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Research paper

A comparison of hydraulic architecture in three similarly sized woody species differing in their maximum potential height

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The physiological mechanisms underlying the short maximum height of shrubs are not understood. One possible explanation is that differences in the hydraulic architecture of shrubs compared with co-occurring taller trees prevent the shrubs from growing taller. To explore this hypothesis, we examined various hydraulic parameters, including vessel lumen diameter, hydraulic conductivity and vulnerability to drought-induced embolism, of three co-occurring species that differed in their maximum potential height. We examined one species of shrub, one short-statured tree and one taller tree. We worked with individuals that were approximately the same age and height, which was near the maximum for the shrub species. A number of variables correlated with the maximum potential height of the species. For example, vessel diameter and vulnerability to embolism both increased while wood density declined with maximum potential height. The difference between the pressure causing 50% reduction in hydraulic conductance in the leaves and the midday leaf water potential (the leaf's hydraulic safety margin) was much larger in the shrub than the other two species. In general, trends were consistent with understory shrubs having a more conservative life history strategy than co-occurring taller species.

Keywords: height, hydraulic architecture, hydraulic conductivity, vulnerability to embolism.

Introduction

Increases in plant height were a crucial evolutionary advance that changed the landscape of terrestrial environments and initiated an arms race in the competition for light. Despite the importance of height for carbon gain (Falster and Westoby 2003), most forests contain plants of a variety of heights. It is clear that the strategy of shorter plants is more conservative than their canopy-exposed neighbors; understory plants tend to have lower maximum photosynthetic rates, light saturation and compensation points, and stomatal conductance than sun-exposed trees (Givnish 1988, Kitajima 1994, Valladares and Niinemets 2008). The shrub growth form is really a collection of forms that probably result from many ecological factors as well as some ecological canalization. Shrubs commonly compose the

overstory in arid, permafrost, wetland, windy, derelict or estuarine habitats, but they are also common as understory plants beneath tree canopies.

The question of what factors contribute to keep a shrub short can be addressed from many different perspectives. From an evolutionary perspective, there may be phylogenetic limitations on the maximum height of plants in genera or families that are predominately short-statured. From an ecological perspective, understory shrubs fill a niche by capturing light that filters through the overstory canopy. They may also fill other niches that have not been as well-understood, related to other aspects of the understory environment (e.g., a more equable microclimate, higher probability of debris fall, different types of herbivore interactions). Arguably, though, one or more physiological

or mechanistic explanations underlie these different perspectives, because they would identify the reason that shrubs are unable to grow taller. Physiological constraints on plant stature could result from a variety of factors, which are not mutually exclusive, including differences in allocation patterns, longevity, differences in hormonal distribution or sensitivity and hydraulic architecture. Although many or all of these factors may contribute to the maximum height of plants, here we focused solely on how various hydraulic parameters differ in similarly sized individuals from three woody species that vary in their maximum potential height in an attempt to determine if there are distinct differences between these species in these parameters.

One of the reasons we were interested in exploring a hydraulic explanation for the maximum height in shrubs is that recent work has linked the maximum height in trees to limitations imposed by the xylem network (Koch et al. 2004, Woodruff et al. 2004, Domec et al. 2008, Ishii et al. 2008), although it has recently been suggested that allometry also plays an important role (Givnish et al. 2014). At extreme heights, the hydrostatic decline in water potential limits the abilities of growing cells to fully expand (Woodruff and Meinzer 2011). This limitation on cell expansion limits growth in all tissues (Koch et al. 2004, Woodruff et al. 2004, 2008), including the xylem (Domec et al. 2008). At the heights of shrubs, the decline in water potential imposed by the hydrostatic gradient is trivial, but declines in the hydraulic conductivity of the xylem in the distal portions of the shrub may still affect its ability to grow taller by increasing gradients in xylem tension.

In order to compare the hydraulic architecture of species that differed in their maximum heights, we selected ramets of shrubs and saplings of trees that were approximately the same basal diameter, height and age. The heights selected were near the maximum heights for the shortest species we collected. Our theory was that if hydraulics correlates with the maximum height to which the species could grow, then short-statured species would exhibit the largest differences from taller species when the shrubs were near their maximum height and the trees were far from theirs. Furthermore, for these co-occurring species, the light environment is similar when they are all the same size. Later in development, the trees have greater exposure to light, which is known to positively correlate with various measures of water transport efficiency (Brodribb and Feild 2000).

We hypothesized that if shrubs were limited in their height because of their hydraulic architecture, they would exhibit lower hydraulic conductivity on a xylem area basis (specific conductivity, k_s) than neighboring trees. Previous work comparing the hydraulic architecture of tree and shrub habits in one species, *Juniperus communis* L. (Beikircher and Mayr 2008), found much greater k_s in basal stems of trees than the shrubs, and we expected to find the same result across our species. We also hypothesized that the anatomical change underlying this functional trend would be greater increases in the vessel diameter from the branches to the trunks in trees than in shrubs.

Recent work comparing the anatomy and morphology of shrubs spanning habitats with a range of mean annual precipitation found strong positive correlations between aridity and both wood density and smaller vessel lumen areas (Martinez-Cabrera et al. 2009). This work was then extended to include trees in the comparison and negative correlations were found between wood density and both maximum height and vessel diameter (Martinez-Cabrera et al. 2011). These correlations of wood density and various factors are likely to have a mechanistic basis, because of the biologically important correlations of wood density with a variety of functional traits (Lachenbruch and McCulloh 2014). For example, wood density has been shown to negatively correlate with vulnerability to embolism (Hacke et al. 2001, Jacobsen et al. 2005) and hydraulic capacitance (Scholz et al. 2007, 2011, Meinzer et al. 2008) in many cases. Thus we hypothesize that wood density will be negatively related to the species' maximum potential height and that vulnerability to embolism will be positively correlated with maximum potential height in a comparison of a shrub, a short tree, and a taller tree.

Materials and methods

Plant material

Individuals of three angiosperm species co-occurred and were collected from the McDonald Forest near Corvallis, OR, USA (44°N, 123°W). In order of maximum height they can achieve (Hitchcock and Cronquist 1998), the species were *Alnus rubra* Bong. (25 m), *Rhamnus purshiana* DC. (10 m) and *Corylus cornuta* Marsh. (3, rarely to 5 m; shrubs in our study site did not reach more than ~3.5 m). All sampled individuals or ramets were 4–7 years old and the ages did not differ among species ($P = 0.42$).

Four saplings or ramets from different shrubs (hereafter all will be referred to as shoots) were collected for each species and were 2–3 m tall. All species were growing in sites that received direct sun part of the day, but were not fully exposed. Shoots were harvested in the morning by cutting them as close to the ground as possible, measuring the distance from the ground to the highest leaf, and bagging them for transport back to the laboratory. In the laboratory, we selected stem segments to be used for measurement, selecting segments that spanned the range of diameters available within the shoot and generally fell into diameter categories of around 7, 15 and 25 mm, and then counted the leaves distal to each segment. Four to six stem segments were used from each shoot. Samples were removed from the main stem by cutting under water.

Stem hydraulic conductivity and vulnerability to embolism

The small, medium and large diameter segments were used for measurements of hydraulic conductivity and vulnerability to drought-induced embolism. Diameter size classes of segments did not differ among species and the under-bark means (standard error (SE)) were 7.0 mm (0.2), 14.8 mm (1.6) and

24.7 mm (1.6), respectively for small, medium and large segments. If the stems were too wide to fit into our air-injection sleeve (see below), we chiseled a wedge from the segment for the hydraulic conductivity measurements (Domec and Gartner 2002). The axial ends of all stem segments were re-cut under water with fresh razor blades. Cut segments were submerged in a filtered perfusion solution of water mixed with a small amount of concentrated hydrochloric acid, which reduced the pH to 2 in order to retard microbial growth. A partial vacuum was applied overnight to the stems and solution to refill air-filled vessels with water.

After segments were vacuum infiltrated, hydraulic conductivity measurements were begun to construct vulnerability curves, alternately measuring hydraulic conductivity and then radially injecting air at known, high pressures. The 'air-injection' method (Sperry and Saliendra 1994) assumes that air seeds between vessels at a pressure that has the same magnitude when pushed into vessels as it does in situ when the negative pressures pull air into vessels. Air pressures imposed on stems between each measurement of k_h were increased in 1.0 MPa increments. Segments were held at each pressure for at least 2 min, and samples were left underwater after pressurization until air no longer bubbled from the axial ends before the next k_h measurement.

To measure hydraulic conductivity, we determined the volume flow rate of water through stems 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 this volume flow rate by the pressure gradient used to induce flow across the sample. The pressure gradient across the length of the stem was created by a hydrostatic pressure head, which was kept ~40 cm high (~4 kPa). Vulnerability curves were graphed by comparing the percent loss in k_h relative to the maximum k_h (immediately after vacuum infiltration) vs the applied pressure. Xylem-area specific conductivity (k_s) was determined by dividing maximum k_h by the xylem cross-sectional area of the branch after bark had been removed using digital calipers.

Leaf hydraulic conductance

Hydraulic conductance was measured in *Alnus* and *Rhamnus* leaves using the rehydration kinetics method (Brodribb and Holbrook 2003). Data for *Corylus* were taken from Johnson et al. (2012). Leaf-bearing shoots were cut from the main shoot under water and allowed to rehydrate for ~2 h. After rehydration, shoots were either ready for measurement (see below) or allowed to transpire for various lengths of time to achieve a range of water potentials at which to measure hydraulic conductance. Once shoots were ready for measurement, they were bagged and placed in the dark for at least 1 h to allow water potentials to equilibrate throughout the shoot. After equilibration, 3–4 leaves were selected randomly and the water potentials measured using a pressure chamber (PMS Inc., Albany, OR,

USA) to confirm that the water status was consistent (within 0.1 MPa) throughout the shoot. Pairs of neighboring leaves were then selected, which were assumed to have the same water potential. From each pair, the water potential was measured from the first leaf (Ψ_i) immediately after removing it from the shoot. After the second leaf was removed, its petiole was recut under water and the leaf was allowed to rehydrate in water for t seconds, after which the leaf water potential was determined (Ψ_f). Leaf hydraulic conductance (K_{leaf}) was then calculated as

$$K_{\text{leaf}} = \frac{C \ln(\Psi_i / \Psi_f)}{t}, \quad (1)$$

where C is the leaf capacitance, which was calculated using the pressure–volume (P – V) curves (Tyree and Hammel 1972) made on 3–5 separate leaves. These curves are made by alternately determining leaf mass and water potential as the leaf dries. The leaf water potential at the turgor loss point is the inflection point on a graph of the inverse of the water potential vs relative water content (RWC). C values on a RWC basis were calculated before and after the turgor loss point as the slope of relationship between leaf water potential and RWC. C values were converted to a leaf area basis using the mean leaf area for each species based on scanned images of ~20 leaves, which were analyzed in ImageJ version 1.47 (NIH, Bethesda, MD, USA, <http://rsb.info.nih.gov/ij/>).

Field water potential measurements

To allow us to estimate the safety factor for hydraulic failure of leaves, we needed to know the actual leaf water potential at the driest period. Water potential measurements were made on leaves of all species before dawn and at midday on three separate days during late summer (August and September) of 2011. On each of these days and measurement period, two leaves from each of 3–4 shoots per species were measured using a pressure chamber. While we were in the forest for the predawn measurements, we placed aluminum foil-covered plastic bags over each of three shoots. Leaves within the bags remained equilibrated with the subtending branches, and allowed us to estimate the branch xylem water potentials by measuring covered, non-transpiring leaves.

Anatomical and morphological measurements

Vessel lumen diameters were measured for the stem segments used for hydraulic measurements. To do this, cross-sections were made near the midpoint of the segments using a sliding microtome. Sections were mounted on slides with glycerin and photographed using a digital camera (Q Imaging MicroPublisher 5.0 RTV camera, Q Imaging, Inc., Surrey, BC, Canada) mounted on a Nikon Eclipse E400 microscope connected to a PC using Q Capture Pro 6.0 Image capture software (Q Imaging, Inc.). Images from the sections were then analyzed using the software

program ImageJ version 1.47 (NIH). Vessels were measured along radial files from the cambium to pith (for small diameter stems) or for the length of the section (for large diameter, chiseled segments) until a radial file was complete and we had measured a minimum of ~100 vessels. Vessel lumen diameters were determined by measuring the area of the lumen and calculating the diameter of a circle with the same area. The frequency of vessels was determined by measuring the total area of the radial file of vessels and dividing the number of vessels within the file by the area (i.e., mm^{-2}).

Wood density was determined as the grams fresh volume per cm^3 of dry weight. Shoot segments were used that were ~1 cm long, and adjacent and distal to the segments used for hydraulics. Bark and pith were removed from the segments, which were then hydrated overnight under partial vacuum. Fresh volume was estimated by dipping each segment into a beaker of water on a scale and recording the mass of water replaced. The mass was converted to cm^3 using the density of water at the temperature of the water (measured using a thermometer). Segments were then placed in a drying oven at 100 °C for 2–3 days and weighed to determine dry weight.

Data analyses

Relationships between various measured parameters (e.g., stem diameter, hydraulic conductivity) were established using standard major axis regressions with the program SMATR (Warton et al. 2006). Sigma Plot (Systat Software, Inc., San Jose, CA, USA) was used to fit three-parameter sigmoid curves to the raw vulnerability curve data for each individual. P_{50} values (pressure that results in a 50% decrease in hydraulic conductance (for leaves) or conductivity (for stems)) among species and organs (leaf and stem size class) were determined from the fitted curves and averaged for the species and organ. P_{50} values and safety margins (the difference between the P_{50} and the minimum measured water potential for leaves and small stems) among species were compared using an analysis of variation in R version 3.0.3 (R Project for Statistical Computing, <http://www.r-project.org/>) and differences between species were assessed using Tukey's honest significance test ('lsmeans' package, Lenth and Hervé 2015). Differences were considered significant at P -values below 0.05 and marginally different below 0.1.

Results

The slopes of the relationships between vessel diameters and vessel frequency did not differ among species (Figure 1). However, the elevation (i.e., y -intercept) of the line did differ among the species, with *Corylus* having a higher vessel frequency for a given vessel diameter than *Rhamnus* ($P = 0.01$) or *Alnus* ($P = 0.07$). In addition, the vessel diameters of *Corylus* were narrower than the other two species ($P < 0.05$ for comparisons with both *Rhamnus* and *Alnus*). Consistent with this, the slope

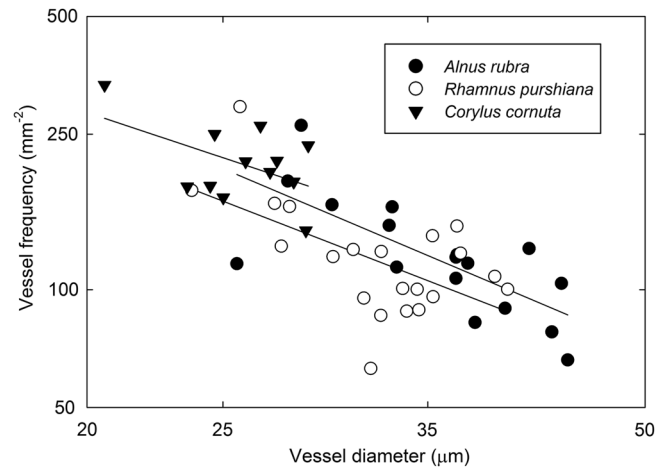


Figure 1. The relationship between vessel diameter and vessel frequency (the number of vessels per area) for three species that differ in their maximum potential height. Slopes within species were not different from one another, but the y -intercept for *Corylus* was significantly higher than *Rhamnus* ($P = 0.01$).

comparing vessel diameter with stem diameter was shallower in *Corylus* than in *Alnus* (Figure 2a). As hypothesized, there was a trend of greater shoot base-to-tip vessel tapering with maximum potential height (Table 1). *Rhamnus*, which is intermediate in maximum height, exhibited a slope that was not different from either of the other species, which were distinct from one another.

As we hypothesized and as was expected based on their anatomical differences, the specific hydraulic conductivity increased with stem diameter to a greater extent in *Alnus* than in *Corylus* (Figure 2b, Table 1). The slopes of the relationships between these two variables in *Rhamnus* did not differ from either of the other species. What was not predicted by the narrower vessel diameters in *Corylus*, was the overlap of specific conductivity among the species, which we would have expected to be lower in the shrub based solely on vessel diameters.

Various leaf biophysical properties such as the components of the pressure–volume curve analyses were also consistent with the more conservative growth strategy implied by the hydraulic network of the *Corylus* leaves. The average turgor loss point (SE) was more negative in *Corylus* (−2.5 (0.1) MPa) than in *Rhamnus* (−1.6 (0.1) MPa) or *Alnus* (−1.3 (0.1) MPa) at $P = 0.001$. Similarly, the average osmotic potential at full turgor (SE) was significantly more negative in *Corylus* (−2.0 (0.1) MPa) than in *Rhamnus* (−1.1 (0.1) MPa) or *Alnus* (−1.2 (0.1) MPa) at $P = 0.001$. The bulk leaf modulus of elasticity was only marginally different among the species ($P = 0.08$), but the trend was for *Corylus* to have more flexibility to deal with a wider range of water potential values (i.e., it had the lowest modulus of elasticity among the species). The average values (SE) were 5.5 (0.4) MPa for *Corylus*, 10.1 (0.9) MPa for *Rhamnus* and 7.8 (1.5) MPa for *Alnus*.

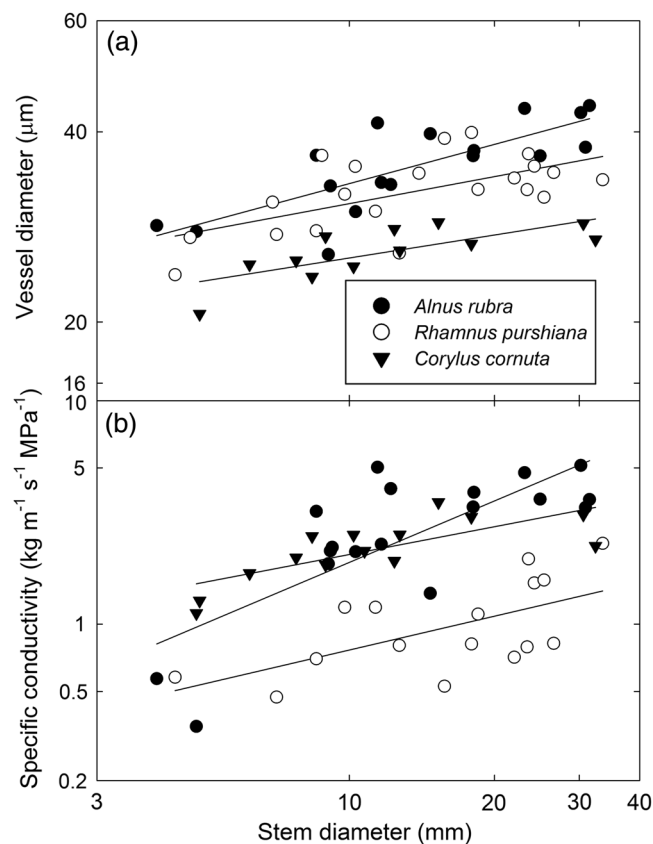


Figure 2. The relationship between stem diameter and vessel diameter (a) and specific hydraulic conductivity (b) for three species that differ in their maximum potential height. Values for the slopes and the relationships among them are shown in Table 1. Values of r^2 and P for (a) are 0.60, <0.001 for *Alnus*, 0.55, 0.006 for *Rhamnus* and 0.38, 0.003 for *Corylus*, respectively. Values of r^2 and P for (b) are 0.58, <0.001 for *Alnus*, 0.37, 0.013 for *Rhamnus* and 0.57, 0.002 for *Corylus*, respectively.

Table 1. Slope values for standard major axis relationships between diameter (D) of stems and both vessel diameter and specific hydraulic conductivity (k_s). Superscript letters indicate slopes that differ statistically at the 0.05 level. Species are listed in descending order of maximum potential height.

Species	Stem D vs vessel D	Stem D vs k_s	Maximum pot. ht (m)
<i>Alnus</i>	0.63 ^a	1.20 ^a	25
<i>Rhamnus</i>	0.52 ^{ab}	0.83 ^{ab}	10
<i>Corylus</i>	0.26 ^b	0.55 ^b	3

Wood density was different among the three species ($P < 0.001$), and declined as maximum potential height increased from *Corylus* to *Rhamnus* to *Alnus* (Figure 3). Greater wood density was also associated with smaller vessel diameters and lower specific conductivity across all species (Figure 3). The relationship between wood density and vessel diameter across all three species was highly significant ($P < 0.0001$; $r^2 = 0.29$), and the relationship remained significant within *Alnus* ($P = 0.019$; $r^2 = 0.32$) and *Corylus* ($P = 0.03$; $r^2 = 0.41$), but not *Rhamnus* ($P = 0.32$; $r^2 = 0.06$). Similarly, the relationship between wood

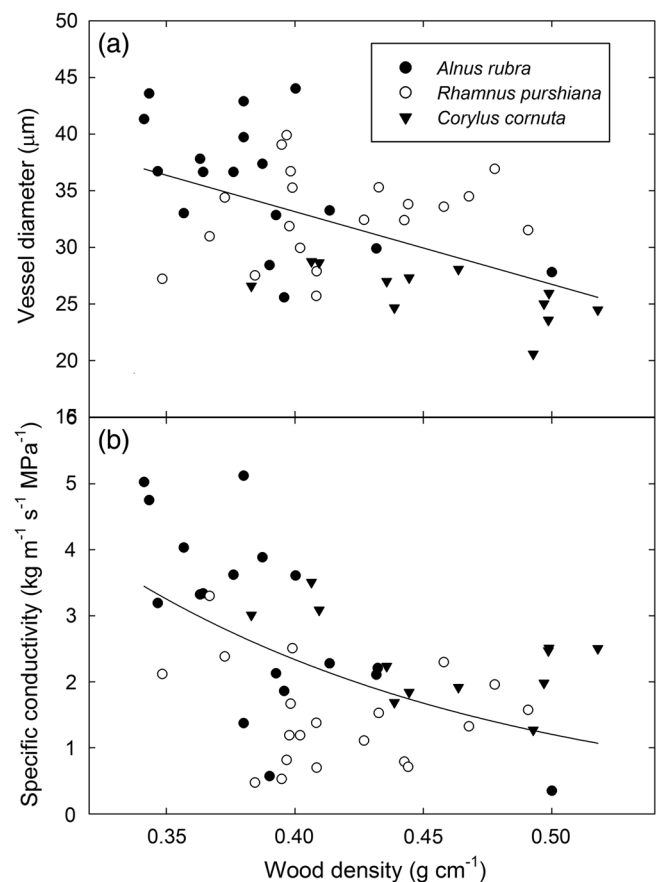


Figure 3. The relationship between wood density and vessel diameter (a) and specific hydraulic conductivity (b) for three species that differ in their maximum potential height. Only common slopes are shown because the relationships were not significant for *Rhamnus* in either panel and *Corylus* in (b). For the slope in (a), $P < 0.001$ and $r^2 = 0.29$. For the slope in (b), $P = 0.009$ and $r^2 = 0.22$.

density and specific conductivity was strong among all species ($P = 0.009$; $r^2 = 0.22$) and within *Alnus* ($P = 0.02$; $r^2 = 0.49$), but not significant in *Corylus* ($P = 0.42$, $r^2 = 0.28$) and *Rhamnus* ($P = 0.88$; $r^2 = 0.04$). The exponential decay function was expected for the relationship between wood density and specific conductivity because of the exponential relationship between vessel diameter and hydraulic conductivity.

The vulnerabilities to embolism of the leaves and stems were compared for each species using their P_{50} values (Figure 4). As we had hypothesized, there were significant differences among species ($P < 0.001$) with the trend of vulnerability to embolism increasing with maximum potential height (i.e., *Corylus*, *Rhamnus*, *Alnus*). Pairwise comparisons between species comparing all ranks showed *Corylus* was significantly more resistant than *Alnus* ($P < 0.001$) as well as *Rhamnus* ($P = 0.002$), while *Rhamnus* and *Alnus* were not different from one another ($P = 0.61$). Midday stem and leaf water potential values did not differ among the species (Table 2). However, the predawn values of *Rhamnus* were more negative than for the other species. The safety margins (the midday water potential—the P_{50}) of the leaves were

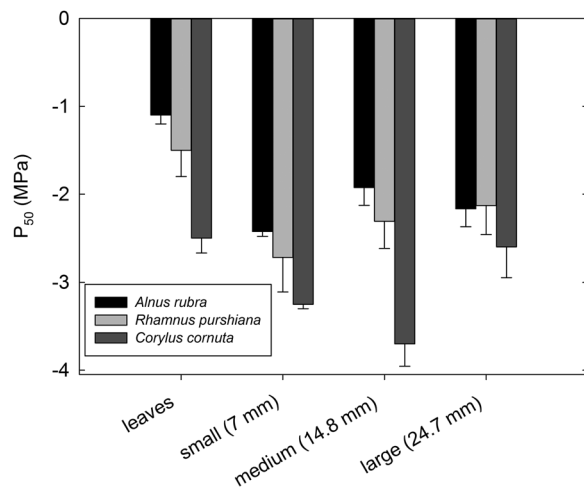


Figure 4. The P_{50} , which is the pressure causing a 50% loss of hydraulic conductance (in leaves) or conductivity (in stems), for leaves and stems of three different size classes in three species that differ in their maximum potential height. Error bars indicate SE.

Table 2. Water potential values (MPa) measured on leaves before dawn and at midday, and on leaves equilibrated with subtending stems at midday. Superscript letters indicate values that differ statistically within a column at the 0.05 level. No letters are shown when values did not differ.

Species	Predawn	Midday leaf	Midday stem
<i>Alnus</i>	-0.4 (0.04) ^a	-1.2 (0.07)	-0.9 (0.06)
<i>Rhamnus</i>	-0.9 (0.09) ^b	-1.4 (1.00)	-1.2 (0.08)
<i>Corylus</i>	-0.5 (0.05) ^a	-1.4 (1.44)	-1.1 (1.54)

much smaller than that of the small diameter stems ($P = 0.03$), particularly for *Alnus* and *Rhamnus* (Figure 5). Midday water potentials were not measured in larger stem size classes, so we were unable to compare how safety margins changed throughout the plants. *Corylus* stem safety margins were significantly higher than *Alnus* ($P = 0.02$) and *Rhamnus* ($P = 0.05$). *Corylus* leaf safety margins were also significantly higher than both *Alnus* and *Rhamnus* ($P < 0.01$), which were not significantly different from each other in either organ.

The P_{50} values for the stems increased with the vessel diameter across species (Figure 6; $P = 0.007$; $r^2 = 0.68$). This relationship corresponded with increases in vessel diameter and P_{50} values as maximum potential height increased among the species.

Discussion

Despite all sampled individuals being approximately the same height and age among the three species, trends were apparent among many of the variables measured and maximum potential height. For example, as species' maximum potential height increased, vessel diameter and specific conductivity both increased with stem diameter more quickly, wood density

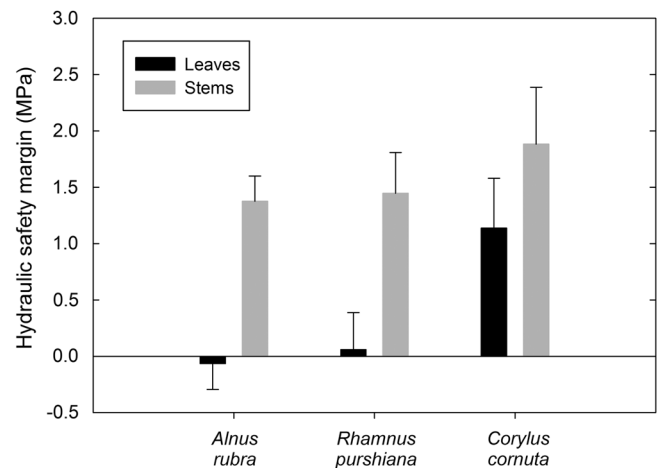


Figure 5. The hydraulic safety margins (pressure causing 50% reduction in hydraulic conductance (for leaves) or conductivity (for stems) minus the midday water potential of that organ) for leaves and stems from three species that differ in their maximum potential height.

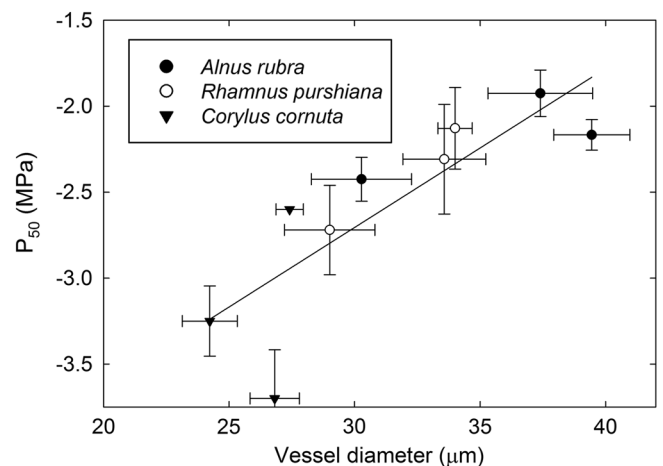


Figure 6. The vessel diameter vs the pressure causing 50% loss of hydraulic conductivity (P_{50}) for three size classes of stems in three species that differ in their maximum potential height. Only the common slope is shown because the individual relationships among species were not significant. For the slope, $P = 0.007$ and $r^2 = 0.68$. Error bars indicate SE.

declined, and leaf safety margins dropped. Functionally, the patterns with stem diameter predict that the whole-plant hydraulic conductance, which integrates the conductivities along the length of the plant, would increase with maximum potential height. Hydraulic conductance is an important parameter, because it has been linked to photosynthetic rate (Hubbard et al. 2001, Santiago et al. 2004) and productivity (Mencuccini and Grace 1996, Poorter et al. 2010). Higher hydraulic conductance may allow the trees to continue their axial growth and attain greater heights than the co-occurring shrub species.

There are several other differences between these three species that could also be influencing the hydraulic architecture. For example, *Alnus* is less tolerant of shade than the other two species

(Franklin and Dyrness 1973), and various hydraulic parameters have been linked to the light environment (Brodribb and Feild 2000). However, at the time of collection individuals of all three species were growing in similar light environments: near the edge of small gaps and beside forest roads and trails. It is possible the hydraulic traits of *Corylus* and *Rhamnus* are not particularly plastic and cannot adjust when individuals grow in areas of higher light than in the deep understory. If we assume that these species can acclimate their hydraulic architecture to their light environment, then it is unlikely that the differences we observed can be attributed to the light environment. A second potential difference between the species that could contribute to the trends in hydraulic properties that we observed is differences in rooting depth. We were unable to directly determine the rooting depths of these species, but the predawn water potential values indicate that *Rhamnus* may have had shallower or more restricted rooting area. This restriction could have limited *Rhamnus*' hydraulic architecture. However, the predawn water potential values of *Corylus* and *Alnus* were not different from one another, suggesting that rooting depth likely did not result in the observed differences in hydraulic properties between those two species. Other potential differences between the species that could also contribute to the observed hydraulic parameters include differences in phenology, hormone distribution patterns and sensitivity, and habitat preferences.

The more negative P_{50} of leaves (Figure 4) and the larger difference between the P_{50} and the midday leaf water potential (the safety margin; Figure 5) in *Corylus* may indicate a greater investment in the xylem of the leaves of this species vs the other species. All three species experienced similar midday water potentials, which indicates that the differences in safety margins was due to the greater resistance to embolism in *Corylus*. Drought-induced losses of leaf hydraulic conductance have been shown to largely be the result of embolism formation within the xylem network (Johnson et al. 2012), and the potential that the leaf vulnerability curves are artifactually vulnerable because of the introduction of emboli during sample preparation (Wheeler et al. 2013) is unlikely given recent work indicating that leaves do not seem to be affected by this issue (Scoffoni and Sack 2015). Costs of making a more drought resistant network in leaves have not been as thoroughly studied as in stems, but include the construction costs associated with building a high density of major veins (Sack and Holbrook 2006, Scoffoni et al. 2011, Sack and Scoffoni 2013) and thicker walls for a given lumen diameter (Blackman et al. 2010).

A striking difference between *Corylus* and the two other species was the amount of leaf hydraulic conductance they are predicted to lose at midday. *Alnus* and *Rhamnus* both had safety margins near zero, which mean that ~50% of the leaf hydraulic conductance is expected to be lost at midday. In order to recover their K_{leaf} , these species would need to refill the embolized vessels in their leaves overnight. Many species exhibit loss and recovery of leaf hydraulic conductance on a daily basis and this

strategy may serve as an additional mechanism (beyond stomatal control) to protect upstream portions of the plant from hydraulic dysfunction (Bucci et al. 2003, Johnson et al. 2009). Although vessel dimensions within the leaves were not measured, only extremely small diameter vessels (i.e., $<1 \mu\text{m}$) would be able to refill passively based on the predawn water potential values in *Alnus* (Yang and Tyree 1992), which had a more favorable predawn water status than *Rhamnus*. Although the process of actively refilling xylem conduits is not well understood, it likely involves metabolic costs (Zwieniecki and Holbrook 2009, Nardini et al. 2011).

Consistent with its more conservative hydraulic behavior, the leaf biophysical properties indicated that *Corylus* was able to tolerate a greater range of water stress than the other two species. Both the turgor loss point and the osmotic potential at full turgor were more negative in *Corylus*, which represents a sizeable energy cost to take up and maintain solutes in the symplast. In species with relatively constant turgor loss points (Meinzer et al. 2014), as would be expected from the species examined here, this value represents the water potential at which the leaf would wilt and would no longer be able to maintain gas exchange (Mitchell et al. 2008, Blackman et al. 2010, Bartlett et al. 2012). Midday water potential measurements were made at the peak of the summer dry season, and yet the *Corylus* leaves appear to be substantially overbuilt in terms of maintaining turgor based on the amount of water stress the leaves were experiencing that summer.

The stems of *Corylus* also exhibited a more conservative strategy by having greater wood density than the other two species, consistent with our hypothesis. Higher wood density is associated with lower growth rates (Muller-Landau 2004, King et al. 2005, Poorter et al. 2008, Chave et al. 2009, Wright et al. 2010) and is often correlated with more negative P_{50} s (Hacke et al. 2001, Jacobsen et al. 2005). As we found, higher wood density has also been associated with lower k_s values (Domec and Gartner 2003, Bucci et al. 2004, Pratt et al. 2007, Meinzer et al. 2008, McCulloh et al. 2011) caused by narrower conduits, yet *Corylus* had higher k_s values than would have been predicted from vessel diameter alone because of the greater number of vessels per area than the other two species. In contrast, Martinez-Cabrera et al. (2011) found that shrubs packed fewer vessels per area at a given vessel diameter than trees did when they compared the anatomy of many species of shrubs and trees. In that study, though, only one point within each plant was examined and it is unclear how xylem anatomy changed axially within those individuals. The strategy exhibited here by *Corylus* seems to be to have small diameter vessels that may permit more negative P_{50} values (Figure 4) but then to pack more vessels per area (Figure 1) to minimize the effect of narrower conduits on hydraulic conductivity.

In their work that examined shrub vs tree growth forms of *J. communis*, Beikircher and Mayr (2008) found that the hydraulic architecture of stems of the shrub form was more similar to large diameter branches than trunks in the tree form. This result

is consistent with our finding that vessel diameters were smaller at a given stem diameter in *Corylus* than the other two species and that specific conductivity increased more with stem diameter in *Corylus* vs *Alnus*. The restrictions on the hydraulic architecture of shrubs may be linked to the increased need for protection from breakage for understory species, which may be one of the reasons why the wood density was highest in *Corylus* (Aide 1987, Gartner 1989).

To return to our fundamental question: is the height of shrubs limited by their hydraulic architecture? Although it would be difficult to directly demonstrate a hydraulic constraint on height growth in *Corylus*, a number of the variables measured across species were consistent with a hydraulic limitation along the continuum of maximum potential height. While *Corylus* exhibited a more conservative growth strategy than the other species, the overlap of the specific conductivities among the species suggests that *Corylus* is not limited by its hydraulic architecture at the plant sizes examined here. However, the increase in the steepness of the slopes in the relationship between specific conductivity and stem diameter as maximum potential height increases indicates that as the tree species continue to grow wider trunks, the specific conductivity of their basal segments would quickly exceed the values observed in *Corylus*. Thus, it seems likely that although hydraulics appeared not to limit the growth of the shrubs when compared with the trees when they were short, the xylem network of the trees will eventually exceed that of the shrubs. It is also worth noting the limitation of this study in terms of the number of species examined. Ideally, this type of work would be carried out on a large number of closely related species that co-occur. It would also be useful to extend this work into other habitats and include species with other wood types, because our results point to the importance of hydraulics in limiting potential stature in woody species.

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Conflict of interest

None declared.

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References

- Aide TM (1987) Limbfalls: a major cause of sapling mortality for tropical forest plants. *Biotropica* 19:284–285.
- Bartlett MK, Scoffoni C, Sack L (2012) The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. *Ecol Lett* 15:393–405.
- Beikircher B, Mayr S (2008) The hydraulic architecture of *Juniperus communis* L. ssp. *communis*: shrubs and trees compared. *Plant Cell Environ* 31:1545–1556.
- Blackman CJ, Brodribb TJ, Jordan GJ (2010) Leaf hydraulic vulnerability is related to conduit dimensions and drought resistance across a diverse range of woody angiosperms. *New Phytol* 188:1113–1123.
- Brodribb TJ, Feild TS (2000) Stem hydraulic supply is linked to leaf photosynthetic capacity: evidence from New Caledonian and Tasmanian rainforests. *Plant Cell Environ* 23:1381–1388.
- 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, Sternberg LDASL (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 Environ* 26:1633–1645.
- Bucci SJ, Goldstein G, Meinzer FC, Scholz FG, Franco AC, Bustamante M (2004) Functional convergence in hydraulic architecture and water relations of tropical savanna trees: from leaf to whole plant. *Tree Physiol* 24:891–899.
- Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE (2009) Towards a worldwide wood economics spectrum. *Ecol Lett* 12:351–366.
- Domec J-C, Gartner BL (2002) How do water transport and water storage differ in coniferous earlywood and latewood? *J Exp Bot* 53:2369–2379.
- 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, Lachenbruch B, Meinzer FC, Woodruff DR, Warren JM, McCulloh KA (2008) Maximum height in a conifer is associated with conflicting requirements for xylem design. *Proc Natl Acad Sci USA* 105:12069–12074.
- Falster DS, Westoby M (2003) Plant height and evolutionary games. *Trends Ecol Evol* 18:337–343.
- Franklin JF, Dyrness CT (1973) Natural vegetation of Oregon and Washington. Pub No: 1773.
- Gartner BL (1989) Breakage and regrowth of *Piper* species in rain forest understory. *Biotropica* 21:303–307.
- Givnish TJ (1988) Adaptation to sun and shade: a whole-plant perspective. *Aust J Plant Physiol* 15:63–92.
- Givnish TJ, Wong SC, Stuart-Williams H, Holloway-Phillips M, Farquhar GD (2014) Determinants of maximum tree height in *Eucalyptus* species along a rainfall gradient in Victoria, Australia. *Ecology* 95:2991–3007.
- Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloh KA (2001) Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* 126:457–461.
- Hitchcock CL, Cronquist A (1998) Flora of the Pacific Northwest. University of Washington Press, Seattle and London, 730.
- Hubbard RM, Ryan MG, Stiller V, Sperry JS (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant Cell Environ* 24:113–121.
- Ishii HT, Jennings GM, Sillett SC, Koch GW (2008) Hydrostatic constraints on morphological exploitation of light in tall *Sequoia sempervirens* trees. *Oecologia* 156:751–763.
- Jacobsen AL, Ewers FW, Pratt RB, Paddock WA III, Davis SD (2005) Do xylem fibers affect vessel cavitation resistance? *Plant Physiol* 139:546–556.
- Johnson DM, Woodruff DR, McCulloh KA, Meinzer FC (2009) Leaf hydraulic conductance, measured in situ, declines and recovers daily: leaf hydraulics, water potential and stomatal conductance in four temperate and three tropical tree species. *Tree Physiol* 29:879–887.

- Johnson DM, McCulloh KA, Woodruff DR, Meinzer FC (2012) Evidence for xylem embolism as a primary factor in dehydration-induced declines in leaf hydraulic conductance. *Plant Cell Environ* 35:760–769.
- King DA, Davies SJ, Nur Supardi MN, Tan S (2005) Tree growth is related to light interception and wood density in two mixed dipterocarp forests of Malaysia. *Funct Ecol* 19:445–453.
- Kitajima K (1994) Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia* 98:419–428.
- Koch GW, Sillett SC, Jennings GM, Davis SD (2004) The limits to tree height. *Nature* 428:851–854.
- Lachenbruch B, McCulloh KA (2014) Traits, properties, and performance: how woody plants combine hydraulic and mechanical functions in a cell, tissue, or whole plant. *New Phytol* 204:747–764.
- Lenth RV, Hervé M (2015) lsmeans: least-squares means. R package version 2.15. <http://CRAN.R-project.org/package=lsmeans>.
- Martinez-Cabrera HI, Jones CS, Espino S, Schenk HJ (2009) Wood anatomy and wood density in shrubs: responses to varying aridity along transcontinental transects. *Am J Bot* 96:1388–1398.
- Martinez-Cabrera HI, Schenk HJ, Cevallos-Ferriz SRS, Jones CS (2011) Integration of vessel traits, wood density, and height in angiosperm shrubs and trees. *Am J Bot* 98:915–922.
- McCulloh KA, Johnson DM, Meinzer FC, Lachenbruch B (2011) An annual pattern of native embolism in upper branches of four tall conifer species. *Am J Bot* 98:1007–1015.
- Meinzer FC, Campanello PI, Domec JC, Genoveva Gatti M, Goldstein G, Villalobos-Vega R, Woodruff DR (2008) Constraints on physiological function associated with branch architecture and wood density in tropical forest trees. *Tree Physiol* 28:1609–1617.
- 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.
- Mencuccini M, Grace J (1996) Hydraulic conductance, light interception and needle nutrient concentration in Scots pine stands and their relations with net primary productivity. *Tree Physiol* 16:459–468.
- Mitchell PJ, Veneklaas EJ, Lambers H, Burgess SSO (2008) Leaf water relations during summer water deficit: differential responses in turgor maintenance and variation in leaf structure among different plant communities in south-western Australia. *Plant Cell Environ* 31:1791–1802.
- Muller-Landau HC (2004) Interspecific and inter-site variation in wood specific gravity of tropical trees. *Biotropica* 36:20–32.
- Nardini A, Lo Gullo MA, Salleo S (2011) Refilling embolized xylem conduits: Is it a matter of phloem unloading? *Plant Sci* 180:604–611.
- Poorter L, Wright SJ, Paz H et al. (2008) Are functional traits good predictors of demographic rates? Evidence from five neotropical forests. *Ecology* 89:1908–1920.
- Poorter L, McDonald I, Alarcón A, Fichtler E, Licona J-C, Peña-Claros M, Sterck F, Villegas Z, Sass-Klaassen U (2010) The importance of wood traits and hydraulic conductance for the performance and life history strategies of 42 rainforest tree species. *New Phytol* 185:481–492.
- Pratt RB, Jacobsen AL, Ewers FW, Davis SD (2007) Relationships among xylem transport, biomechanics and storage in stems and roots of nine Rhamnaceae species of the California chaparral. *New Phytol* 174:787–798.
- Sack L, Holbrook NM (2006) Leaf hydraulics. *Annu Rev Plant Biol* 57:361–381.
- Sack L, Scoffoni C (2013) Leaf venation: structure, function, development, evolution, ecology and applications in the past, present and future. *New Phytol* 198:983–1000.
- Santiago LS, Goldstein G, Meinzer FC, Fisher JB, Machado K, Woodruff D, Jones T (2004) Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia* 140:543–550.
- Scholz FG, Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Miralles-Wilhelm F (2007) Biophysical properties and functional significance of stem water storage tissues in Neotropical savanna trees. *Plant Cell Environ* 30:236–248.
- Scholz FG, Phillips NG, Bucci SJ, Meinzer FC, Goldstein G (2011) Hydraulic capacitance: biophysics and functional significance of internal water sources in relation to tree size. In: Meinzer FC, Lachenbruch B, Dawson TE (eds) Size- and age-related changes in tree structure and function. Springer, Dordrecht, pp 341–361.
- Scoffoni C, Sack L (2015) Are leaves ‘freewheelin’? Testing for a Wheeler-type effect in leaf xylem hydraulic decline. *Plant Cell Environ* 38:534–543.
- Scoffoni C, Rawls M, McKown A, Cochard H, Sack L (2011) Decline of leaf hydraulic conductance with dehydration: relationship to leaf size and venation architecture. *Plant Physiol* 156:832–843.
- Sperry JS, Saliendra NZ (1994) Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. *Plant Cell Environ* 17:1233–1241.
- 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.
- Valladares F, Niinemets U (2008) Shade tolerance, a key plant feature of complex nature and consequences. *Annu Rev Ecol Evol Syst* 39:237–257.
- Warton DI, Wright IJ, Falster DS, Westoby M (2006) Bivariate line-fitting methods for allometry. *Biol Rev* 81:259–291.
- Wheeler JK, Huggett BA, Toft AN, Rockwell FE, Holbrook NM (2013) Cutting xylem under tension or supersaturated with gas can generate PLC and the appearance of rapid recovery from embolism. *Plant Cell Environ* 36:1938–1949.
- Woodruff DR, Meinzer FC (2011) Size-dependent changes in biophysical control of tree growth: the role of turgor. In: Meinzer FC, Lachenbruch B, Dawson TE (eds) Size- and age-related changes in tree structure and function. Springer, Dordrecht, pp 363–384.
- Woodruff DR, Bond BJ, Meinzer FC (2004) Does turgor limit growth in tall trees? *Plant Cell Environ* 27:229–236.
- Woodruff DR, Meinzer FC, Lachenbruch B (2008) Height-related trends in leaf xylem anatomy and shoot hydraulic characteristics in a tall conifer: safety versus efficiency in water transport. *New Phytol* 180:90–99.
- Wright SJ, Kitajima K, Kraft NJB et al. (2010) Functional traits and the growth–mortality trade-off in tropical trees. *Ecology* 91:3664–3674.
- Yang S, Tyree MT (1992) A theoretical model of hydraulic conductivity recovery from embolism with comparison to experimental data on *Acer saccharum*. *Plant Cell Environ* 15:633–643.
- Zwieniecki MA, Holbrook NM (2009) Confronting Maxwell’s demon: biophysics of xylem embolism repair. *Trends Plant Sci* 14:530–534.