must be narrow enough to meet the requirements of La Place's law. For example, at the top of a 50 m tree, the least negative water potential possible should be -0.5 MPa due to the gravitational gradient. The bubble diameter, and therefore conduit diameter, that could be refilled at this pressure is less than 1 μ m if the bubble is composed of air (less pressure is needed to refill bubbles composed of water vapor, so slightly wider conduits could be refilled at this pressure). Second, the water potential gradient must be decoupled from the gravitational gradient. For example, if water from dew or fog could be absorbed by the leaves (Burgess and Dawson, 2004; Limm et al., 2009; Simonin et al., 2009), the xylem pressure could rise above the pressure predicted based on the height of the tree. This scenario would allow xylem pressures that could refill wider conduits. Third, the embolized conduits must somehow be isolated from the surrounding xylem so the pressure can be raised and the conduit refilled via "novel" refilling (Salleo et al., 1996; Bucci et al., 2003; Hacke and Sperry, 2003; Clearwater and Goldstein, 2005; Zwieniecki and Holbrook, 2009; Secchi and Zwieniecki, 2010). Novel refilling is not well understood (Zwieniecki and Holbrook, 2009; Secchi and Zwieniecki 2010), but has been shown to allow embolism refilling at average xylem pressures more negative than predicted possible by La Place's law.

Given that increased tree height simulates an aridity gradient and that the climatic conditions of the Pacific Northwest can be hydraulically stressful in both summer and winter, we asked whether the small diameter branches at the tops of tall conifers experience seasonal changes in native embolism, which is a

measure of the in situ hydraulic conductivity relative to the potential maximum conductivity. Because of our assumption that it would be very difficult for embolized conduits high in trees to refill, we hypothesized that healthy upper branches would not develop high levels of native embolism throughout the summer or winter.

MATERIALS AND METHODS

Site and species—Branches from four species of conifer were examined throughout 2009: grand fir [*Abies grandis* (Dougl.) Forbes], Douglas-fir [*Pseudotsuga menziesii* (Mirbel) Franco.], western redcedar [*Thuja plicata* Donn.], and western hemlock [*Tsuga heterophylla* (Raf.) Sarg.]. These species are the four main overstory conifers growing in an old growth Douglas fir–western hemlock forest in southern Washington at the Wind River Canopy Crane Research Facility (45°49'13.76"N, 121°57'06.88"W). This facility has a 75-m-tall canopy crane with an 85-m-long jib to which a gondola is attached to provide access to the crowns of the trees. Four (for grand fir) to seven representatives from each species were selected based on height, availability, and a healthy appearance.

Material collection—Beginning in January 2009, two replicate branches were collected each month (except February) from within 2–3 m of the tops of each of three individuals from each species before or near dawn. Thus, the mean measurement heights ranged from ~ 47 m in grand fir to ~ 58 m in Douglas-fir (Table 1). A severe snowstorm in late February prevented us from using the crane on our scheduled date, so two collections were made on 3 and 26 March. Where possible, the same individuals were not sampled during successive months to avoid overharvesting distal branches on these slowly growing trees. During the rainy winter months, excess water or snow was shaken from the branches before bagging the samples in sealable plastic bags. Despite our efforts to eliminate excess water, the samples remained wet during the transport to the laboratory. In the summer, a wetted paper towel was added to the bags containing the samples to prevent further drying. All samples were stored in a cooler to minimize temperature changes during the several-hour transport to the laboratory at Oregon State University for analysis. Keeping the samples cool and away from light also delayed potential embolism refilling during transport to the laboratory.

Water potential measurements—We measured predawn and midday water potentials on sampling dates when there was no precipitation and temperature was above 0°C. Water potential was not measured during sub-0°C days because of the difficulty interpreting values from frozen or partially frozen samples. The water potential of ice is much lower than the water potential of liquid water (Pearce, 2001). The weather permitted us to measure water potentials from 20 May 2009 to 22 September 2009. On each of these dates, the water potential was measured on small shoots (diameter ~ 2 –3 mm including bark) from each individual from which branches were taken (three individuals per month from each species) using a pressure chamber (PMS Instrument Co., Corvallis, Oregon, USA). During the predawn visit, one branch on each tree was bagged with a sealable plastic bag that had been covered with aluminum foil. This bag allowed the water potential of the enclosed part of the branch to equilibrate and permitted the branch water potential to be estimated from easily measureable shoots. Measurements were made on unbagged samples before dawn and on bagged and unbagged branches at midday (1100–1300 hours PDT). Samples were taken from the same branches as those from which the hydraulic samples were taken.

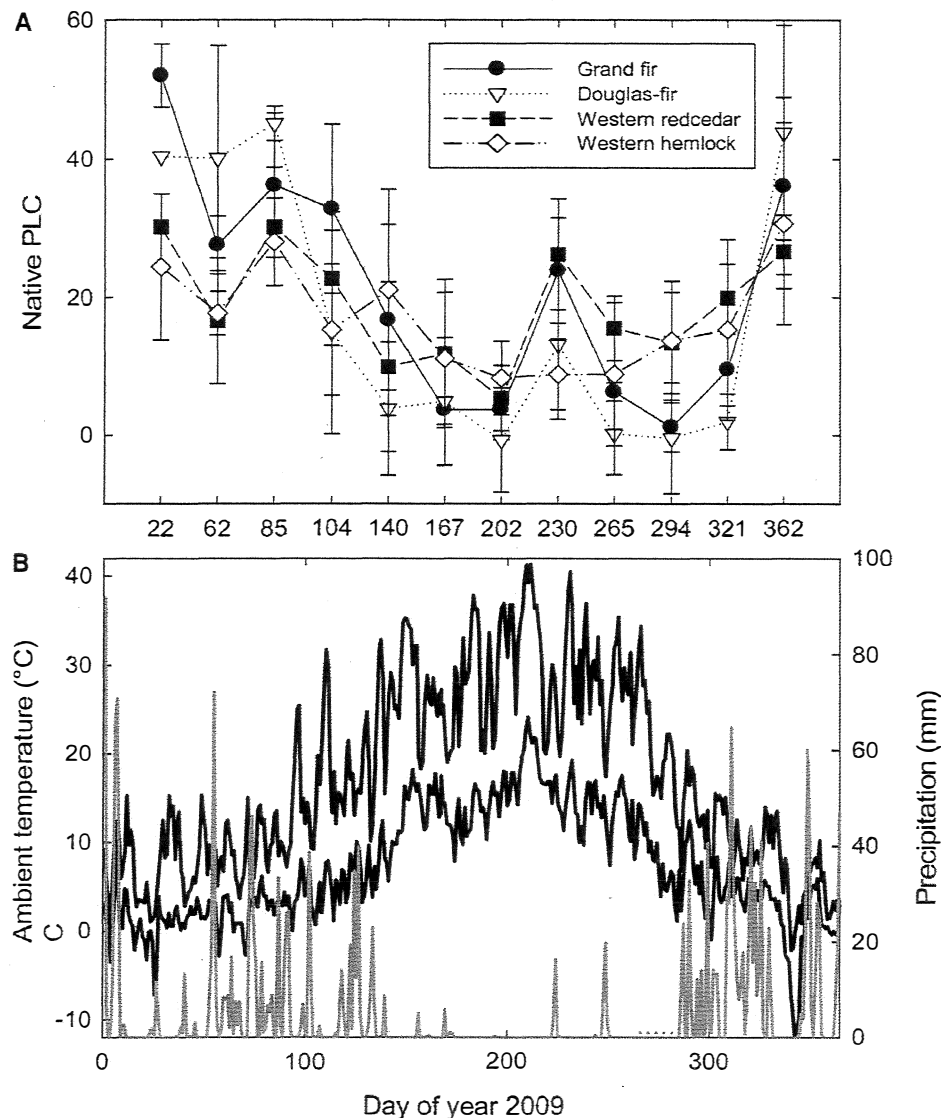


Fig. 1. (A) Native percent loss in hydraulic conductivity (PLC) vs. sampling date of 2009 for small-diameter branches from four conifer species. Symbols show means of six samples per species and error bars are one standard deviation. Days shown on the x-axis are the actual day samples were taken. (B) Meteorological data for 2009 for the Wind River Canopy Crane Research Facility. Daily maximum and minimum temperatures (black lines, left axis) are from a weather station just above the canopy (70 m height). Precipitation data (gray lines, right axis) are from an open field station 0.8 km from the crane. The gray dots between days 200 and 300 indicate a short period during which the precipitation station was not functioning.

Native embolism measurements—Each month, the native hydraulic conductivity relative to the maximum value was measured for six branches from each species (see *Material collection* section for details on sampling). Sun-exposed branches were selected near the tops (within 1.5 m) of healthy, tall trees and were clipped in air from the tree approximately 3 cm basally from the segment that would be used for hydraulic measurements. This distance should be long enough to avoid introducing embolisms to the segment on which we would measure hydraulic conductivity. In the laboratory, stem segments 5–8 cm long and ~3 mm in diameter (Table 1) were cut under water to avoid tracheids that may have embolized during collection, and the edges were trimmed (under water) using a fresh razor blade. The stems were then fitted to solution-filled tubing attached on the upstream end to a solution reservoir approximately 90–100 cm above the sample and on the downstream end to tubing attached to a graduated pipette. The perfusion solution was filtered and deionized water acidified to pH 2 with HCl. The native specific hydraulic conductivity (K_{nat}) was then measured by dividing the volume flow rate of water

through the pipette by the pressure gradient driving flow (derived from the height of the reservoir relative to the sample and sample length), and dividing by xylem cross-sectional area. After K_{nat} had been measured, all samples were submerged in perfusion solution under a partial vacuum for ~20 h to refill any embolized tracheids. The next morning, the samples were inspected for bubbles on the cut ends (none were ever seen), and the specific hydraulic conductivity was remeasured and termed the maximum hydraulic conductivity (K_{max}). The native percent loss in conductivity, or native embolism value, was calculated as $[(K_{\text{max}} - K_{\text{nat}})/K_{\text{max}}] \times 100$.

Wood density and anatomical measurements—Wood density was determined for each branch used for hydraulic measurements and a subset of branches was sampled for anatomical measurements. Wood density ($\text{g}\cdot\text{cm}^{-3}$) was calculated from the fresh volume of the sample, determined using Archimedes' principle (Smith, 1955), divided by the dry mass, which was measured after drying samples in an oven at 70°C for 2 d.

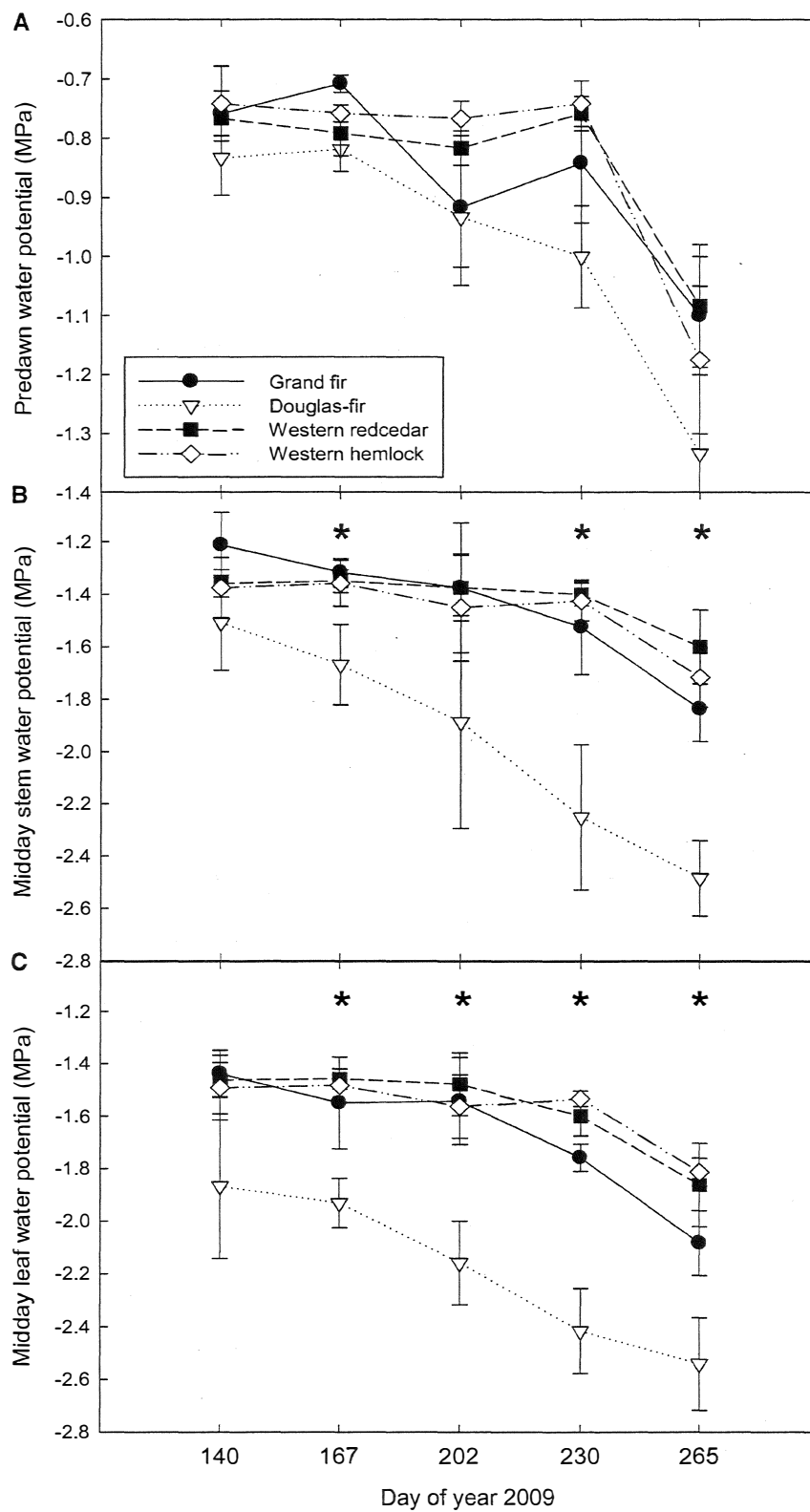


Fig. 2. Monthly (A) predawn, (B) midday stem, and (C) midday leaf water potentials for four conifer species during summer 2009. Symbols indicate means of three measurements per species, and error bars show standard deviations. Asterisks specify months that Douglas-fir water potentials were significantly lower than the other species ($P < 0.05$).

For each species, two of the hydraulics samples were used for anatomical measurements. From each of these samples, cross sections were made using a sliding microtome and mounted in glycerin. Images of the cross sections were taken using a digital camera mounted on a Nikon Eclipse E400 compound light microscope. The image-analysis program ImageJ (National Institutes of Health, USA; <http://rsb.info.nih.gov/ij/>) was used to measure the lumen area of all tracheids in three to four lines running radially from the bark for the youngest four years. At least 300 tracheids were measured from each branch. Lumen cross-sectional area was converted to diameter (d) by assuming the tracheids were square using $d = A^{1/2}$ where A is the cross-sectional lumen area. The total area of the lines of tracheids (measured by tracing approximately through the middle of the tangential walls of neighboring tracheids) was determined to calculate the number of tracheids per area (conduit frequency). The mean tracheid hydraulic diameter, which is the diameter of a tracheid with the average Hagen–Poiseuille lumen conductivity, was estimated as $(\Sigma d^4/n)^{1/4}$, where n is the number of conduits measured in the sample.

Statistical analyses—Measurements of native PLC were compared throughout the year with an ANOVA with measurement day as a categorical variable and the mean native PLC for each tree as the dependent variable. Species was treated as a random factor. Autocorrelation of repeated measures within individuals across measurement days was assessed and determined to be marginal (0.17). A similar ANOVA was used to compare the change in stem, and leaf midday and predawn water potentials over time. These ANOVAs were performed in SAS version 9.2 (SAS Institute, Cary, North Carolina, USA). For the comparison of maximum specific conductivity vs. wood density, lines were fitted and compared with the standard major axis (SMA) approach using SMATR freeware (Warton et al., 2006; website <http://www.bio.mq.edu.au/ecology/SMATR/>).

RESULTS

All species showed marked seasonal changes in the degree of native loss of conductivity in their branches, reflecting seasonal differences in the amount of embolism during 2009 (Fig. 1A). In spite of the year's weather (Fig. 1B) being warmer and drier than average, the native percent loss of conductivity (PLC) was much higher in the winter months than in the summer months. This result was supported by type 3 tests of fixed effects of the ANOVA, which indicated a strong species $\times$ day interaction ($F_{33,77} = 2.77$, $P = 0.0001$). Further examination of this interaction showed that for each species the mean native PLC from the months with freeze–thaw events was significantly higher than the months without freezes (Table 2). The exception to the low values during the summer months was August, which showed significant increases in native PLC relative to July in grand fir ($t = -4.25$, $df = 10$, $P = 0.002$) and western redcedar ($t = -4.05$, $df = 10$, $P = 0.003$), and a nonsignificant increase in Douglas-fir ($t = -1.5$, $df = 10$, $P = 0.07$). This spike may have been caused by the extremely high temperatures during the 28 d between the July and August sampling dates: the mean maximum temperature was 31°C, and 10 d exceeded 35°C (Fig. 1B). All three species that showed increases in August had declines in native PLC values in September, but this recovery of hydraulic function was only statistically significant in grand fir ($t = 4.6$, $df = 10$, $P < 0.05$).

The water potentials of these upper branches during the summer months were very similar in all species except Douglas-fir, which exhibited lower values and a greater decline over the summer (Fig. 2). All species showed a decline in all measures of water potential over the summer, and May values were less negative than September values (least significant difference [LSD] $P < 0.005$, df for each species = 4) for all four species. While the predawn water potentials did not differ among the species during any month, in Douglas-fir the values of mid-

TABLE 2. Mean ($\pm$ SD) native percent loss in conductivity (PLC) in small diameter branches in four tall conifer species for months with and without freeze–thaw events, and the associated P value and t statistics from t tests. Each species had 77 degrees of freedom.

Species	Native PLC		P	t
	Freeze–thaw mos.	Nonfreeze–thaw mos.		
<i>Abies grandis</i> (grand fir)	36.5 $\pm$ 14.3	13.0 $\pm$ 15.2	<0.0001	7.27
<i>Pseudotsuga menziesii</i> (Douglas fir)	42.6 $\pm$ 14.5	5.4 $\pm$ 13.1	<0.0001	11.67
<i>Thuja plicata</i> (western redcedar)	26.5 $\pm$ 9.1	16.0 $\pm$ 11.8	0.0008	3.49
<i>Tsuga heterophylla</i> (western hemlock)	24.8 $\pm$ 11.9	13.0 $\pm$ 12.7	0.0006	3.58

day stem and leaf water potential were significantly more negative than the other species during most of the summer months (Fig. 2, asterisks indicate LSD $P < 0.05$, df for each species = 4).

For three of the four species, there was a significant decline in the maximum specific conductivity with increasing wood density (Fig. 3A). Western hemlock showed no relationship between these two variables. Western redcedar exhibited the smallest range of wood densities, had the lowest maximum wood density, and had the steepest decline in hydraulic conductivity with density (Table 3). Douglas-fir had the greatest range and the highest single density. The extremely high wood densities in grand fir and Douglas-fir were caused by large amounts of latewood relative to the earlywood (data not shown), and not by any obvious compression wood.

In contrast, when the native PLC during months without freeze–thaw cycles was compared with wood density, only grand fir showed a within-species relationship (Fig. 3B). Although branches with high wood density (i.e., >0.8 g·cm⁻³) exhibited little or no native PLC for all species, the remaining samples of Douglas-fir, western redcedar and western hemlock spanned the range of native PLC observed. We did not include the native PLC values from months with freeze–thaw cycles because the mechanism of embolism formation from freeze–thaw events should not be affected by wood density (see introduction).

Douglas-fir individuals were the tallest, on average, of the four species examined (Table 1). Douglas-fir wood also had wider tracheids than those of the grand fir, but not western redcedar or western hemlock. Western redcedar branches had tracheids with the widest mean hydraulic diameter and the fewest tracheids per square millimeter, suggesting a greater proportion of the wood was devoted to tracheid lumens than in the other species, which is consistent with the species' generally lower wood density.

DISCUSSION

The tallest trees in the Pacific Northwest undergo cycles of crown dieback and regrowth in their highest branches, which may be the result of transport insufficiencies (Rood et al., 2000). We had hypothesized that because of the difficulty of refilling embolized tracheids in upper branches of these tall trees, the healthy branches would show no change in the native percent loss of conductivity (PLC) throughout the year. Contrary to this

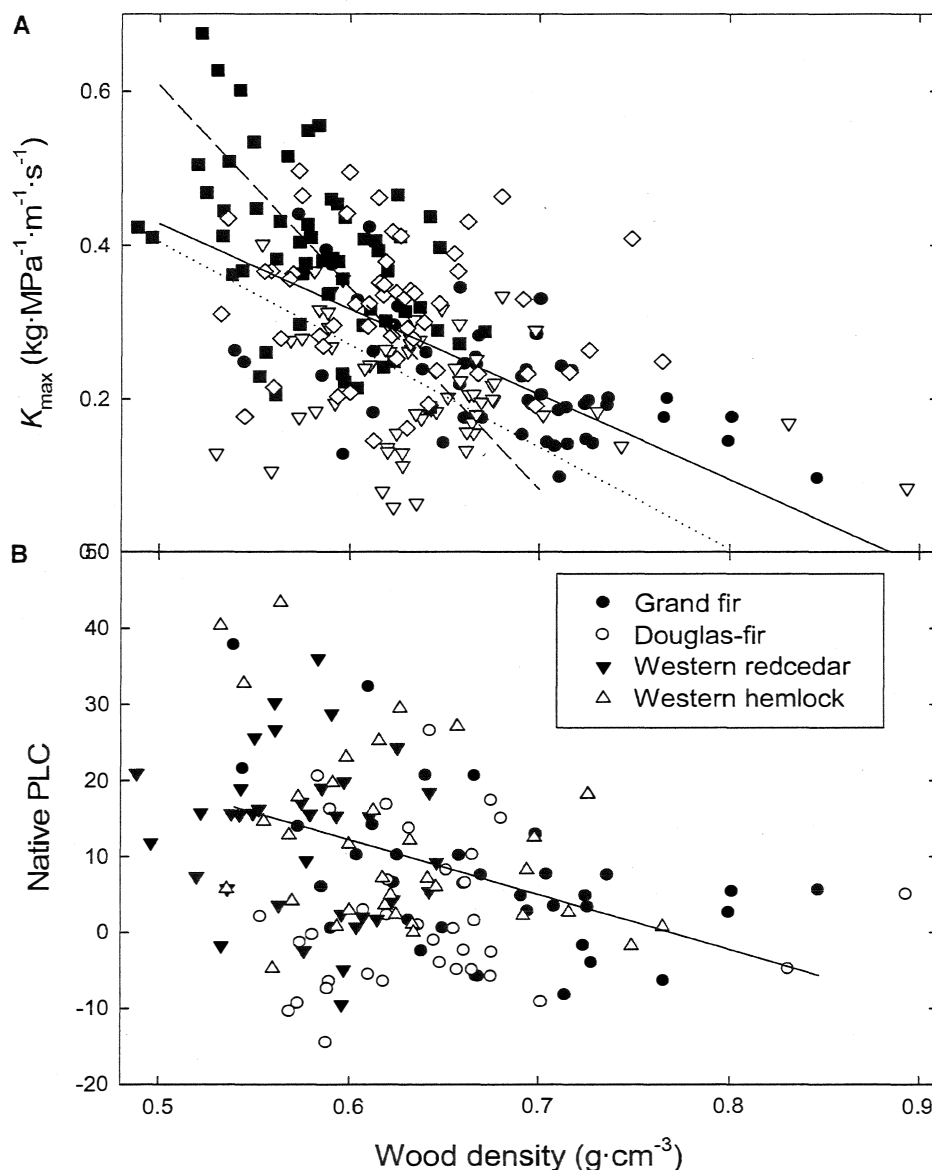


Fig. 3. (A) Maximum specific conductivity ($K_{\max}$) and (B) native percent loss of conductivity (PLC) of small diameter branches vs. their wood density for four conifer species. The native PLC values are only from months without freeze-thaw events. In (A), significant relationships (SMA $P < 0.03$) were found in grand fir (solid line), Douglas-fir (dotted line) and western redcedar (dashed line), but not western hemlock. In (B), a significant relationship (SMA $P = 0.016$) was found only for grand fir.

hypothesis, a significant increase in native PLC was observed in all species in winter relative to summer months (Fig. 1A, Table 2). This result implies that the freeze-thaw cycles during the cold, wet winter were much more hydraulically stressful than the dry, hot summer. In fact, the native monthly PLC for all species pooled (excluding August) was negatively correlated (Fig. 4) with the mean minimum air temperature during the 10 d prior to sampling. Mean native PLC changed little with declining minimum temperature until a threshold of 0 to 2°C, below which PLC increased sharply as minimum temperature declined. The R^2 for the relationship between PLC and temperature did not decline, and the regression parameters were not significantly different when only the minimum temperatures

from the 5 d prior to measurement were compared (data not shown). This drop in hydraulic conductivity occurred despite the tracheid diameters being well below the 44 μm diameter threshold shown to be susceptible to single freeze-thaw events at a mild tension (Table 1) (Sperry and Robson, 2001; Pittermann and Sperry, 2003).

It is known from field measurements that even in branches with small diameter tracheids, multiple freeze-thaw cycles result in greater losses in the native hydraulic conductivity than single freeze-thaw cycles (Sperry et al., 1994; Sparks and Black, 2000; Sparks et al., 2001; Mayr et al., 2003b). In these previous studies, though, the field locations were cold enough to have frozen soils, which would have prevented the trees from

extracting soil water and could have caused severely low water potentials when sun-exposed branchlets thawed during the day (Mayr et al., 2003b). Although freezing temperatures below the soil surface were not a concern at our site (data not shown), soil temperatures were below the 9°C threshold previously found to cause an increase in root hydraulic resistance (Goldstein et al., 1985). It is also possible that larger branches upstream from the ones on which measurements were made could have frozen and created a bottleneck to water flow similar to that caused by frozen soil. Although experimental work has shown that repeated freeze-thaw cycles cause increases in native embolism values in branches with small-diameter tracheids only after 50–100 cycles for species with small-diameter tracheids (Mayr et al., 2003a), wider diameter tracheids, similar in size to those measured here, have been shown to embolize after only one or two cycles (Mayr and Sperry, 2010). Our site experienced 19 cycles in which air temperatures went from below to above 0°C during the 2008–2009 winter and 10 before the final measurement in December 2009. Thus, it is difficult to determine whether the loss of function was caused exclusively by freeze-thaw cycles within the branches of the sizes we measured or whether an upstream bottleneck caused more negative xylem water potentials that, when combined with freeze-thaw events, resulted in increases in native PLC (Pittermann and Sperry, 2006).

As in the current study, previous field measurements on annual cycles of native PLC in conifer branches also observed a spring increase in specific hydraulic conductivity, interpreted as a refilling of conduits (Sperry et al., 1994; Sparks and Black, 2000; Sparks et al., 2001; Mayr et al., 2003b). In the previous work and here, the decrease in native PLC values was too great to be explained solely by growth of new tracheids in the spring. Furthermore, the spring decrease in native PLC we observed occurred too early in the spring to be explained by cambial growth, which usually begins in May in the study region. This temporal disconnect was reconfirmed by calculating the average proportion of the branch radius made up by the most recent year's growth. For all species, this proportion was ~5%, which is insufficient area of xylem to have recovered ~60% of the hydraulic function. In our study, the diameter of the tracheids (Table 1), combined with the extreme heights of the trees, also rules out the possibility that refilling could have occurred through the method of bubble dissolution explained by La Place's law if the sole source of water was from the roots. Thus, the two remaining methods for refilling are water absorption through the needles, which could allow water potentials to reach values required by La Place's law to refill tracheids, and "novel" refilling. At this point, it is unclear which of these two processes occurred or in what way they combined to refill embolized tracheids in the spring. However, it is known that Douglas-fir can absorb water through its needles (Limm et al., 2009).

The functional significance, if any, of this winter-time loss of hydraulic conductivity is also unclear. During these months, generally the leaves are damp, and the humidity is very high, suggesting that a reduced hydraulic conductivity may still be sufficient to replace any water lost from the leaves. This scenario would certainly not pertain to the summer months, when the xylem would need to function at or near the level at which it had been constructed to perform.

The only summer month during which any species showed greater than 20% native PLC was August (Fig. 1A). These high summertime native PLC values were observed in grand fir, Douglas-fir, and western redcedar and declined again in September. This recovery occurred despite further drops in predawn

TABLE 3. Average, maximum, and minimum wood density (WD), maximum and minimum values of the maximum specific hydraulic conductivity ($K_{\max}$), and maximum and minimum values of the native percent loss in conductivity (PLC) for four tall conifer species. The standard deviation of wood density is shown in parentheses.

Species	WD (g·cm ⁻³)	Max WD Min WD (g·cm ⁻³)	Max $K_{\max}$ Min $K_{\max}$ (kg·MPa ⁻¹ ·m ⁻¹ ·s ⁻¹)	Max PLC Min PLC
<i>Abies grandis</i> (grand fir)	0.68 (0.08)	0.93 0.54	0.44 0.10	71.9 -8.4
<i>Pseudotsuga menziesii</i> (Douglas fir)	0.64 (0.06)	0.89 0.53	0.40 0.06	65.8 -14.6
<i>Thuja plicata</i> (western redcedar)	0.58 (0.04)	0.67 0.49	0.68 0.19	41.6 -15.5
<i>Tsuga heterophylla</i> (western hemlock)	0.62 (0.05)	0.76 0.53	0.50 0.15	43.3 -4.8

and midday leaf and stem water potential for all species (Fig. 2). In contrast, western hemlock showed no change in native PLC in August. The stems of western hemlock did not tend to be more resistant to drought-induced embolism than Douglas-fir (K. A. McCulloh and D. M. Johnson, unpublished data), which suggests that the stomata of western hemlock are more conservative with respect to water loss and is consistent with the smaller amount of soil water extracted than Douglas-fir (Meinzer et al., 2007). Given that these species are able to reverse native increases in PLC during the hot, dry summer months, why do they behave so conservatively and maintain most of their hydraulic function throughout most of the summer? This question is particularly relevant for Douglas-fir, which is known to usually close its stomata at water potentials well above those that cause embolism formation in its stems (Domec et al., 2008). If refilling occurs by water absorption through the needles, perhaps the trees may be conservative during the summer because the conditions necessary for refilling may not occur every night. If refilling occurs by novel refilling, there may be

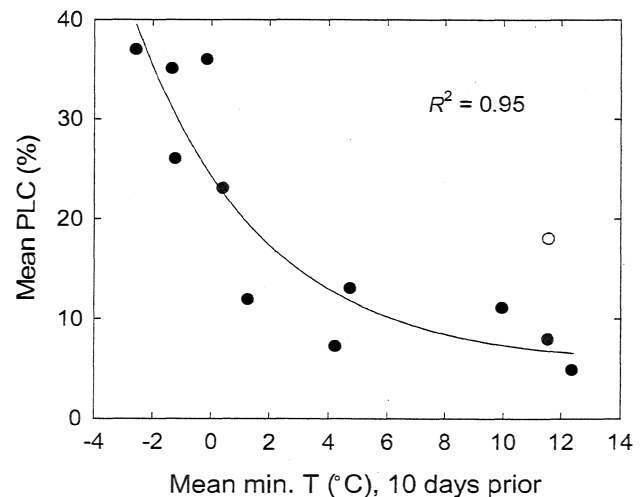


Fig. 4. Mean native percent loss in hydraulic conductivity for all four species vs. mean minimum temperature in the 10 d prior to measurement. The line shows the three-parameter, nonlinear regression fit to the 11 measurement dates (solid symbols) excluding August (open symbol, see Results for details). The R^2 value does not include the August data point.

costs to the process that outweigh the gains of keeping stomata open for longer periods during the day.

For grand fir, Douglas-fir, and western redcedar, greater wood density was associated with lower maximum specific conductivity (Fig. 3A), but denser wood only provided greater safety from water-stress-induced native PLC for grand fir (Fig. 3B). Previous work has found higher density wood to have greater resistance to drought-induced embolism when compared across some species (Hacke et al., 2001), but not others (Jacobsen et al., 2008; Fichot et al., 2010). If the lower conductivity of the higher density branches of Douglas-fir and western redcedar yields a benefit in terms of another functional trait, it is not clear what that trait is. What is also unclear from our measurements is whether this safety from embolism is achieved differently by the low vs. high density branches. Those with low density may avoid hydraulic failure by maintaining relatively moderate xylem tensions by relying on stored water (i.e., capacitance) released throughout the day (Meinzer et al., 2009; Barnard et al., 2011). Those with high density may rely on structural features within the wood (Domec et al., 2008; Hacke et al., 2004).

A possible link between native PLC during the winter months and wood density was considered because higher density wood should be associated with tracheids with thicker walls and smaller diameters. However, no relationships were observed between these variables (data not shown). This discrepancy may arise from the increases in wood density being largely driven by increases in latewood proportions, while hydraulic conductivity changes are more influenced by embolisms in earlywood.

The branches we examined represent only one segment of the trees' entire hydraulic pathway. Previous work on annual patterns of the amount of water in the boles of conifers has reported seasonal shifts in ponderosa pine (*Pinus ponderosa*; Domec et al., 2005), Scots pine (*Pinus sylvestris*; Waring et al., 1979), lodgepole pine (*Pinus contorta*; Sparks et al., 2001) and Douglas-fir (*Pseudotsuga menziesii*; Waring and Running, 1978; Domec and Gartner, 2002), but not in eastern hemlock (*Tsuga canadensis*; Gibbs, 1957). While these studies did not include direct measures of changes in native PLC of boles, relative water content has been linked to conductivity (Domec and Gartner, 2001, 2002). For those species that exhibited an annual pattern, water content in the trunk declined to a minimum in summer and peaked in winter or early spring. Thus, if the same seasonal decrease in hydraulic conductivity occurred in the bole as we observed in the branches, it was not due to lack of water availability.

The commonly held view is that the summer months of the Pacific Northwest are extremely stressful to plants. Recent work has suggested that longer, drier summers could result in species range shifts in the region (van Mantgem et al., 2009). Yet, our results indicate that the winter months are more stressful in terms of hydraulic function and suggest that perhaps an inability to recover from the increase in native embolism rates over the winter may cause greater branch dieback in old growth trees than shifts in summer climate.

LITERATURE CITED

- BARNARD, D., F. C. MEINZER, B. LACHENBRUCH, K. A. MCCULLOH, D. M. JOHNSON, AND D. R. WOODRUFF. 2011. Climate-related trends in sapwood biophysical properties in two conifers: Avoidance of hydraulic dysfunction through coordinated adjustments in xylem efficiency, safety and capacitance. *Plant, Cell & Environment* 34: 643–654.
- BUCCI, S. J., F. G. SCHOLZ, G. GOLDSTEIN, F. C. MEINZER, AND L. D. A. S. L. STERNBERG. 2003. Dynamic changes in hydraulic conductivity in petioles of two savanna tree species: Factors and mechanisms contributing to the refilling of embolized vessels. *Plant, Cell & Environment* 26: 1633–1645.
- BURGESS, S. S. O., AND T. E. DAWSON. 2004. The contribution of fog to the water relations of *Sequoia sempervirens* (D. Don): Foliar uptake and prevention of dehydration. *Plant, Cell & Environment* 27: 1023–1034.
- CLEARWATER, M. J., AND G. GOLDSTEIN. 2005. Embolism repair and long distance water transport. In N. M. Holbrook and M. A. Zwieniecki [ed.], *Vascular transport in plants*. Elsevier Academic Press, London, UK.
- DAVIS, S. D., J. S. SPERRY, AND U. G. HACKE. 1999. The relationship between xylem conduit diameter and cavitation caused by freezing. *American Journal of Botany* 86: 1367–1372.
- DOMEC, J.-C., AND B. L. GARTNER. 2001. Cavitation and water storage in bole xylem segments of mature and young Douglas-fir trees. *Trees* 15: 204–214.
- DOMEC, J.-C., AND B. L. GARTNER. 2002. Age- and position-related changes in hydraulic versus mechanical dysfunction of xylem: Inferring the design criteria for Douglas-fir wood structure. *Tree Physiology* 22: 91–104.
- DOMEC, J.-C., B. LACHENBRUCH, F. C. MEINZER, D. R. WOODRUFF, J. M. WARREN, AND K. A. MCCULLOH. 2008. Maximum height in a conifer is associated with conflicting requirements for xylem design. *Proceedings of the National Academy of Sciences, USA* 105: 12069–12074.
- DOMEC, J.-C., M. L. PRUYN, AND B. L. GARTNER. 2005. Axial and radial profiles in conductivities, water storage and native embolism in trunks of young and old-growth ponderosa pine trees. *Plant, Cell & Environment* 28: 1103–1113.
- EDWARDS, W. R. N., P. G. JARVIS, J. GRACE, AND J. B. MONCRIEFF. 1994. Reversing cavitation in tracheids of *Pinus sylvestris* L. under negative water potentials. *Plant, Cell & Environment* 17: 389–397.
- FICHOT, R., S. B. TÊTÈ, S. CHAMAILLARD, D. L. THIEC, F. LAURANS, H. COCHARD, AND F. BRIGNOLAS. 2010. Common trade-offs between xylem resistance to cavitation and other physiological traits do not hold among unrelated *Populus deltoides* × *Populus nigra* hybrids. *Plant, Cell & Environment* 33: 1553–1568.
- FRANKLIN, J. F., AND R. H. WARING. 1980. Distinctive features of the northwestern coniferous forest: Development, structure, and function. In *Forests: Fresh perspectives from ecosystem analyses*, 40th Annual Biological Colloquium, 59–86, 1979, Corvallis, Oregon, USA. Oregon State University Press, Corvallis, Oregon.
- GIBBS, R. D. 1957. Patterns in the seasonal water content of trees. In K. V. Thimann [ed.], *The physiology of forest trees*, 43–69. Ronald Press, New York, New York, USA.
- GOLDSTEIN, G. H., L. B. BRUBAKER, AND T. M. HINCKLEY. 1985. Water relations of white spruce (*Picea glauca* (Moench) Voss) at tree line in north central Alaska. *Canadian Journal of Forest Research* 15: 1080–1087.
- HACKE, U., AND J. S. SPERRY. 2003. Limits to xylem refilling under negative pressure in *Laurus nobilis* and *Acer negundo*. *Plant, Cell & Environment* 26: 303–311.
- HACKE, U. G., J. S. SPERRY, AND J. PITTMANN. 2004. Analysis of circular bordered pit function. II. Gymnosperm tracheids with torus-margo pit membranes. *American Journal of Botany* 91: 386–400.
- HACKE, U. G., J. S. SPERRY, W. T. POCKMAN, S. D. DAVIS, AND K. A. MCCULLOH. 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* 126: 457–461.
- JACOBSEN, A. L., R. B. PRATT, S. D. DAVIS, AND F. W. EWERS. 2008. Comparative community physiology: Nonconvergence in water relations among three semi-arid shrub communities. *New Phytologist* 180: 100–113.
- LANGAN, S. J., F. W. EWERS, AND S. D. DAVIS. 1997. Xylem dysfunction caused by water stress and freezing in two species of co-occurring chaparral shrubs. *Plant, Cell & Environment* 20: 425–437.
- LIMM, E. B., K. A. SIMONIN, A. G. BOTHMAN, AND T. E. DAWSON. 2009. Foliar water uptake: A common water acquisition strategy for plants of the redwood forest. *Oecologia* 161: 449–459.

- MAYR, S., A. GRUBER, AND H. BAUER. 2003a. Repeated freeze-thaw cycles induce embolism in drought stressed conifers (Norway spruce, stone pine). *Planta* 217: 436–441.
- MAYR, S., U. HACKE, P. SCHMID, F. SCHWEINBACHER, AND A. GRUBER. 2006. Frost drought in conifers at the alpine timberline: Xylem dysfunction and adaptations. *Ecology* 87: 3175–3185.
- MAYR, S., F. SCHWIENBACHER, AND H. BAUER. 2003b. Winter at the alpine timberline: Why does embolism occur in Norway spruce but not in stone pine? *Plant Physiology* 131: 780–792.
- MAYR, S., AND J. S. SPERRY. 2010. Freeze-thaw-induced embolism in *Pinus contorta*: Centrifuge experiments validate the 'thaw-expansion hypothesis' but conflict with ultrasonic emission data. *The New Phytologist* 185: 1016–1024.
- MEINZER, F. C., D. M. JOHNSON, B. LACHENBRUCH, K. A. MCCULLOH, AND D. R. WOODRUFF. 2009. Xylem hydraulic safety margins in woody plants: Coordination of stomatal control of xylem tension with hydraulic capacitance. *Functional Ecology* 23: 922–930.
- MEINZER, F. C., J. M. WARREN, AND J. R. BROOKS. 2007. Species-specific partitioning of soil water resources in an old-growth Douglas-fir-western hemlock forest. *Tree Physiology* 27: 871–880.
- PEARCE, R. S. 2001. Plant freezing and damage. *Annals of Botany* 87: 417–424.
- PITTERMANN, J., AND J. S. SPERRY. 2003. Tracheid diameter is the key trait determining the extent of freezing-induced embolism in conifers. *Tree Physiology* 23: 907–914.
- PITTERMANN, J., AND J. S. SPERRY. 2005. Analysis of freeze-thaw embolism in conifers: The interaction between cavitation pressure and tracheid size. *Plant Physiology* 140: 374–382.
- ROBSON, D. J., W. J. MCHARDY, AND J. A. PETTY. 1988. Freezing in conifer xylem. II. Pit aspiration and bubble formation. *Journal of Experimental Botany* 39: 1617–1621.
- ROOD, S. B., S. PATIÑO, K. COOMBS, M. T. TYREE. 2000. Branch sacrifice: Cavitation-associated drought adaptation of riparian cottonwoods. *Trees—Structure and Function* 14: 248–257.
- SALLEO, S., M. LO GULLO, D. DEPAOLI, AND M. ZIPPO. 1996. Xylem recovery from cavitation-induced embolisms in young plant of *Laurel novelis*: A possible mechanism. *New Phytologist* 132: 357–366.
- SECCHI, F., AND M. A. ZWIENIECKI. 2010. Patterns of PIP gene expression in *Populus trichocarpa* during recovery from xylem embolism suggest a major role for the PIP1 aquaporin subfamily as moderators of refilling process. *Plant, Cell & Environment* 33: 1285–1297.
- SIMONIN, K. A., L. S. SANTIAGO, AND T. E. DAWSON. 2009. Fog interception by *Sequoia sempervirens* (D. Don) crowns decouples physiology from soil water deficit. *Plant, Cell & Environment* 32: 882–892.
- SMITH, D. M. (1955). A comparison of two methods for determining the specific gravity of small samples of second-growth Douglas-fir. USDA Forest Service Forest Products Laboratory Report 2045, Madison, Wisconsin.
- SOBRADO, M. A., J. GRACE, AND P. G. JARVIS. 1992. The limits to xylem embolism recovery in *Pinus sylvestris* L. *Journal of Experimental Botany* 43: 831–836.
- SPARKS, J. P., AND R. A. BLACK. 2000. Winter hydraulic conductivity and xylem cavitation in coniferous trees from upper and lower treeline. *Arctic and Alpine Research* 32: 101–106.
- SPARKS, J. P., G. S. CAMPBELL, AND R. A. BLACK. 2001. Water content, hydraulic conductivity, and ice formation in winter stems of *Pinus contorta*: A TDR case study. *Oecologia* 127: 468–475.
- SPERRY, J. S., K. J. NICHOLS, J. E. M. SULLIVAN, AND S. E. EASTLACK. 1994. Xylem embolism in ring-porous, diffuse-porous and coniferous trees of northern Utah and interior Alaska. *Ecology* 75: 1736–1752.
- SPERRY, J. S., AND D. J. ROBSON. 2001. Xylem cavitation and freezing in conifers. In F. J. Bigras and S. J. Colombo [eds.], *Conifer cold hardiness*, 121–136. Kluwer, Dordrecht, Netherlands.
- SPERRY, J. S., AND J. E. M. SULLIVAN. 1992. Xylem embolism in response to freeze-thaw cycles and water stress in ring-porous, diffuse-porous and conifer species. *Plant Physiology* 100: 605–613.
- SUCOFF, E. 1969. Freezing of conifer xylem and the cohesion-tension theory. *Physiologia Plantarum* 22: 424–431.
- TYREE, M. T., AND M. H. ZIMMERMANN. 2002. Xylem structure and the ascent of sap. Springer-Verlag, Berlin, Germany.
- VAN MANTGEM, P. J., N. L. STEPHENSON, J. C. BYRNE, L. D. DANIELS, J. F. FRANKLIN, P. Z. FULE, M. E. HARMON, ET AL. 2009. Widespread increase of tree mortality rates in the western United States. *Science* 323: 521–524.
- WARING, R. H., AND S. W. RUNNING. 1978. Sapwood water storage: Its contribution to transpiration and effect upon water conductance through the stems of old-growth Douglas-fir. *Plant, Cell & Environment* 1: 131–140.
- WARING, R. H., D. WHITEHEAD, AND P. G. JARVIS. 1979. The contribution of stored water to transpiration in Scots pine. *Plant, Cell & Environment* 2: 309–317.
- WARTON, D. I., I. J. WRIGHT, D. S. FALSTER, AND M. WESTOBY. 2006. Bivariate line-fitting methods for allometry. *Biological Reviews of the Cambridge Philosophical Society* 81: 259–291.
- YANG, S., AND M. T. TYREE. 1992. A theoretical model of hydraulic conductivity recovery from embolism with comparison to experimental data on *Acer saccharum*. *Plant, Cell & Environment* 15: 633–643.
- ZWIENIECKI, M. A., AND N. M. HOLBROOK. 2009. Confronting Maxwell's demon: Biophysics of xylem embolism repair. *Trends in Plant Science* 14: 530–534.