Research paper

A test of the hydraulic vulnerability segmentation hypothesis in angiosperm and conifer tree species

Daniel M. Johnson[1,7], Remi Wortemann[2], Katherine A. McCulloh[3], Lionel Jordan-Meille[4], Eric Ward[5], Jeffrey M. Warren[6], Sari Palmroth[2] and Jean-Christophe Domec[2,4]

[1]Department of Forest, Rangeland and Fire Sciences, University of Idaho, 875 Perimeter Drive MS1133, Moscow, ID 83844, USA; [2]Nicholas School of the Environment, Duke University, Durham, NC 27708, USA; [3]Department of Botany, University of Wisconsin-Madison, Madison, WI 53705, USA; [4]Bordeaux Sciences Agro, UMR INRA-ISPA 1391, 33195 Gradignan, France; [5]Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC 27607, USA; [6]Environmental Sciences Division and Climate Change Science Institute, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA; [7]Corresponding author (danjohnson@uidaho.edu)

Water transport from soils to the atmosphere is critical for plant growth and survival. However, we have a limited understanding about many portions of the whole-tree hydraulic pathway, because the vast majority of published information is on terminal branches. Our understanding of mature tree trunk hydraulic physiology, in particular, is limited. The hydraulic vulnerability segmentation hypothesis (HVSH) stipulates that distal portions of the plant (leaves, branches and roots) should be more vulnerable to embolism than trunks, which are nonredundant organs that require a massive carbon investment. In the current study, we compared vulnerability to loss of hydraulic function, leaf and xylem water potentials and the resulting hydraulic safety margins (in relation to the water potential causing 50% loss of hydraulic conductivity) in leaves, branches, trunks and roots of four angiosperms and four conifer tree species. Across all species, our results supported strongly the HVSH as leaves and roots were less resistant to embolism than branches or trunks. However, branches were consistently more resistant to embolism than any other portion of the plant, including trunks. Also, calculated whole-tree vulnerability to hydraulic dysfunction was much greater than vulnerability in branches. This was due to hydraulic dysfunction in roots and leaves at less negative water potentials than those causing branch or trunk dysfunction. Leaves and roots had narrow or negative hydraulic safety margins, but trunks and branches maintained positive safety margins. By using branch-based hydraulic information as a proxy for entire plants, much research has potentially overestimated embolism resistance, and possibly drought tolerance, for many species. This study highlights the necessity to reconsider past conclusions made about plant resistance to drought based on branch xylem only. This study also highlights the necessity for more research of whole-plant hydraulic physiology to better understand strategies of plant drought tolerance and the critical control points within the hydraulic pathway.

Keywords: cavitation, drought, embolism, transpiration, water relations.

Introduction

Because water transport is critical for photosynthesis and plant survival, plants have evolved a variety of strategies to prevent and potentially repair hydraulic failure in their conductive tissues. To sustain xylem function, and prevent catastrophic embolism and loss of hydraulic conductivity, plants may change the structure and allometry of their vascular systems to alter the fine balance between water supply and water loss over developmental timescales. This is often accomplished through alterations in leaf area (Parker and Pallardy 1985, see also Pallardy 2008), entire branch dieback (Rood et al. 2000) or alteration of root area (van Hees 1997). The tree bole or trunk has less plasticity than these organs for altering hydraulic properties on short (less than annual) timescales (Domec et al. 2012). In the stem xylem

pathway, the complete failure of any segment interrupts the supply of water to all segments distal to it, and thus, plants should be adapted to protect the bole, or potentially the roots (especially in resprouting species; Pratt et al. 2014), from hydraulic failure at all costs (i.e., shedding leaves, branches etc.; Tyree et al. 1993, Tyree and Zimmermann 2002, Pivovaroff et al. 2014). Those findings support Martin Zimmermann's original segmentation hypothesis, which stipulated that plants are hydraulically designed to sacrifice highly vulnerable plant segments to keep the remaining parts hydraulically active (Zimmermann 1983). In other words, distal portions of plants should be more susceptible to hydraulic dysfunction than boles due to high hydraulic resistances at organ connections (i.e., branch–leaf connections, branch–bole connections) resulting in large pressure gradients. In 1991, Tyree and Ewers proposed a modified version of this hypothesis called the hydraulic vulnerability segmentation hypothesis (HVSH; Tyree and Ewers 1991). The HVSH states that more distal portions of trees should be the most vulnerable to embolism (embolizing at less negative pressures, irrespective of the pressure gradient in the plant) than basal portions, and therefore, the trunk should be the most resistant. Although these hypotheses are often assumed to be correct, little work has explicitly addressed or tested the HVSH, likely due to the difficulty of measuring the hydraulic functioning of the whole tree trunk (especially large trunks in adult trees).

Within a specimen, the only way to predict the entire role of hydraulic segmentation is to determine the vulnerability and loss of hydraulic capacity of leaves versus branches versus large stems (i.e., trunks) versus roots (Tyree and Ewers 1991). Furthermore, to assess the HVSH relative to the tree's hydraulic status, one needs to know not only the hydraulic properties throughout the tree but also the most negative water potentials experienced in the field (Tyree and Zimmermann 2002). Unfortunately, the field of plant hydraulics has mostly used branches as hydraulic proxies for overall plant hydraulic properties for decades (Cochard et al. 1992a, Sperry et al. 1994, Choat et al. 2012), and there is a crucial lack of information on the hydraulic vulnerability of tree trunks. It is with this in mind that we have measured hydraulic properties of trees along the hydraulic continuum, from root to trunk to branch to leaf.

The previous work that has addressed the HVSH has resulted in data that both do and do not support the hypothesis. One study found that trunk and roots of two conifer species of the Pacific Northwest USA were more hydraulically vulnerable than branches (Domec et al. 2009a). More recently, in four tall old-growth conifers from the same region, trunks were dramatically more vulnerable than branches (McCulloh et al. 2014). These data are clearly in disagreement with the HVSH. However, results from other studies support the HVSH. For example, Tsuda and Tyree (1997) observed that petioles of *Acer saccharinum* L. were more vulnerable to embolism than stems, but roots were more resistant than stems to embolism. To our knowledge, no study of comparative hydraulic vulnerability to declining water potentials and drought in roots, trunks, branches and leaves across species has ever been performed. Our goals were therefore to assess the validity of the HVSH across multiple angiosperm and gymnosperm tree species. We hypothesized, based on some of our previous work (Domec and Gartner 2001, Domec et al. 2005, McCulloh et al. 2014), that the HVSH would not be supported and that trunks would be less resistant to embolism than branches. Additionally, to test the hypothesis that leaves and roots, the most distal tree organs, can act as hydraulic 'safety valves', we assessed whether or not the leaves, branches, trunks and roots reached water potentials causing significant loss of hydraulic capacity under field conditions. This analysis allowed us to generate curves describing whole-tree vulnerability to hydraulic dysfunction and to demonstrate which organ had the greatest impact on whole-tree hydraulic conductance.

Materials and methods

Species were selected from a temperate eastern North American forest, a semi-arid North American ecosystem and a temperate European ecosystem. Six species were selected from Duke Forest located in Durham, NC, USA (Lat. 35.9782, Long. -79.0942) (*Acer rubrum* L. (Sapindaceae), *Liquidambar styraciflua* L. (Altingaceae), *Liriodendron tulipifera* L. (Magnoliaceae), *Pinus taeda* L. (Pinaceae), *Pinus virginiana* Mill. (Pinaceae) and *Prunus serotina* Ehrh. (Rosaceae)), one species was selected from the Edward's Plateau of Texas, Bend, TX, USA (Lat. 31.3243, Long. -98.5900) (*Juniperus ashei* Buchholz (Cupressaceae)) and one species was selected from southern France, Gradignan, France (Lat. 44.7743, Long. -0.6189) (*Pinus pinea* L. (Pinaceae)). All individuals were mature trees (tree height and diameter at breast height (dbh) in Table 1). All samples were collected in July through November of 2011 and 2013. The trees were selected to have no major forks or injury and to be of similar size and dominance. Trees were felled with a chainsaw. Branches and leaves were removed from the sun-exposed portions of the tree crown and 30–40 cm trunk sections were cut from breast height (what would have been breast height prior to felling). Roots were excavated and traced back to the parent tree to ensure that they were correctly identified to species. Trunk segments were split into quarters and trunk sections, branches with leaves and roots were placed into plastic bags containing damp paper towels and transported back to the laboratory where they were placed in storage at 4 °C until measurements could be made. Measurements took no longer than 5 days to complete, which was too short a time to induce any effect of storage duration on hydraulic parameters (Erickson 1960, Domec and Gartner 2001).

Trunk sections were chiseled out using hand chisels and a mallet and were planed down to small ~15 mm diameter cylindrical dowels that were then trimmed to 20–22 cm in length (see Domec

Table 1. Hydraulic parameters for the study species. Mean tree height (m) and dbh (cm) for sampled trees, maximum xylem hydraulic conductivity ($k_{s\ max}$ of roots, trunks and branches in kg m^{-1} s^{-1} MPa^{-1}), maximum hydraulic conductance in leaves ($K_{leaf\ max}$ in mmol m^{-2} s^{-1} MPa^{-1}) and P_{50} values (−MPa). Values of P_{50} compared between organs were considered statistically different if there was no overlap in 95% confidence intervals and significant differences are indicated by different letters. Numbers in parentheses are SEs.

Species	Tree dbh	Tree height	$k_{s\ max}$ root	$k_{s\ max}$ trunk	$k_{s\ max}$ branch	$K_{leaf\ max}$	Root P_{50}	Trunk P_{50}	Branch P_{50}	Leaf P_{50}
A. rubrum	15.0 (0.8)	9.8 (0.6)	1.04 (0.60)	0.76 (0.11)	0.67 (0.13)	11.02 (0.67)	2.00a	2.23a	3.13b	1.53c
L. styraciflua	16.0 (0.7)	16.7 (1.5)	6.86 (0.89)	1.76 (0.24)	1.47 (0.33)	16.60 (0.98)	0.32a	2.87b	3.26b	1.04c
L. tulipifera	17.2 (1.4)	16.3 (2.2)	8.23 (0.64)	3.09 (0.66)	2.61 (0.30)	9.95 (0.61)	1.78a	2.63b	4.13c	1.20d
Pr. serotina	9.0 (2.0)	7.2 (0.9)	0.82 (0.25)	1.19 (0.25)	0.55 (0.10)	7.73 (0.18)	1.94a	3.56b	4.87c	1.30d
J. ashei	22.4[1] (1.0)	5.1 (0.4)	1.02 (0.36)	0.22 (0.04)	0.13 (0.01)	6.65 (0.30)	9.48a	14.8[2]b	13.1[3]	1.67c
P. pinea	23.5 (2.2)	5.5 (0.5)	2.76 (0.32)	2.65 (0.60)	0.34 (0.09)	6.16 (0.11)	1.51a	2.86b	4.57c	1.05d
P. taeda	24.9 (1.7)	24.2 (0.9)	4.65 (0.95)	2.80 (0.33)	0.83 (0.18)	7.19 (0.25)	1.34a	3.28b	3.90c	0.78a
P. virginiana	17.2 (1.4)	8.4 (0.4)	12.3 (3.16)	1.42 (0.09)	1.42 (0.42)	24.1 (0.98)	1.12a	2.87b	3.65c	1.84d

[1]Basal diameter; *J. ashei* trunks often split into multiple stems just above the ground.
[2]Estimate based on extrapolated vulnerability curve.
[3]From Willson et al. (2008).

and Gartner 2001). The outside portions of the dowels (cambium and bark) were maintained through preparation until the final shaping of the dowels. This was done to ensure that the most recent growth rings were represented in each dowel. The mean number of growth rings in each dowel was 7.4 (standard error (SE) = 0.9). The mean lengths of angiosperm branches and roots were 18.1 (SE = 0.8) and 36.5 (SE = 6.2) cm, respectively. The mean length of conifer branches and roots was 15.0 (SE = 0.8) and 17.1 (SE = 0.7) cm, respectively. Diameters of branch and root samples ranged from ~8 to 14 and ~6 to 11 mm, respectively. To test for open vessels in branch, trunk and root segments for the angiosperms, we used the compressed air method of Ewers and Fisher (1989), where air is forced into the proximal end of the segment at 50–100 kPa and the distal end is submerged under water. Only segments with no open vessels were used for hydraulic measurements. To test for potential artifacts due to pressurization with air for open vessel tests, we compared sample maximum specific hydraulic conductivity and vulnerability to embolism in samples that had been pressurized and those that had not and found no significant differences ($n = 5$–16, t-test $P = 0.57$). Segments with no open vessels were then either placed in water (at a pH of 2, to inhibit microbial growth) under a partial vacuum overnight or were flushed at a pressure of 50 kPa for 30 min with pH 2 water. Conifers were not tested for open conduits because maximum tracheid sizes of the species studied never exceed >5 mm (Panshin and De Zeeuw 1980) and were placed in pH 2 water under a partial vacuum overnight.

To measure maximum hydraulic conductivity ($k_{h\ max}$), a hydrostatic pressure head of 6–9 kPa was used to induce flow through the branch, root and trunk segments. The resulting volume flow rate was measured by timing the intervals for water to reach successive gradations on a pipette attached with tubing to the distal end of the segment. Hydraulic conductivity (k_h) was calculated by dividing the volume flow rate of water flowing through the segment by the hydrostatic pressure gradient driving the flow. Specific conductivity (k_s) was calculated by dividing

k_h by the cross-sectional area of the section being measured. Trunk sections were wrapped in Parafilm (Parafilm M, Bemis Inc., Oshkosh, WI, USA) to prevent leaks from open conduits. The temperature of the solution was recorded before and after each specific conductivity measurement, and all conductivity calculations were corrected to 20 °C to account for changes in fluid viscosity with temperature.

Vulnerability curves in root, trunk and branch samples were constructed using the air injection method (Sperry and Tyree 1990, Cochard et al. 1992b, Salleo et al. 1992, Sperry and Saliendra 1993). Previous work has shown that reliable measurements of hydraulic vulnerability can be obtained by using this method even on long-vesseled species, especially when using a small pressure sleeve and ensuring that there are no open vessels (Domec et al. 2006, Choat et al. 2010, Ennajeh et al. 2011). Briefly, after determining $k_{h\ max}$, a stem was placed in a double-ended pressure sleeve (8 cm in length) and pressurized for 2 min. The stem was then removed from the pressure sleeve and k_h was measured using the same method used for $k_{h\ max}$. This process was repeated at 0.5 or 1.0 MPa increments (depending on species and organ) of increasing pressure until k_h had fallen to <10% of its maximum value (with the exception of *J. ashei* because this value was beyond the capabilities of the instrument). The percentage loss in hydraulic conductivity at a given applied pressure (PLC(Ψ)) was calculated as:

$$PLC = 100 \times \left(1 - \frac{k_h}{k_{h\ max}}\right) \tag{1}$$

Leaf hydraulic conductance (mmol m^{-2} s^{-1} MPa^{-1}) was determined using a timed rehydration method described in Brodribb and Holbrook (2003), which is based on an analogy between rehydrating a leaf and discharging a capacitor:

$$K_{leaf}(\Psi_o) = \frac{C \ln(\Psi_o / \Psi_f)}{t} \tag{2}$$

where C is the capacitance, Ψ_o the leaf water potential prior to partial rehydration, Ψ_f the leaf water potential after partial rehydration and t is the duration of rehydration. For measurement of K_{leaf}, sampled branches (~10–20 cm in length) were rehydrated with distilled water until Ψ was close to −0.5 MPa and then placed on the laboratory bench to dry for different amounts of time to reach a range of leaf water potentials. Branches were then bagged, placed in the dark and allowed to equilibrate for at least 2 h. Leaves were then excised for determination of Ψ_o and leaf samples from the same branch were rehydrated for a period of t seconds and Ψ_f was measured. Leaf water potential was measured using a pressure chamber (PMS Instrument Company, Corvallis, OR, USA).

Leaf capacitance was estimated from pressure–volume curves (Scholander et al. 1965, Tyree and Hammel 1972) using the methods described by Brodribb and Holbrook (2003). Briefly, the Ψ_L corresponding to turgor loss was estimated as the inflection point of the graph of Ψ_L versus relative water content (RWC). The slope of the curve prior to, and following, turgor loss provided C in terms of RWC (C_{rwc}) for pre-turgor loss and post-turgor loss, respectively. Five to six leaves of each species were used to construct pressure–volume curves and estimate C. Pressure–volume curve measurements were conducted on individual leaves for the broadleaf species, on fascicles of needles for the *Pinus* species and on small shoot tips (~2 cm length) for *Juniperus*. Branch samples of ~30–50 cm length, from the same individuals that were used for rehydration and measurement of K_{leaf}, were excised early in the morning, shipped overnight back to the laboratory and rehydrated the next day for ~1 h. This rehydration time was chosen to prevent 'over-rehydration' artifacts (see Meinzer et al. 2014). The curves were created by plotting the inverse of Ψ_L against RWC with alternate determinations of fresh mass and Ψ_L repeated during slow dehydration of the twig or leaf on the laboratory bench until values of Ψ_L neared −4.0 MPa. For normalizing C on a leaf area basis, leaf areas for the all species were obtained with a leaf area meter (LI-3100C, LI-COR, Lincoln, NE, USA).

Using the Ohm electrical analogy applied to a hydraulic circuit where all the resistances to water flow are in series, whole-tree hydraulic conductance on a leaf area basis at a given water potential ($K_{tree}(\Psi)$) was calculated from each organ conductance as:

$$K_{tree}(\Psi) = \left[\frac{1}{K_{root}(\Psi)} + \frac{1}{K_{trunk}(\Psi)} + \frac{1}{K_{branch}(\Psi)} + \frac{1}{K_{leaf}(\Psi)} \right]^{-1} \quad (3)$$

or

$$K_{tree}(\Psi) =$$
$$\left[\frac{P_{Root}(0)}{K_{tree}(0) \times (1 - LC_{root}(\Psi))} + \frac{P_{Trunk}(0)}{K_{tree}(0) \times (1 - LC_{trunk}(\Psi))} \right.$$
$$+ \frac{P_{Branch}(0)}{K_{tree}(0) \times (1 - LC_{branch}(\Psi))}$$
$$\left. + \frac{P_{Leaf}(0)}{K_{tree}(0) \times (1 - LC_{leaf}(\Psi))} \right]^{-1} \quad (4)$$

where $P_{Root}(0)$, $P_{Trunk}(0)$, $P_{Branch}(0)$ and $P_{Leaf}(0)$ represent the partitioning of the total tree resistance in roots, trunk, branches and leaves, respectively. $LC_{root}(\Psi)$, $LC_{trunk}(\Psi)$, $LC_{branch}(\Psi)$ and $LC_{leaf}(\Psi)$ represent the loss of hydraulic conductivity (Eq. (1)) at a given water potential (vulnerability curves) in roots, trunk, branches and leaves, respectively. Applying a monotonic decline in predawn water potentials, the conductivity in each organ of Eq. (4) was decreased proportionally to the loss in conductivity observed in their respective vulnerability curves (Figures 1 and 2). Note that in Eq. (4), the absolute value of $K_{tree}(0)$, which corresponds to the initial value of K_{tree}, i.e., the maximum whole-tree hydraulic conductance, does not affect the whole-tree vulnerability curves when expressed as a percentage loss of conductivity. When the trees were fully hydrated, we assumed that $P_{Root}(0)$ and $P_{Leaf}(0)$ would represent 50 and 25% of the whole-tree resistance to water flow ($1/K_{tree}(0)$), respectively (Nardini and Tyree 1999, Engelbrecht et al. 2000, Cruiziat et al. 2002, Tyree and Zimmermann 2002, Sack and Holbrook 2006, Domec et al. 2009b, Pratt et al. 2010). The remaining aboveground hydraulic resistance represented 10 and 15% in trunk and branches ($P_{Trunk}(0)$ and $P_{Branch}(0)$), respectively, which reflected the differences in sapwood conductivity measured in those two organs (Table 1). For comparison, additional scenarios using water potential gradients measured in the field and with different resistance partitioning were calculated (see Table S1 available as Supplementary Data at *Tree Physiology* Online).

Leaf water potential measurements were performed at predawn and midday (14:00–15:00 h local time) on fully sun-exposed south-facing shoots. All water potentials were measured during the seasonal dry period (August–September) except for *J. ashei*, which was also measured during a severe drought (see Johnson et al. 2014). Because large disequilibria can exist between stem and leaf water potentials, especially at midday (Bucci et al. 2004), measurements of stem water potential were performed at midday to estimate the amount of maximum native embolism in stems. To measure branch water potential, we covered shoots with a sealable plastic bag covered in aluminum foil before dawn and then measured the midday water potential of bagged leaves (Turner and Long 1980, Meinzer 2002). Under these conditions, leaf water potential is generally agreed to equilibrate to that of the adjacent xylem (Richter 1997). Since trunk water potential was not measured, midday trunk water potentials were assumed to be between predawn leaf and midday branch water potential values (e.g., Hellkvist et al. 1974, Domec et al. 2005). Hydraulic safety margins were calculated as the midday water potential measured in a particular organ minus the P_{50} for that organ. In organs where midday water potentials were not measured (trunk and roots), the predawn leaf water potential or the range of water potentials from predawn leaf to midday branch were used for roots and trunks, respectively.

For comparing leaf hydraulic safety margins (leaf Ψ_{MIN} − leaf P_{50}) with leaf to branch hydraulic vulnerability
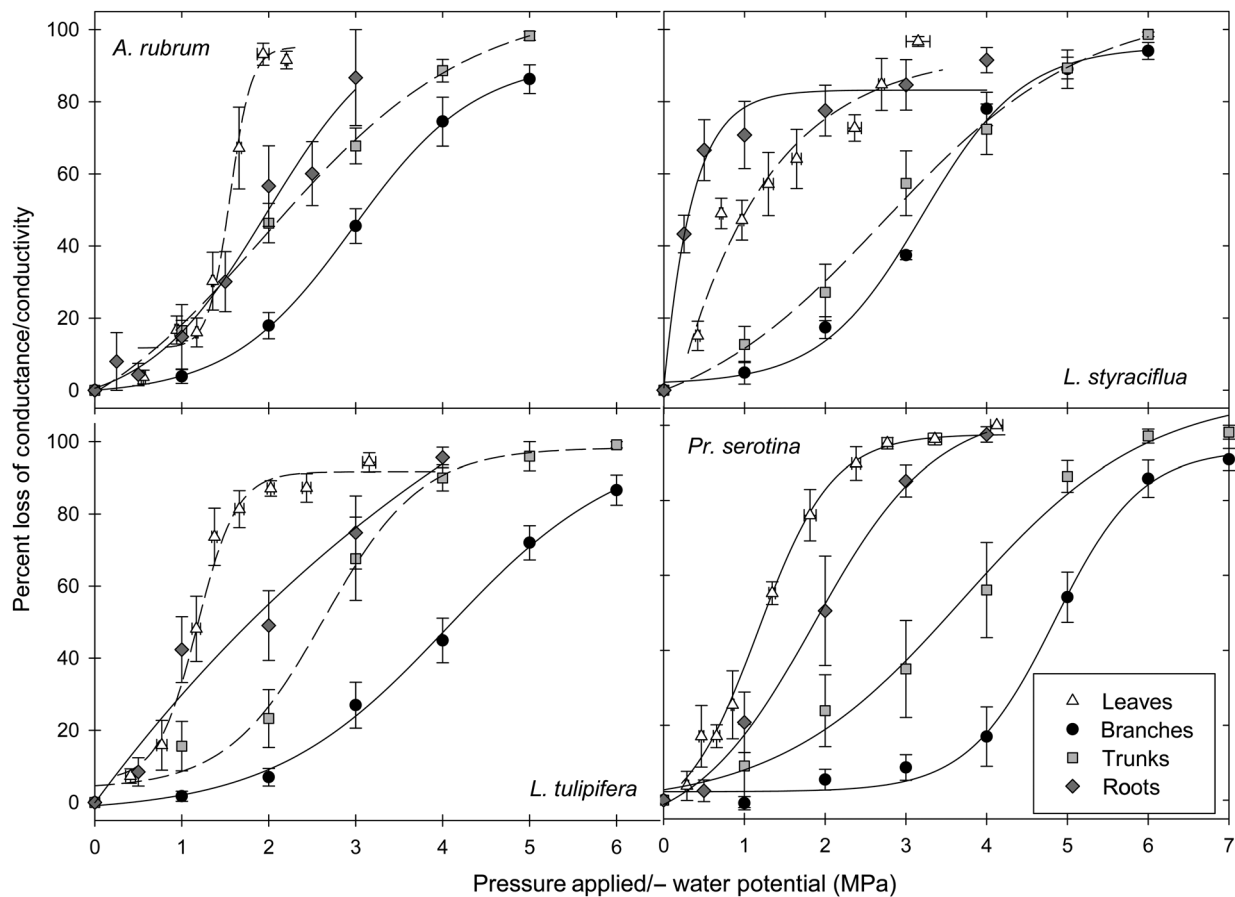
Figure 1. Percent loss of leaf (open triangle), branch (filled circle), trunk (gray square) and root (dark grey diamond) hydraulic conductivity/conductance in four angiosperm species: *A. rubrum*, *L. styraciflua*, *L. tulipifera* and *Pr. serotina*. Error bars are SEs and sample sizes are five to six for each organ.

segmentation (leaf P_{50} – branch P_{50}), leaf Ψ_{MIN}, leaf P_{50} and branch P_{50} data were compiled from Hao et al. (2008), Domec et al. (2009a), Nardini et al. (2012, 2013), Johnson et al. (2011, 2013), McCulloh et al. (2012, 2014, 2015) and from the current study.

Sigmoid functions were fit to vulnerability data and 95% confidence intervals were calculated using Sigmaplot (version 12.5, Systat Software Inc., San Jose, CA, USA). Values of P_{50} were considered statistically different if the 95% confidence intervals did not overlap. Student's *t*-tests were used for comparisons of branches and trunks in *J. ashei*.

Results

Branches were, in general, more resistant to loss of hydraulic conductivity than any other organ tested (Figures 1 and 2, Table 1). Overall, branches had between 0.8 and 1.7 MPa greater (i.e., more resistant, pressures expressed as positive or applied pressures) P_{50} values than trunks, depending on species. The only species in which branches were not significantly more resistant than trunks was *L. styraciflua*, where the 95% confidence intervals for P_{50}s of branches and trunks overlapped.

Although it was not possible to compare P_{50}s for *J. ashei*, the percent loss of conductivity at 10 MPa (the highest pressure we were able to apply) was significantly different in branches and trunks (Figure 2). At 10 MPa, branches of *J. ashei* experienced only 2% loss of conductivity, whereas trunks experienced a 16% loss of conductivity (*t*-test, $P = 0.017$). Overall, leaves were most vulnerable to hydraulic dysfunction (mean $P_{50} = 1.3$ MPa), but one species, *L. styraciflua*, had exceptionally vulnerable roots with a P_{50} of 0.32 MPa. Whole-tree hydraulic vulnerability was greater than branch or trunk vulnerability and was driven primarily by leaf and root hydraulic dysfunction (Table 2, see Figures S1 and S2 available as Supplementary Data at *Tree Physiology* Online). Even when changing resistance partitioning between roots, trunks, branches and leaves, whole-tree hydraulic vulnerability only changed by 0.1 MPa (see Table S1 available as Supplementary Data at *Tree Physiology* Online). When using measured gradients of water potential between soil and leaf, the predicted soil water potentials resulting in a 50% loss of whole-plant conductance were between −0.3 and −0.9 MPa.

Based on vulnerability curves and water potentials measured in the field, leaves were the organ predicted to experience the
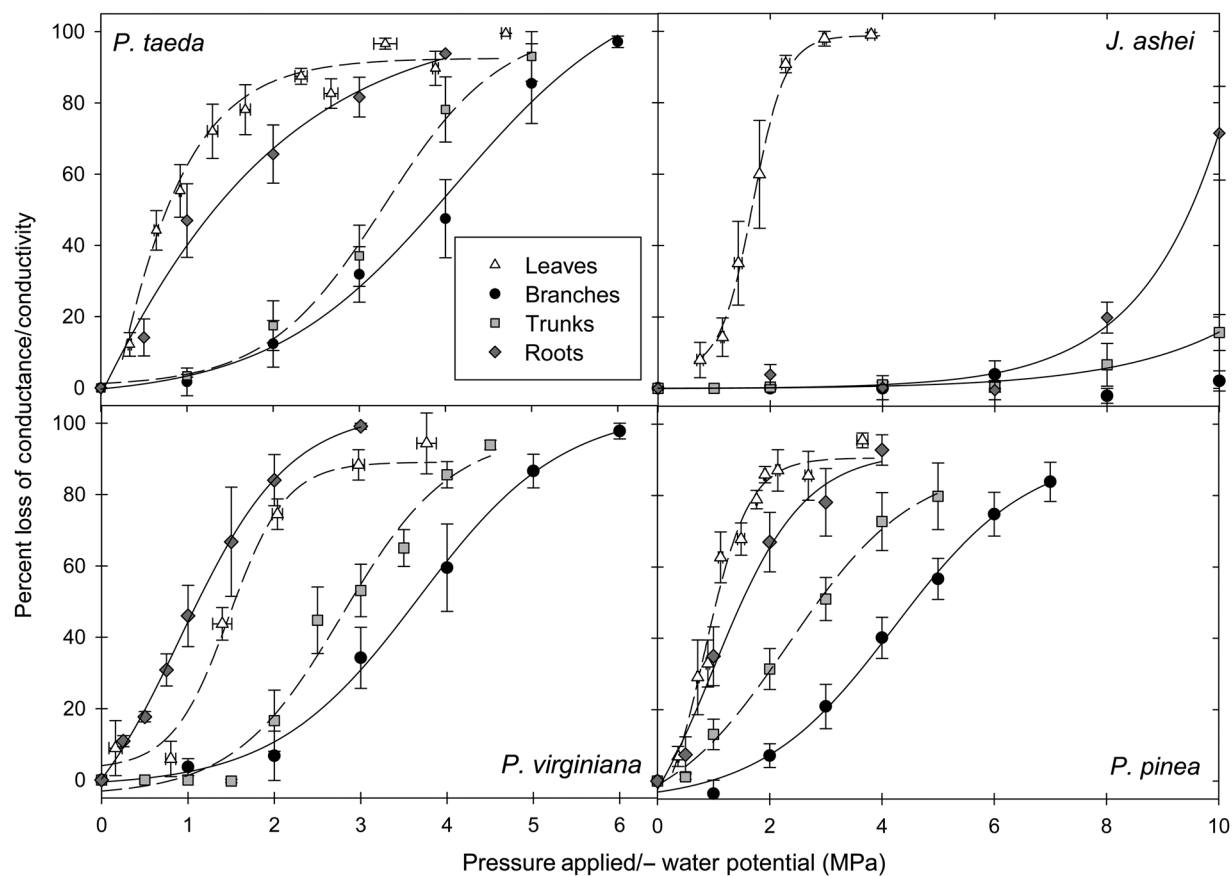
Figure 2. Percent loss of leaf (open triangle), branch (filled circle), trunk (grey square) and root (dark grey diamond) hydraulic conductivity/conductance in four gymnosperm species: *P. taeda*, *P. virginiana*, *J. ashei* and *P. pinea*. Note the different scales between the *x*-axis in left and right panels. Error bars are SEs and sample sizes are five to six for each organ.

Table 2. Predawn water potential resulting in a 50% loss of whole-tree conductivity (P_{50WT}; MPa) and percent whole-tree conductivity remaining at minimum measured predawn water potentials (%$K_{WT \Psi MIN}$), and for *J. ashei* percent remaining under a severe drought ('sd' in parentheses). Whole-tree vulnerability curves are shown in Figures S1 and S2 available as Supplementary Data at *Tree Physiology* Online.

Species	P_{50WT} (MPa)	%$K_{WT \Psi MIN}$
A. rubrum	−1.7	86
L. styraciflua	−0.5	12
L. tulipifera	−1.5	66
Pr. serotina	−1.7	76
J. ashei	−2.2	28 (0 sd)
P. pinea	−1.4	69
P. taeda	−1.1	41
P. virginiana	−1.3	85

largest declines in hydraulic function in situ (Table 3). Averaged across all species, K_{leaf} was reduced at midday to 13% of its maximum values. Roots of *L. styraciflua* were predicted to experience large losses in hydraulic conductance (79%), but the other species in the study were predicted to retain the majority of their root hydraulic conductivity. Branches and trunks of all species in the study were not predicted to have

large losses of hydraulic conductivity. Similarly, hydraulic safety margins ($\Psi_{MIN} - P_{50}$) in branches and trunks of all study species were positive (Figures 3 and 4), and safety margins in leaves were negative. *Juniperus ashei* had a particularly large positive trunk safety margin (6.3–11.0 MPa) and a particularly large negative leaf safety margin (−5.1 MPa); however, measurements of water potentials in this species were also made during a drought. When nondrought values of water potentials were used to calculate safety margins in *J. ashei*, leaf safety margins were much less negative (−1.5 MPa) and trunk safety margins were even greater (11.6–13 MPa). Hydraulic safety margins in roots were generally positive, but were negative in *L. styraciflua*. Again, *J. ashei* had the largest positive safety margin in roots (3.8 MPa during drought and 7.6 MPa under nondrought conditions).

We observed a negative relationship between leaf hydraulic safety margins and leaf to branch vulnerability segmentation (Figure 5). Although when the regression was run without the conifers, the r^2 decreased to 0.26 ($P = 0.016$). Species with greater leaf to branch segmentation tended to have greater leaf hydraulic dysfunction. This was especially apparent in conifers, which were the most strongly segmented and had the most leaf hydraulic dysfunction.

Table 3. Minimum predawn (Ψ_{pd}) and midday (Ψ_{md}) water potentials (in MPa; SEs in parentheses) and the predicted amount of conductivity/conductance remaining (expressed as a percentage and as actual values in parentheses) at the measured water potentials during the normal dry part of the growing season and during an extreme drought in *J. ashei*. Note that actual values of hydraulic conductivity (roots, trunks and branches) are expressed in kg m^{-1} s^{-1} MPa^{-1} and hydraulic conductance in leaves is expressed in mmol m^{-2} s^{-1} MPa^{-1}.

| Species	Ψ_{pd}	$\Psi_{md\,br}$	$\Psi_{md\,lf}$	$k_{s\,root}$ remaining	$k_{s\,trunk}$ remaining	$k_{s\,branch}$ remaining	K_{leaf} remaining
A. rubrum	−0.80 (0.09)	−1.39 (0.10)	−1.98 (0.02)	89% (0.9)	73–86% (0.6–0.7)	93% (0.6)	8% (0.9)
L. styraciflua	−1.09 (0.05)	−2.05 (0.10)	−2.23 (0.13)	21% (1.4)	69–95% (1.2–1.7)	86% (1.3)	22% (3.7)
L. tulipifera	−1.09 (0.07)	−2.18 (0.12)	−2.32 (0.10)	68% (5.6)	68–90% (2.1–2.8)	89% (2.3)	9% (0.9)
Pr. serotina	−1.04 (0.06)	−1.94 (0.06)	−2.13 (0.03)	81% (5.5)	83–91% (4.5–5.5)	98% (1.8)	14% (2.8)
J. ashei	−1.83 (0.10)	−3.07 (0.02)	−3.17 (0.07)	>99% (0.5)	>99% (0.2)	>99% (0.2)	2% (0.1)
P. pinea	−0.98 (0.05)	−1.41 (0.14)	−2.08 (0.12)	69% (1.9)	82–89% (2.2–2.4)	97% (0.3)	14% (0.9)
P. taeda	−1.35 (0.03)	−1.92 (0.05)	−2.33 (0.04)	49% (0.8)	88–94% (1.7–2.4)	80% (0.7)	12% (0.9)
P. virginiana	−0.70 (0.02)	−1.16 (0.07)	−2.09 (0.13)	70% (8.6)	97–99% (1.4)	97% (1.4)	22% (1.0)
Mean % remaining across all species				68% (12.0)	82–93% (2.0–5.5)	93% (2.8)	13% (2.9)
J. ashei severe drought	−5.67 (0.19)	−6.70 (0.18)	−6.77 (0.18)	97% (1.0)	97–98% (0.2)	99–100% (0.1)	~1% (0.1)
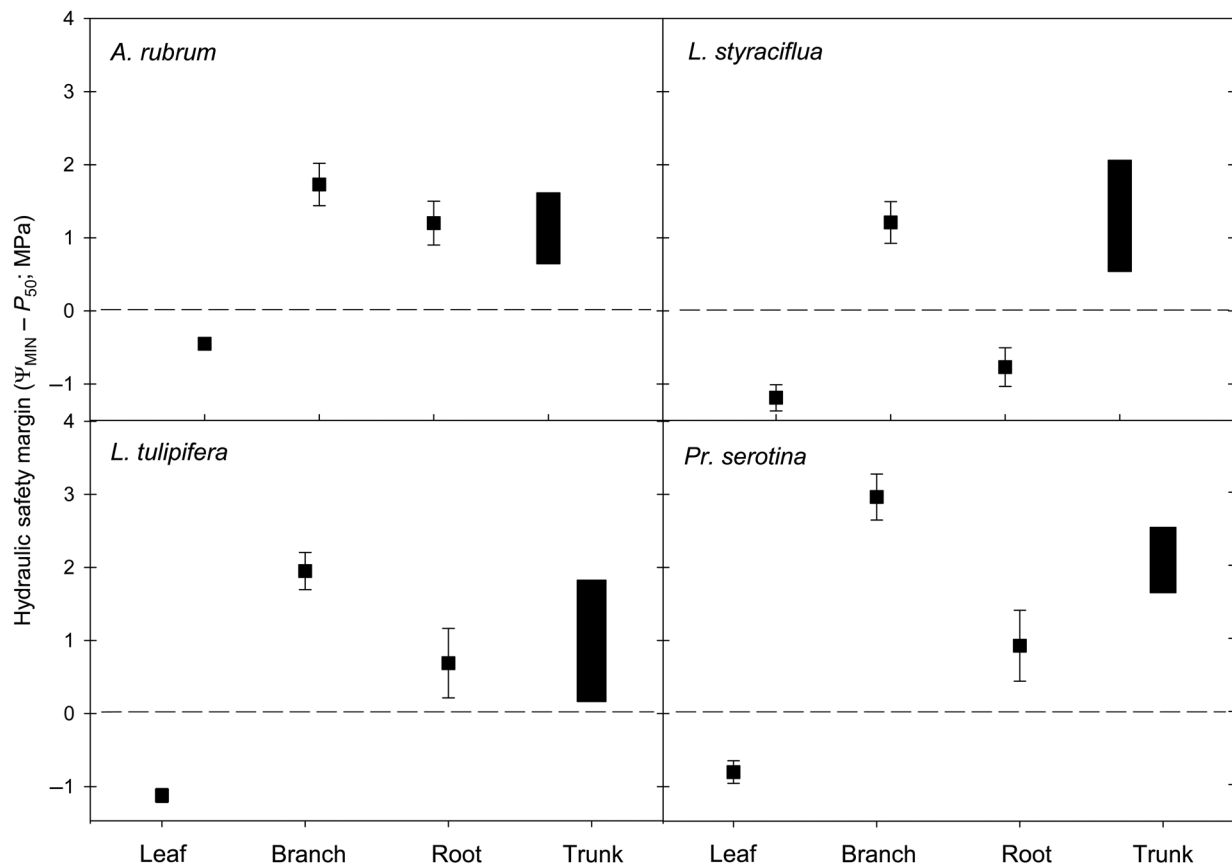
Figure 3. Leaf, branch, root and trunk hydraulic safety margins (expressed as minimum measured water potential − P_{50}) in four angiosperm species: *A. rubrum*, *L. styraciflua*, *L. tulipifera* and *Pr. serotina*. A range of potential trunk safety margins were estimated by using the range of water potentials corresponding to predawn leaf and midday branch and assuming that the trunk water potential would be between those two values. Error bars are SEs and sample sizes are five to six for each organ.

Discussion

We found strong support for the HVSH, especially in distal organs. Leaves and roots were more vulnerable to hydraulic dysfunction than branches or trunks. Previous work has found similar results, especially in leaves and petioles compared with stems. Tyree et al. (1993) and Tsuda and Tyree (1997) found that branches were ~0.8 and 1.0 MPa more resistant to embolism (comparing P_{50}s of branch and petiole) than petioles in
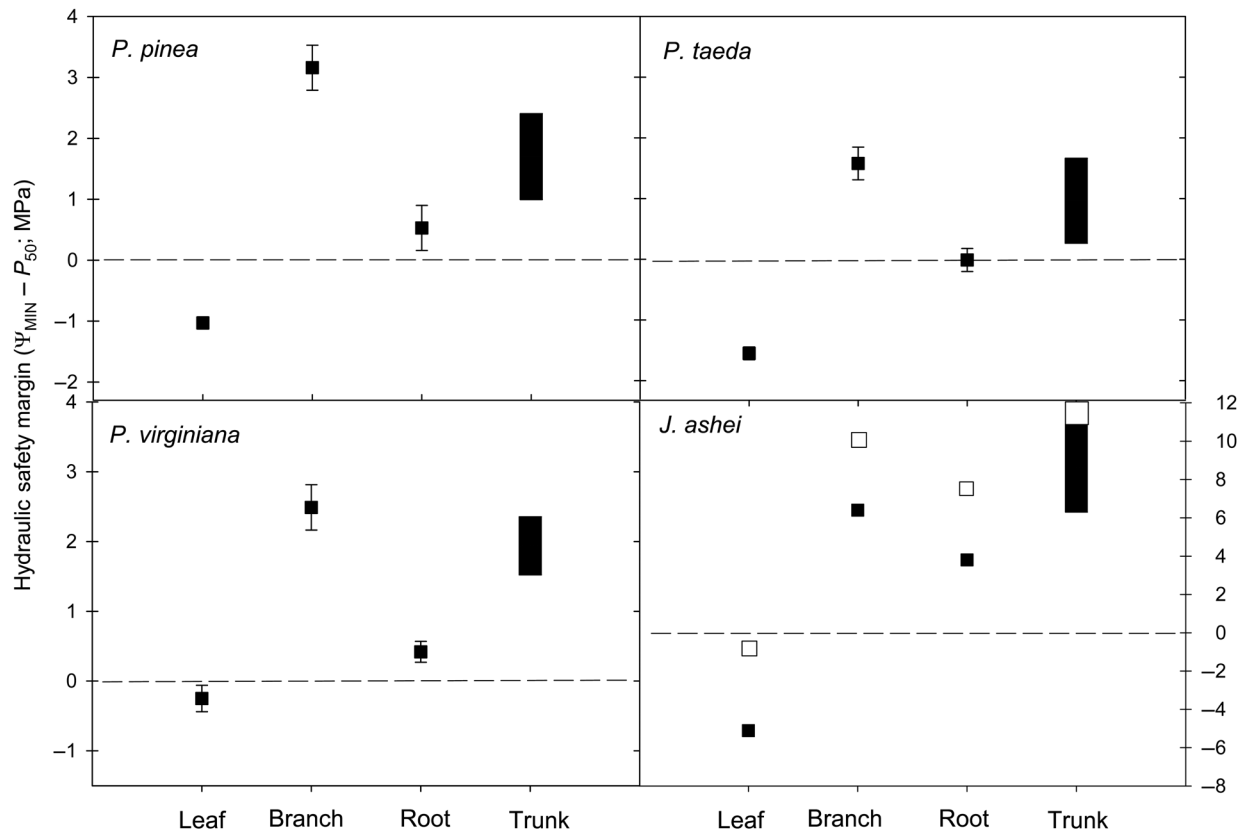
Figure 4. Leaf, branch, root and trunk hydraulic safety margins (expressed as minimum measured water potential $- P_{50}$) in four conifer species: *P. taeda*, *P. virginiana*, *J. ashei* and *P. pinea*. Open symbols in *J. ashei* panel represent nondrought conditions. Error bars are SEs and sample sizes are five to six for each organ.
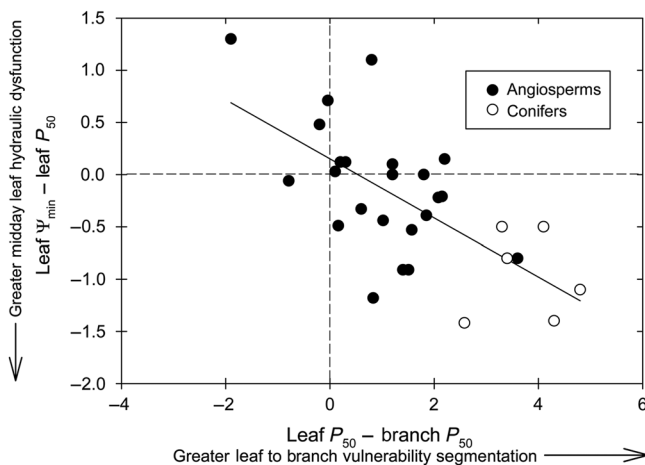

Figure 5. A comparison of leaf hydraulic safety margins (leaf minimum water potential $- P_{50}$) and leaf to branch vulnerability segmentation (leaf $P_{50} -$ branch P_{50}). Species with stronger segmentation (i.e., larger stem to leaf P_{50} differences) tend to lose more leaf hydraulic conductance midday ($r^2 = 0.45$, $P < 0.0001$). See Materials and methods for data references.

Juglans regia L. and *A. saccharinum*, respectively. More recently, Scholz et al. (2014) found that leaves of *Nothofagus dombeyi* Mirb. Oerst. and *Austrocedrus chilensis* Florin & Boutelie were 1.8 and 3.1 MPa more vulnerable to loss of hydraulic function than branches, respectively. This greater

leaf to branch hydraulic vulnerability segmentation (P_{50} leaf $-$ P_{50} branch) in conifers compared with angiosperms is supported by the current study (Figure 5) and is documented in the literature (Chen et al. 2009, Johnson et al. 2011, 2012, McCulloh et al. 2014).

Both the current study and multiple additional studies indicate that in general, roots were more vulnerable to embolism than branches within the same species (e.g., Hacke and Sauter 1996, McElrone et al. 2004, Maherali et al. 2006, Willson et al. 2008). The root diameters (and likely age classes) used in the cited studies above and in the current study were in the same diameter ranges: between 6 and 11 mm; therefore, it is not surprising that the results between studies were similar. The difference between root and branch vulnerability to embolism is typically greater in conifers than in angiosperms (Table 1, Johnson et al. 2012), and in the current study, mean root $P_{50} -$ branch P_{50} was 2.1 MPa for angiosperms and 3.1 MPa for conifers; however, this difference was not significant (*t*-test, $P = 0.07$). Taken together, the above studies and the current study show broad support for the HVSH when comparing branches and roots.

Much less is known about trunk hydraulic conductivity and vulnerability to embolism than branches. McCulloh et al. (2014) found that in four species of tall, old-growth conifers, branches were dramatically more resistant (by 3–4 MPa) to embolism than

trunks of the same individuals. Similarly, in *Pseudotsuga menziesii* (Mirb.) Franco and *Pinus ponderosa* Douglas ex. C. Lawson, branches were 1–2 MPa more resistant to embolism than trunks, and this difference in xylem embolism resistance appeared to be greatest when comparing branches with samples taken from the bases of older trees (Domec and Gartner 2003, Domec et al. 2009a). However, other studies have found no difference between branch and trunk vulnerability to embolism. Hao et al. (2013) found no evidence for differences in branch and trunk vulnerability in *Betula papyrifera* Marsh. trees, and Choat et al. (2005) found no difference in air-seeding pressures between trunks and branches of *Acer saccharum* Marsh. In the current study, branches were consistently more resistant to hydraulic dysfunction than trunks, leaves or roots, and therefore, these data do not support the HVSH as it pertains to branches. Additionally, whole trees were quite vulnerable to embolism (see Figures S1 and S2 available as Supplementary Data at *Tree Physiology* Online), especially when compared with branches. Branches were on average 3.7 MPa more resistant to hydraulic dysfunction than whole trees (2.6 MPa more resistant when *J. ashei* was not included). Even when changing the resistance partitioning between roots, trunks, branches and leaves, whole-tree hydraulic vulnerability remained essentially unchanged (see Table S1 available as Supplementary Data at *Tree Physiology* Online). These data suggest that branches may be a poor proxy for whole-tree hydraulic vulnerability, particularly when compared with small roots and leaves.

Safety margins express the degree of hydraulic conservatism exhibited by a plant (e.g., Meinzer et al. 2009, Johnson et al. 2012, Skelton et al. 2015). Plants and organs vary widely in their hydraulic safety margins, with conifer woody tissues tending to have wider safety margins than angiosperm woody tissues (Choat et al. 2012, Johnson et al. 2012). It has been postulated that the reason for this difference between woody tissues of angiosperms and conifers is that conifers may be less efficient at recovery of hydraulic function once it is lost than angiosperms (Johnson et al. 2012). Additionally, the ability to resprout after trunk mortality is rarely found in North American conifer adult trees, but is found in the majority of North American angiosperm tree species (Del Tredici 2001, Bond and Midgley 2003). The lack of ability to resprout in the majority of conifer trees makes protection of the trunk even more critical for individual tree survival.

The species in the current study exhibited positive safety margins for branches and trunks and negative safety margins in leaves, indicating that leaves of all species in the current study lose the majority of their hydraulic conductance at the measured minimum water potentials. Data from species in the current study, as well as data from the literature, indicate that leaves of species that have greater branch to leaf hydraulic segmentation lose more leaf hydraulic conductance than species with less branch to leaf segmentation (Figure 5). This supports previous work

suggesting that leaves can act as hydraulic 'safety valves' and that this effect may be greater in conifers than in angiosperms (Johnson et al. 2011, Zufferey et al. 2011). We have observed in the current study (*J. ashei*) and in a previous study (Johnson et al. 2011, *P. virginiana*) that some conifers lose all or nearly all of their leaf hydraulic conductance daily (98–100%). This could represent an alternate strategy (compared with strict stomatal regulation preventing leaf hydraulic dysfunction) whereby the leaf hydraulic dysfunction cuts off upstream portions of the transpiration stream from the atmosphere and thus keeps the branches and trunk from experiencing dysfunction. Root safety margins were more variable, with *Liquidambar* having negative safety margins and the roots of the other species in the study having positive (or near-zero) safety margins. Roots often have high vulnerability to embolism and resulting narrow or negative safety margins (Alder et al. 1996, Sperry and Ikeda 1997, Martínez-Vilalta et al. 2002). It could be that this is an adaptive trait that would allow the plant to be decoupled from drying soil during droughts (Johnson et al. 2014).

An understanding of hydraulic vulnerabilities throughout a plant is critical to our understanding of whole-plant function. Our study found support for the HVSH in leaves and roots and supports the notion that measurement of one type on a single organ or at a single scale may not accurately reflect the overall hydraulic strategy of a whole plant (Meinzer et al. 2010). Indeed, most of the accumulated information on plant hydraulics comes from branches, which our study suggests are the least hydraulically constrained organ in many species. Therefore, we recommend that future studies of tree hydraulic properties focus on multiple organs, including roots and leaves, which show the greatest hydraulic failure in the species we studied. Additionally, the many vegetation models that rely on branch hydraulic vulnerability to predict species distributions should consider incorporating a more holistic approach (e.g., Sperry et al. 1998, 2000, McCulloh et al. 2014) for predictions of species distributions under climate change.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

Conflict of interest

None declared.

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References

Alder NN, Sperry JS, 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.

Bond WJ, Midgley JJ (2003) The evolutionary ecology of sprouting in woody plants. Int J plant Sci 164:S103–S114.

Brodribb TJ, Holbrook NM (2003) Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. Plant Physiol 132:2166–2173.

Bucci SJ, Scholz FG, Goldstein G, Meinzer FC, Hinojosa JA, Hoffmann WA, Franco AC (2004) Processes preventing nocturnal equilibration between leaf and soil water potential in tropical savanna woody species. Tree Physiol 24:1119–1127.

Chen J-W, Zhang Q, Li X-S, Cao K-F (2009) Independence of stem and leaf hydraulic traits in six Euphorbiaceae tree species with contrasting leaf phenology. Planta 230:459–468.

Choat B, Lahr EC, Melcher PJ, Zwieniecki MA, Holbrook NM (2005) The spatial pattern of air seeding thresholds in mature sugar maple trees. Plant Cell Environ 28:1082–1089.

Choat B, Drayton WM, Brodersen C, Matthews MA, Shackel KA, Wada H, Mcelrone AJ (2010) Measurement of vulnerability to water stress-induced cavitation in grapevine: a comparison of four techniques applied to a long-vesseled species. Plant Cell Environ 33:1502–1512.

Choat B, Jansen S, Brodribbn TJ et al. (2012) Global convergence in the vulnerability of forests to drought. Nature 491:752–755.

Cochard H, Bréda N, Granier A, Aussenac G (1992a) Vulnerability to air embolism of three European oak species (*Quercus petraea* (Matt) Liebl, *Q pubescens* Willd, *Q robur* L). Ann For Sci 49:225–233.

Cochard H, Cruziat P, Tyree MT (1992b) Use of positive pressures to establish vulnerability curves. Further support for the air-seeding hypothesis and implications for pressure-volume analysis. Plant Physiol 100:205–209.

Cruiziat P, Cochard H, Améglio T (2002) Hydraulic architecture of trees: main concepts and results. Ann For Sci 59:723–752.

Del Tredici P (2001) Sprouting in temperate trees: a morphological and ecological review. Bot Rev 67:121–140.

Domec J-C, Gartner BL (2001) Cavitation and water storage capacity in bole xylem segments of mature and young Douglas-fir trees. Trees 15:204–214.

Domec J-C, Gartner BL (2003) Relationship between growth rates and xylem hydraulic characteristics in young, mature and old-growth ponderosa pine trees. Plant Cell Environ 26:471–483.

Domec J-C, Pruyn ML, Gartner BL (2005) Axial and radial profiles in conductivities, water storage and native embolism in trunks of young and old-growth ponderosa pine trees. Plant Cell Environ 28:1103–1113.

Domec J-C, Scholz FG, Bucci SJ, Meinzer FC, Goldstein G, Villalobos-Vega R (2006) Diurnal and seasonal variation in root xylem embolism in neotropical savanna woody species: impact on stomatal control of plant water status. Plant Cell Environ 29:26–35.

Domec J-C, Warren JM, Meinzer FC, Lachenbruch B (2009a) Safety factors for xylem failure by implosion and air-seeding within roots, trunks and branches of young and old conifer trees. IAWA J 30:101–120.

Domec J-C, Noormets A, King JS, Sun G, McNulty SG, Gavazzi MJ, Boggs J, Treasure EA (2009b) Decoupling the influence of leaf and root hydraulic conductances on stomatal conductance and its sensitivity to vapour pressure deficit as soil dries in a drained loblolly pine plantation. Plant Cell Environ 32:980–991.

Domec J-C, Pruyn ML, Lachenbruch B, Spicer R (2012) Effects of age-related increases in sapwood area, leaf area, and xylem conductivity on height-related hydraulic costs in two contrasting coniferous species. Ann For Sci 69:17–27.

Engelbrecht BMJ, Velez V, Tyree MT (2000) Hydraulic conductance of two co-occuring neotropical understory shrubs with different habitat preferences. Ann For Sci 57:201–208.

Ennajeh M, Simões F, Khemira H, Cochard H (2011) How reliable is the double-ended pressure sleeve technique for assessing xylem vulnerability to cavitation in woody angiosperms? Physiol Plant 142:205–210.

Erickson HD (1960) The effects of storage conditions and time upon permeability of green sapwood. Proc Am Wood Preserv Assoc 56:156–165.

Ewers FW, Fisher JB (1989) Techniques for measuring vessel lengths and diameters in stems of woody plants. Am J Bot 76:645–656.

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

Hao G-Y, Hoffmann WA, Scholz FG, Bucci SJ, Meinzer FC, Franco AC, Cao K-F, Goldstein G (2008) Stem and leaf hydraulics of congeneric tree species from adjacent tropical savanna and forest ecosystems. Oecologia 155:405–415.

Hao G-Y, Wheeler JK, Holbrook NM, Goldstein G (2013) Investigating xylem embolism formation, refilling and water storage in tree trunks using frequency domain reflectometry. J Exp Bot 64:2321–2332.

Hellkvist J, Richards GP, Jarvis PG (1974) Vertical gradients of water potential and tissue water relations in Sitka spruce trees measured with the pressure chamber. J Appl Ecol 11:637–667.

Johnson DM, McCulloh KA, Meinzer FC, Woodruff DR, Eissenstat DM (2011) Hydraulic patterns and safety margins, from stem to stomata, in three eastern US tree species. Tree Physiol 31:659–668.

Johnson DM, McCulloh KA, Woodruff DR, Meinzer FC (2012) Hydraulic safety margins and embolism reversal in stems and leaves: why are conifers and angiosperms so different? Plant Sci 195:48–53.

Johnson DM, Domec J-C, Woodruff DR, McCulloh KA, Meinzer FC (2013) Contrasting hydraulic strategies in two tropical lianas and their host trees. Am J Bot 100:374–383.

Johnson DM, Sherrard ME, Domec J-C, Jackson RB (2014) Role of aquaporin activity in regulating deep and shallow root hydraulic conductance during extreme drought. Trees 28:1323–1331.

Maherali H, Moura CF, Caldeira MC, Willson CJ, Jackson RB (2006) Functional coordination between leaf gas exchange and vulnerability to xylem cavitation in temperate forest trees. Plant Cell Environ 29:571–583.

Martínez-Vilalta J, Prat E, Oliveras I, Piñol J (2002) Xylem hydraulic properties of roots and stems of nine Mediterranean woody species. Oecologia 133:19–29.

McCulloh KA, Johnson DM, Meinzer FC, Voelker SL, Lachenbruch B, Domec J-C (2012) Hydraulic architecture of two species differing in wood density: opposing strategies in co-occurring tropical pioneer trees. Plant Cell Environ 35:116–125.

McCulloh KA, Johnson DM, Meinzer FC, Woodruff DR (2014) The dynamic pipeline: hydraulic capacitance and xylem hydraulic safety in four tall conifer species. Plant Cell Environ 37:1171–1183.

McCulloh KA, Johnson DM, Petitmermet J, McNellis B, Meinzer FC, Lachenbruch B (2015) A comparison of hydraulic architecture in three similarly sized woody species differing in their maximum potential height. Tree Physiol 35:723–731.

McElrone AJ, Pockman WT, Martínez-Vilalta J, Jackson RB (2004) Variation in xylem structure and function in stems and roots of trees to 20 m depth. New Phytol 163:507–517.

Meinzer FC (2002) Co-ordination of vapour and liquid phase water transport properties in plants. Plant Cell Environ 25:265–274.

Meinzer FC, Johnson DM, Lachenbruch B, McCulloh KA, Woodruff DW (2009) Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. Funct Ecol 23:922–930.

Meinzer FC, Lachenbruch B, McCulloh KA, Woodruff DW, Johnson DM (2010) The blind men and the elephant: the impact of context and scale in evaluating conflicts between plant hydraulic safety and efficiency. Oecologia 164:287–296.

Meinzer FC, Woodruff DR, Marias DE, McCulloh KA, Sevanto S (2014) Dynamics of leaf water relations components in co-occurring iso- and anisohydric conifer species. Plant Cell Environ 37:2577–2586.

Nardini A, Tyree MT (1999) Root and shoot hydraulic conductance of seven Quercus species. Ann For Sci 56:371–377.

Nardini A, Pedà G, La Rocca N (2012) Trade-offs between leaf hydraulic capacity and drought vulnerability: morpho-anatomical bases, carbon costs and ecological consequences. New Phytol 196:788–798.

Nardini A, Battistuzzo M, Savi T (2013) Shoot desiccation and hydraulic failure in temperate woody angiosperms during an extreme summer drought. New Phytol 200:322–329.

Pallardy SG (2008) Physiology of woody plants. Academic Press, Oxford, UK, pp 330–331.

Panshin AJ, De Zeeuw C (1980) Textbook of wood technology, 4th edn. McGraw-Hill, New York, p 134.

Parker WC, Pallardy SG (1985) Drought-induced leaf abscission and whole-plant drought tolerance of seedlings of seven black walnut families. Can J For Res 15:818–821.

Pivovaroff AL, Sack L, Santiago LS (2014) Coordination of stem and leaf hydraulic conductance in southern California shrubs: a test of the hydraulic segmentation hypothesis. New Phytol 203:842–850.

Pratt RB, North GB, Jacobsen AL, Ewers FW, Davis SD (2010) Xylem root and shoot hydraulics is linked to life history type in chaparral seedlings. Funct Ecol 24:70–81.

Pratt RB, Jacobsen AL, Ramirez AR, Helms AM, Traugh CA, Tobin MF, Heffner MS, Davis SD (2014) Mortality of resprouting chaparral shrubs after a fire and during a record drought: physiological mechanisms and demographic consequences. Glob Change Biol 20:893–907.

Richter H (1997) Water relations of plants in the field: some comments on the measurement of selected parameters. J Exp Bot 48:1–7.

Rood SB, Patiño S, Coombs K, Tyree MT (2000) Branch sacrifice: cavitation-associated drought adaptation of riparian cottonwoods. Trees 14:248–257.

Sack L, Holbrook NM (2006) Leaf hydraulics. Annu Rev Plant Biol 57:361–381.

Salleo S, Hinckley TM, Kikuta SB, Lo gullo MA, Weilgony P, Yoon T-M, Richter H (1992) A method for inducing xylem emboli in situ: experiments with a field-grown tree. Plant Cell Environ 15:491–497.

Scholander PF, Hammel HT, Bradstreet ED, Hemmington EA (1965) Sap pressure in vascular plants. Science 148:339–346.

Scholz FG, Bucci SJ, Goldstein G (2014) Strong hydraulic segmentation and leaf senescence due to dehydration may trigger die-back in Nothofagus dombeyi under severe droughts: a comparison with the co-occurring Austrocedrus chilensis. Trees 28:1475–1487.

Skelton RP, West AG, Dawson TE (2015) Predicting plant vulnerability to drought in biodiverse regions using functional traits. Proc Natl Acad Sci USA 112:5744–5749.

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

Sperry JS, Pockman WT (1993) Limitation of transpiration by hydraulic conductance and xylem cavitation in Betula occidentalis. Plant Cell Environ 16:279–287.

Sperry JS, Tyree MT (1990) Water-stress-induced xylem embolism in three species of conifers. Plant Cell Environ 13:427–436.

Sperry JS, Nichols KL, Sullivan JEM, Eastlack SE (1994) Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of northern Utah and interior Alaska. Ecology 75:1736–1752.

Sperry JS, Adler FR, Campbell GS, 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, Hacke UG, Oren R, Comstock JP (2002) Water deficits and hydraulic limits to leaf water supply. Plant Cell Environ 25: 251–263.

Tsuda M, Tyree MT (1997) Whole-plant hydraulic resistance and vulnerability segmentation in Acer saccharinum. Tree Physiol 17:351–357.

Turner NC, Long MJ (1980) Errors arising from rapid water loss in the measurement of leaf water potential by the pressure chamber technique. Aust J Plant Physiol 7:527–537.

Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. New Phytol 119:345–360.

Tyree MT, Hammel HT (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. J Exp Bot 23:267–282.

Tyree MT, Zimmermann MH (2002) Xylem structure and the ascent of sap. Springer Science and Business Media, Berlin, pp 171–174.

Tyree MT, Cochard H, Cruiziat P, Sinclair B, Ameglio T (1993) Drought-induced leaf shedding in walnut: evidence for vulnerability segmentation. Plant Cell Environ 16:879–882.

van Hees AFM (1997) Growth and morphology of pedunculate oak (Quercus robur L) and beech (Fagus sylvatica L) seedlings in relation to shading and drought. Ann For Sci 54:9–18.

Willson CJ, Manos PS, Jackson RB (2008) Hydraulic traits are influenced by phylogenetic history in the drought-resistant, invasive genus Juniperus (Cupressaceae). Am J Bot 95:299–314.

Zimmermann MH (1983) Xylem structure and the ascent of sap. Springer, Berlin, pp 80–82.

Zufferey V, Cochard H, Ameglio T, Spring J-L, Viret O (2011) Diurnal cycles of embolism formation and repair in petioles of grapevine (Vitis vinifera cv. Chasselas). J Exp Bot 62:3885–3894.