

A case-study of water transport in co-occurring ring- versus diffuse-porous trees: contrasts in water-status, conducting capacity, cavitation and vessel refilling

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Summary Recent work has suggested that the large earlywood vessels of ring-porous trees can be extraordinarily vulnerable to cavitation making it necessary that these trees maintain a consistent and favorable water status. We compared cavitation resistance, vessel refilling, transport capacity and water status in a study of ring-porous *Quercus gambelii* Nutt. (oak) and diffuse-porous *Acer grandidentatum* Nutt. (maple). These species co-dominate summer-dry foothills in the western Rocky Mountains of the USA. Native embolism measurements, dye perfusions and balance pressure exudation patterns indicated that the large earlywood vessels of 2–3-year-old oak stems cavitated extensively on a daily basis as predicted from laboratory vulnerability curves, resulting in a more than 80% reduction in hydraulic conductivity. Maple branches showed virtually no cavitation. Oak vessels refilled on a daily basis, despite negative xylem pressure in the transpiration stream, indicating active pressurization of embolized vessels. Conductivity and whole-tree water use in oak were between about one-half and two-thirds that in maple on a stem-area basis; but were similar or greater on a leaf-area basis. Oak maintained steady and modest negative xylem pressure potentials during the growing season despite little rainfall, indicating isohydric water status and reliance on deep soil water. Maple was markedly anisohydric and developed more negative pressure potentials during drought, suggesting use of shallower soil water. Although ring porosity may have evolved as a mechanism for coping with winter freezing, this study suggests that it also has major consequences for xylem function during the growing season.

Keywords: *Acer grandidentatum*, drought stress, Granier sensors, hydraulic architecture, *Quercus gambelii*, sap flux, vulnerability curves, xylem embolism.

Introduction

Diffuse- and ring-porous temperate trees are defined by qualitative differences in the plumbing of their woody stems. The probably ancestral diffuse-porous pattern has narrow vessels

evenly dispersed across a growth ring (Wheeler and Baas 1993). Ring-porous stems produce a band of wide vessels in the earlywood followed by much narrower and more dispersed vessels in latewood. The earlywood vessels are highly vulnerable to cavitation by freezing and do not appear to function for more than one summer (Zimmermann 1983). Bud break in ring-porous trees is delayed for weeks compared with bud break in diffuse-porous trees, and does not occur until the new earlywood is produced, a delay that minimizes cavitation caused by late-spring freezing (Zimmermann and Brown 1971, Wang et al. 1992).

Recent work suggests that ring-porous xylem is much more vulnerable to cavitation caused by water stress in the summer than xylem of diffuse-porous xylem; so much so that stems can lose over 90% of their maximum hydraulic conductivity in response to normal midday water stress (Li et al. 2008). However, because the maximum conductivity is much greater in ring-porous stems than in diffuse-porous stems, a 90% loss in conductivity can result in a conductivity similar to that of non-embolized diffuse-porous stems (Hacke et al. 2006). The two wood types may reflect different evolutionary responses to a trade-off between conductivity and vulnerability. Compared with ring-porous trees, diffuse-porous trees have narrower vessels, which are packed more densely per unit wood area and generally stay functional longer. Ring-porous trees have wide vessels, but necessarily sacrifice some vessels to cavitation. In theory, the ring-porous strategy should work if the trees are isohydric and maintain similar water status through the season. Isohydricity would be less essential to diffuse-porous function (Hacke et al. 2006). Consistent with this expectation, ring-porous trees appear to show tighter stomatal regulation of transpiration in response to extremes in vapor pressure deficit than diffuse-porous trees (Bush et al. 2008).

We compared xylem function in two naturally co-occurring species that can dominate locally in the winter-deciduous “chaparral” zone between 2000 and 3000 m in the central and western Rocky Mountains of the United States (Barbour and Billings 2000). Ring-porous *Quercus gambelii* Nutt. is the pervasive scrub oak of this foothills vegetation. Its seedlings are

rarely successful on the summer-dry foothills, and it owes its persistence to a root-sprouting habit that forms extensive and long-lived clones (Neilson and Wullstein 1985, Tiedermann et al. 1987). Diffuse-porous *Acer grandidentatum* Nutt. also forms clonal clusters, though not as extensively as oak. The two species grow side-by-side and together constitute virtually all of the woody vegetation on many foothill slopes. This species pair makes an ideal comparison of how two very different plumbing systems function successfully in the same habitat.

Our study was designed to answer four questions. (1) Can we be certain that ring-porous xylem is as extraordinarily vulnerable to water stress as laboratory vulnerability curves suggest? Vulnerability curves measure the loss of conductivity induced by either negative pressure (often generated in a centrifuge) or by air-injection. The long vessels of ring-porous species may cause artifacts (Cochard et al. 2005), although in our recent work none were detected (Li et al. 2008). (2) Given the apparent vulnerability of ring-porous vessels, do they exhibit refilling, and if so, does refilling occur while the transpiration stream is under negative pressure (Tyree et al. 1999)? (3) How does the capacity for water transport differ between the species at the stem, whole-tree and leaf levels? And (4) is the ring-porous oak more isohydric in its water status than the diffuse-porous maple? These questions were addressed by a variety of measurements including native embolism measurement, dye perfusions of stems, examination of exudation from vessels at their balance pressure in a pressure chamber, and whole-tree sap flux measurement coupled with continuous monitoring of water potential.

Materials and methods

Plant materials

Ring-porous *Quercus gambelii* Nutt. (oak) and diffuse-porous *Acer grandidentatum* Nutt. (maple) were studied in natural populations in the Red Butte Canyon Research Natural Area near the University of Utah in Salt Lake City, UT. The study populations (one per species) consisted of adjacent roadside stands of 4–7-year-old, vigorously growing juvenile trees exposed to the sun at midday. Their vigorous growth provided suitably long (28 cm) 2–3-year-old branches free of side-shoots for measurements of native embolism, vulnerability curves, dye perfusions and related parameters detailed in the following sections.

Vulnerability curves

Vulnerability curves, which show loss of hydraulic conductivity as a function of xylem pressure, were determined on branch segments (hereafter vulnerability curve segments) that were 2–3 years old, 27.4 cm long and free of side-shoots. Branch segment length was dictated by the size of the centrifuge rotor used to induce negative pressure (Alder et al. 1997). Segments were cut under water from larger branches that had been harvested and brought to the laboratory in plastic bags to minimize dehydration. The segment was flushed at 100 kPa with filtered 20 mM KCl solution for more than 30 min to refill any

xylem conduits that were embolized in situ or during collection. Filtered 20 mM KCl solution was used for measuring maximum hydraulic conductivity, to ensure a constant ionic strength that was at least as high as that of native sap, thereby controlling for possible effects of ionic strength on conductivity (Zwieniecki et al. 2001). Stems were fitted to a tubing system where a hydraulic head drove flow through the stem and into a reservoir on an electronic balance. In oak, the mass flow rate was measured at a hydraulic head of 2 kPa, which was low enough to prevent refilling by the displacement of air from vessels open at both ends of the stem. A higher hydraulic head of 4–7 kPa was used for maple, which had much shorter vessels. To correct for mass changes unrelated to pressure-driven flow (evaporation, stem absorption), the mass flow rate was measured with no hydraulic head. This background rate was measured before and after the pressurized flow, averaged, and subtracted from the pressurized flow to obtain the net flow rate. Net flow rate was divided by the pressure gradient to obtain the segment's maximum hydraulic conductivity.

The stem was secured in a custom-built centrifuge rotor and centrifuged to generate a negative pressure for 15 min. Segments were then removed from the rotor and their hydraulic conductivity remeasured. The process was repeated at progressively more negative pressures until conductivity was reduced by over 90% of its maximum. Six stems per species were measured to obtain a mean curve. A Weibull function was used for curve fitting (Neufeld et al. 1992).

Native embolism

Loss of conductive capacity due to embolism by native xylem pressure was measured by the flushing method (Sperry et al. 1988). The day before the measurements, six segments (hereafter conductivity segments) per species and per measurement time were identified on trees of the study population. Similar to the vulnerability curve material, the segments were 28-cm long and located on 2–3-year-old portions of branches that were free of side-shoots. Three leaves on side-branches below the conductivity segment were covered with foil tape to promote equilibration of their xylem pressure with that of the parent stem. On the following day, at the appointed hour, these foil-covered leaves were excised from the tree and their xylem pressure potential measured in a pressure chamber. The mean of the three measurements per conductivity segment gave an estimate of the xylem pressure in the segment. Branches attached below the conductivity segment were then cut from the tree to remove desiccating leaf area without embolizing vessels in the conductivity segment (vessels are rarely continuous between distal forks of a branch junction). The shoot bearing the conductivity segment was cut at about 1 m below its basal end. Vessel length measurements on similar material (Hacke et al. 2006) indicated that the 1 m distance made it unlikely that vessels in the conductivity segment were embolized by the cut. The excised branch was wrapped in a large plastic bag to prevent water loss and transported to the laboratory within an hour.

In the laboratory, a 27.4-cm-long segment was cut from the larger branch under water. Its conductivity was measured as

described for the vulnerability curve segments, except that it was not flushed first. This measurement represented the segment's native or in situ conductivity. The segment was then flushed as described above and the maximum conductivity determined. The native embolism of the segment was calculated as the percentage loss of conductivity (PLC) for the native conductivity relative to the maximum conductivity. In a subset of experiments, we centrifuged the flushed segments to their native xylem pressure for 15 min and remeasured their conductivity to see whether it corresponded with the native value. If the centrifuge method does not cause artifacts, the conductivity should be equal to or greater than the native conductivity.

Native embolism was measured at predawn (0630 to 0700 h) and midday (1430 to 1500 h) for both species in early and late summer. In a subset of measurements, we also measured the leaf area supplied by the stem segments, allowing comparison of native and maximum conductivities on a leaf area basis (leaf-specific conductivity).

Dye perfusion experiments

The dye perfusion experiments were generally done in parallel with the native embolism measurements, using segments (hereafter dye perfusion segments) of the same size and age that were collected in the same manner as segments for the native embolism measurements. In the laboratory, 28-cm-long segments were cut under water from the larger harvested branch. One end was connected to a partial vacuum and the other end was immersed in filtered 0.5% safranin solution. Dye uptake was driven by 2 to 4 kPa of vacuum. The pressure was released 30 min after the stain was observed at the upper end and the stem was immersed in water and vacuum pressure reapplied to chase dye from the conducting vessels and thereby minimize dye spread during sectioning.

The 28-cm-long segment was cut transversely at about 13 cm from the upstream end. The earlywood vessels of the oak formed a single band that is typically one vessel wide in the radial plane. To quantify the amount of conducting earlywood, the circumferential length of the earlywood band that was stained was expressed as a percentage of the total circumferential length. The stained and total earlywood perimeter was measured for each segment. The staining was compared between the times of predawn and midday native embolism measurement.

Balance pressure observations

The distribution of functional versus nonfunctional xylem vessels was determined by the exudation pattern of stems at their balance pressure in a pressure chamber. At the balance pressure, functional vessels will exude water but embolized vessels should not. At both the predawn and midday times of native embolism measurement, 2–3-year-old shoots from the study populations were cut and placed in plastic bags for 0.5 to 1 h to allow xylem pressure to equilibrate. The entire shoot was inserted in a 0.8-m-long pressure chamber, with the 2–3-year-old cut surface protruding. The cut end was trimmed with a razor and stained with 0.5% safranin solution. Staining the stem improved the contrast with the exuding sap, making

the pattern easier to observe. The balance pressure was determined by increasing the pressure in the chamber slowly. Once exudation was observed, the pressure was dropped back below the balance pressure and then returned to it and the stem end photographed. For comparison, we perfused the complementary length of stem immediately proximal to the cut with dye as described above.

Sap flux measurements

Two older trees of about 5–6 cm diameter at ground level and about 4 m tall (one per species) were selected for continuous sap flux and root water potential measurements, to determine if the difference in pattern of embolism between species was reflected in a difference in daily water use and water potential regimes. Only two trees were instrumented because of limitations on the number of stem psychrometers available for continuous water potential measurements (see below). The two trees were within a meter of each other, exposed to midday sun, and within 1 km of the roadside populations used in the other experiments. Several additional trees of this size were used in additional tests of the sap flux calibration constants. Sap flux was measured with 2-cm-long Granier-type sensors (Granier 1987). The heated sensor was inserted in the trunk at 1 m above the stem base and the reference sensor was inserted 15 cm below the heated sensor. The sensors were covered with aluminum foil to protect them from solar heating. The voltage difference between the sensors was recorded by a datalogger (CR7, Campbell Scientific, Logan, UT). Voltage difference at zero sap flux was assumed to be the daily minimum which usually occurred between midnight and dawn.

At the end of the measurement period, we harvested the trees with sensors intact and calibrated the relationship between sap flux and voltage difference in the laboratory as described by Clearwater et al. (1999). The trunk was cut at about 1 m above and below the heated sensor. Trunks were wrapped in plastic bags and brought to the laboratory. A portion of the trunk, which included the sensors and was about 60 cm long, was cut underwater and the cut ends trimmed with a razor. The upper end was attached with tubing to a vacuum pump and the lower end was placed in filtered 20 mM KCl solution on a balance. Flow rate was measured by the balance at a series of reduced pressures. Pressures were held long enough for the flow measurements to stabilize (about 10 min). Voltage difference at zero sap flow was measured for each segment about 20 min after the applications of vacuum ended. Following the flow measurements, 0.5% safranin dye was pulled through the trunk segments to measure the conducting xylem area at the probe level. This allowed the conversion of volume flow rate to mean sap velocity (= sap flux density; K). After the voltage differences were converted to temperature differences, K was calculated as:

$$K = \frac{\Delta T_{\max} - \Delta T}{\Delta T_{\max}} \quad (1)$$

where ΔT is temperature ($^{\circ}\text{C}$) difference at the observation

time and ΔT_{\max} is the largest temperature difference corresponding to zero flow (Granier 1987). Best-fit regressions of an exponential function between mean sap velocity (J , m s^{-1}) and K yielded the calibration curve used to estimate sap flow from voltages measured in the field:

$$J = aK^b \quad (2)$$

where a and b are curve fit parameters. These were compared with $a = 1.19 \times 10^{-4} \text{ m s}^{-1}$ and $b = 1.23$ constants reported by Granier (1987). Four additional trunks of each species were similarly calibrated in the laboratory to determine within-species variability and its relationship to the standard calibration. The calibration assumes that ΔT_{\max} in the field corresponds with zero flow, which may not be the case if there was significant nighttime transpiration or recharge. If so, our fluxes would be underestimated. The leaf area of the two study trees was measured with a leaf area meter (Li-Cor, Lincoln, NE), and sap flow calculated on a leaf area basis.

Shallow roots of both trees were fitted with three temperature corrected stem psychrometers (Dixon and Tyree 1984). Excavation around the trunk exposed intact roots of about 1 cm diameter. These were cut to stop sap flow, and pre-calibrated psychrometers were installed on the part of the root still attached to the tree. Sap flow was stopped because it causes temperature fluctuations that reduce accuracy. To install a psychrometer, a sharp razor blade was used to expose a strip of cambium, and the psychrometer chamber clamped against this surface and the contact sealed with vacuum grease. After installation of the psychrometer, the root excavation was back-filled in with soil and covered with leaf litter. Psychrometer readings were recorded with the same CR7 used to record sap flux voltages. Psychrometers were also installed on branches in the canopy and heavily insulated, but these failed to give realistic readings because of extreme temperature fluctuations caused by wide variations in air temperature, wind and sun exposure.

Results

Vulnerability curves indicated that oak was much more vulnerable to drought-induced embolism than maple (Figure 1). The pressure at 50 PLC was -0.25 MPa for oak and -4.79 MPa for maple. The xylem pressure–PLC relationship was somewhat misleading because the maximum conductivity per stem cross-sectional area averaged 2.9 times higher in oak than in maple (Figure 1b). Oak conductivity fell to the maple value at a xylem pressure of about -0.4 MPa , at which point oak was at about 60 PLC relative to only 2 PLC for maple. Minimum midday stem pressure measured in the trees studied for native embolism was $-1.04 \pm 0.08 \text{ MPa}$ in oak versus $-1.07 \pm 0.18 \text{ MPa}$ in maple. This predicted that oak would be about 90% embolized at midday versus less than 10% for maple, and that oak should have a lower absolute stem conductivity.

These predictions were validated by the native embolism measurements (solid and gray symbols, Figure 1). Depending on the sampling date, oak stems were between 81.3 ± 14.9 and

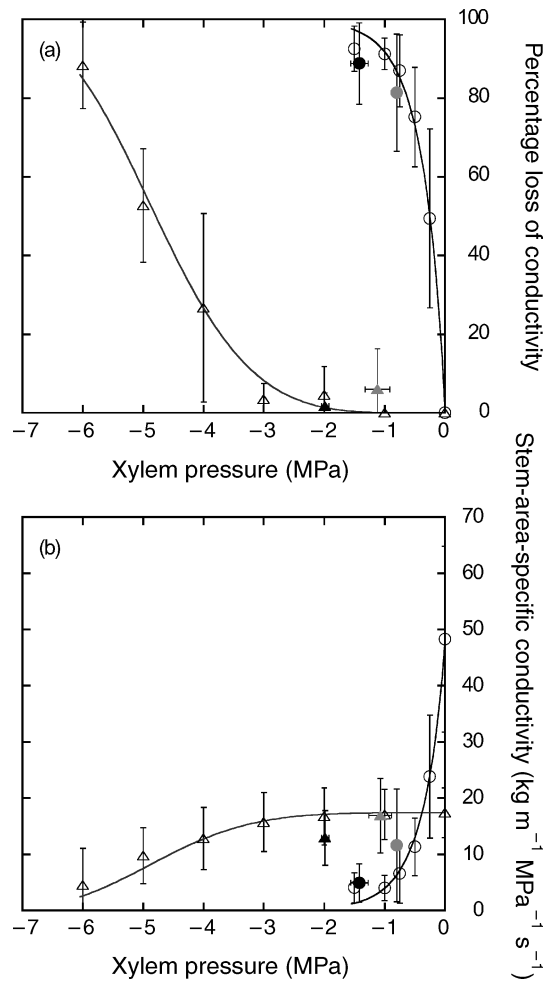


Figure 1. Vulnerability curves and native conductivity of oak (*Quercus gambelii*, \circ , \bullet) and maple (*Acer grandidentatum*, \triangle , \blacktriangle). Conductive capacity is expressed as percentage loss of conductivity for (a), and conductivity per cross section for (b). Open symbols show the laboratory vulnerability curve with a Weibull function fit. Filled symbols are native values measured at midday on June 29 for oak and July 4 (gray) and on August 11 for oak and August 28 for maple (black). Horizontal and vertical bars are ± 1 SD for $n = 4$ –6 samples.

88.8 ± 10.3 PLC at midday versus only 2.98 ± 10.3 and 1.68 ± 3.36 PLC for maple (Figure 1a). Likewise, the absolute values of the native stem-area-specific conductivity were lower in oak than in maple (Figure 1b). In all cases, the native measurements were statistically consistent with vulnerability curve predictions based on t -tests between native PLC and Weibull function PLC obtained from the stem vulnerability curves.

The native conductivities of oak and maple were similar when expressed on a leaf-area basis (leaf-specific conductivity; Figure 2) because there was a greater area of stem per leaf ($3.88 \text{ cm}^2 \text{ m}^{-2}$) in oak than in maple ($2.21 \text{ cm}^2 \text{ m}^{-2}$), although the maximum leaf-specific conductivities for flushed stems were much greater in oak than in maple (Figure 2).

Several lines of evidence indicated that oak refilled embolized vessels overnight throughout most of the summer. When the native embolism was measured at predawn on the

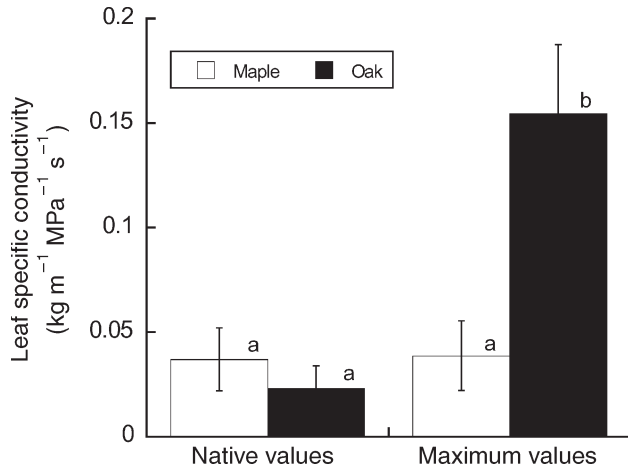


Figure 2. Midday and maximum values of leaf specific conductivity for oak (*Quercus gambelii*, solid) and maple (*Acer grandidentatum*, open). Maximum leaf specific conductivity was measured after the native embolism in the segment was reversed by flushing. Vertical bars are ± 1 SD for $n = 4-6$ samples. Different letters above the columns indicate significant difference between treatments by Tukey's test ($P = 0.05$).

day following a midday sampling, the xylem pressure was less negative (-0.34 ± 0.062 MPa versus -1.28 ± 0.079 MPa; Figure 3a), but the PLC had also dropped from 86.1 ± 6.33 at the previous midday to 34.6 ± 21.9 PLC at predawn (Figure 3b). Native PLC at both times was statistically identical to the PLC caused by centrifugation at the native pressure. This provides direct validation of the centrifuge method for predicting native embolism, and suggests that the refilling was complete for all vessels embolized during the previous day. The dye perfusion experiments also indicated refilling. The percentage of unstained earlywood decreased from 73.6 ± 6.0 to $33.9 \pm 13.0\%$ overnight (Figure 3c).

Vessel refilling occurred despite the high probability that xylem pressures never increased above the predawn value. Continuous recordings from the root psychrometers of the nearby oak, which was also instrumented for sap flux measurements, indicated that, for 21 days running, the maximum xylem pressure occurred at 0630 h; about the same time that we made the predawn native embolism measurements. The psychrometer predawn measurements averaged -0.72 ± 0.036 MPa, which was a little more negative than the predawn

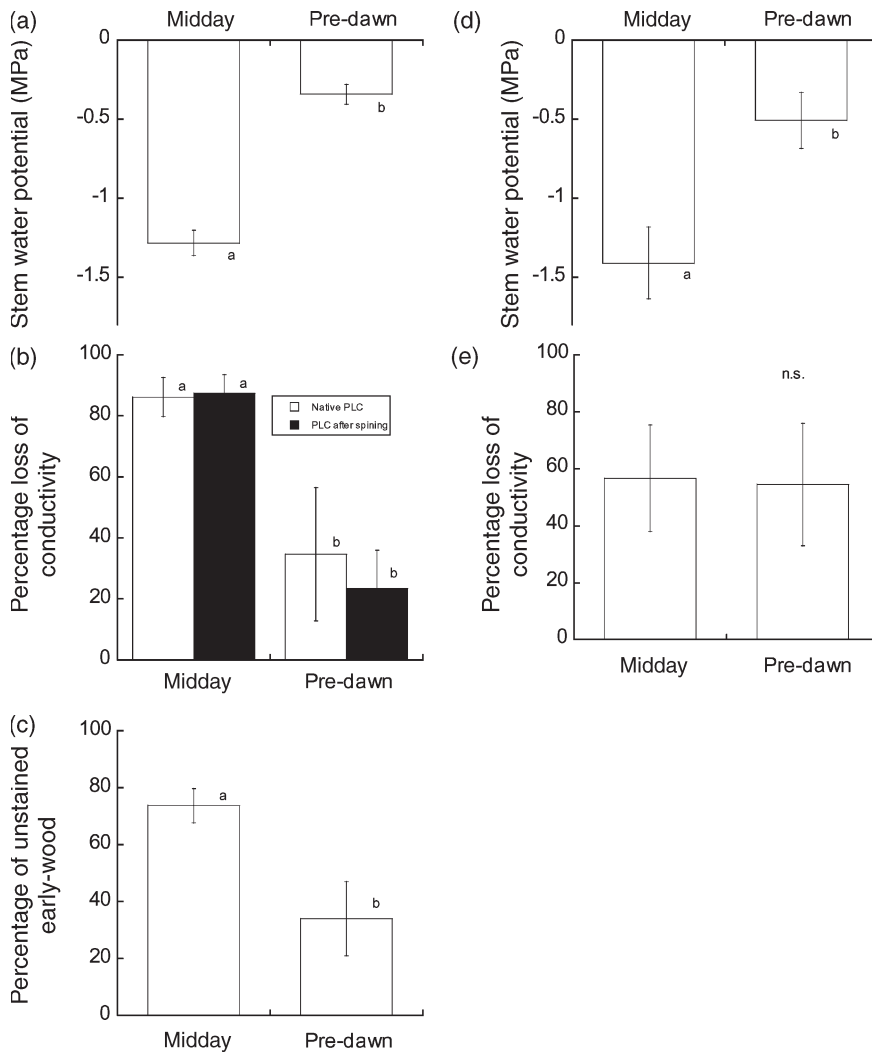


Figure 3. Evidence for refilling of oak (*Quercus gambelii*) vessels. (a) Midday and predawn water potentials measured on two successive days in July. (b) Corresponding values for the percentage loss in conductivity (PLC). Open columns denote stems immediately after harvest, solid columns denote stems flushed to reverse embolism, and then stressed to the native water potential in the centrifuge. (c) Percentage of earlywood that did not conduct dye at midday and the following predawn; 26 days after the measurements in (a) and (b). (d) Midday and predawn water potentials for two successive days in September. (e) Corresponding values for percentage loss in conductivity shown for stems after harvest. Vertical bars are ± 1 SD for $n = 4-6$ samples. Different letters above the columns indicate significant difference between treatments by t -test ($P = 0.05$) for (a), (c), (d) and (e) and by Tukey's test ($P = 0.05$) for (b).

value for the native embolism measurements made between 0630 and 0700 h (Figure 3a) on the nearby stand of juvenile trees.

Balance pressure observations indicated the same refilling behavior. Oak shoots pressurized at midday showed spotty exudation from earlywood vessels that approximately matched dye perfusions of the same branch proximal to the cut (Figures 4c and 4d). Shoots pressurized at predawn showed more even exudation from earlywood vessels, and also more uniform staining (Figures 4e and 4f). Exudation patterns also indicated that the narrow latewood vessels were functional at both times. Dye perfusions generally failed to stain the narrow latewood vessels, suggesting that the perfusion period was only long enough to reliably stain the wider earlywood vessels. Staining of maple shoots was uniform across the secondary xylem as was balance pressure exudation (Figures 4a and 4b) with no diurnal pattern evident, consistent with its low native embolism (Figure 1).

Oak vessels apparently stopped refilling in early fall. A pair of midday and predawn measurements in mid-September showed no drop in predawn PLC despite the higher predawn xylem pressures (Figures 4d and 4e). These PLC values were lower than those of early summer but were not significantly different from those derived from a vulnerability curve measured within a week of the September sampling (t -test, $P = 0.06$). This late-summer curve (not shown) showed greater resistance to cavitation than the early-season curve (Figure 1), suggesting the development of greater resistance to cavitation over the summer.

Psychrometer measurements indicated that the oak was much more isohydric than the maple, exhibiting little decline in root–crown water potentials over a 3-week period (Figure 5a). There was a single rain event (Figure 5a, arrow on July 12), which was not reflected in an increase in root–crown water potential. In contrast, maple began the period at a higher root–crown water potential than oak (predawn = -0.07 MPa,

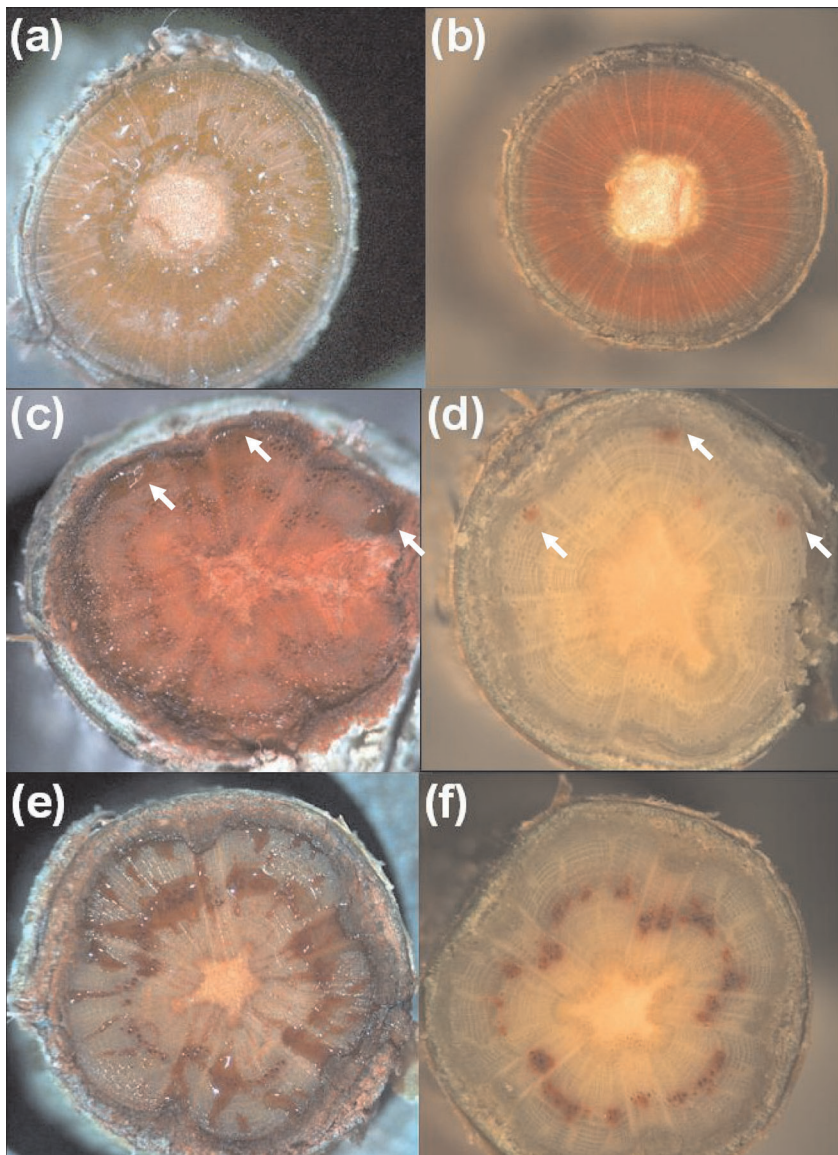


Figure 4. Exudation patterns at shoot balance pressure (left) and dye-stained patterns in the complementary portion of the branch (right) for maple (*Acer grandidentatum*) (a, b) and oak (*Quercus gambelii*) (c–f). Pictures (a) to (d) were taken at midday and pictures (e) and (f) at predawn. Arrows in (c) and (d) highlight exuding and dye-stained earlywood vessels in oak.

midday = -0.75 MPa) but its water potential steadily declined over the rainless period to much lower values than in oak, reaching a minimum of -1.5 MPa at predawn and -2.6 MPa at midday. Also in contrast to oak, the July 12 rain event caused a significant increase in predawn water potential in maple. These contrasting root–crown water potentials strongly imply that oak roots tap a deeper and more stable water supply than maple.

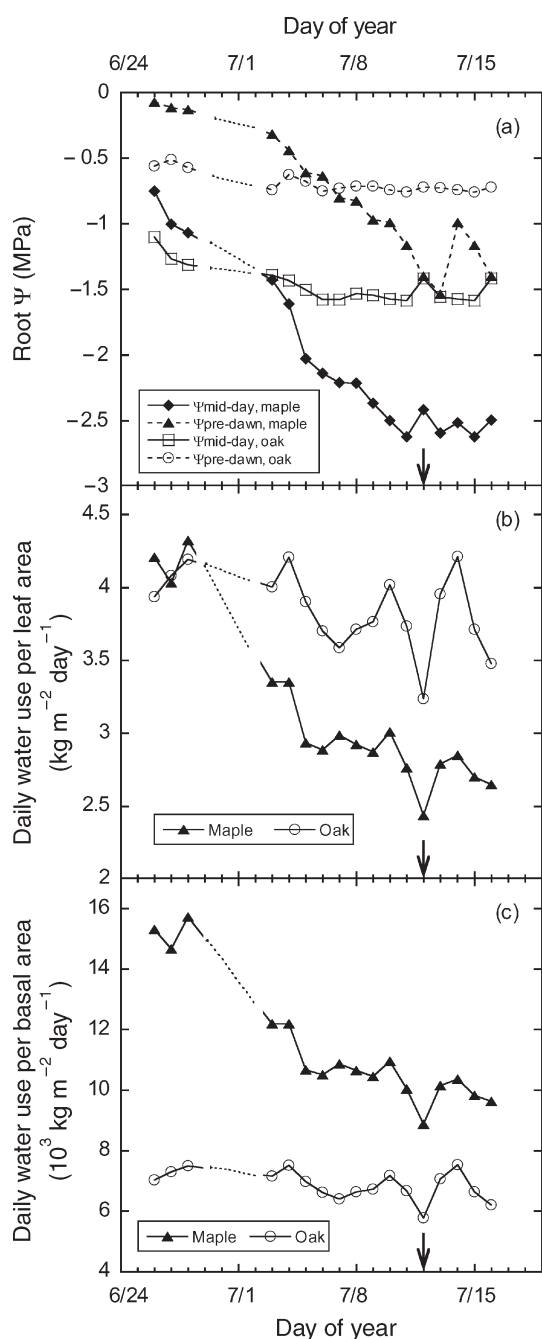


Figure 5. Seasonal changes in (a) root water potential (Ψ) and (b, c) daily water use in an oak (*Quercus gambelii*, \circ , \square) and a maple (*Acer grandidentatum*, \blacktriangle , \blacklozenge) growing within 1 m of each other. The only rain event occurred on July 12 (arrow).

Mean sap velocity (J) versus the K derived from the voltage difference was calibrated on five trees of each species. The fitted exponential equations, however, differed considerably among species and individuals. The mean a coefficient (Equation 2 for mean velocity rather than volume flow rate) was $9.99 \times 10^{-3} \pm 6.18 \times 10^{-3} \text{ m s}^{-1}$ in oak (range: 2.38×10^{-3} to $1.81 \times 10^{-2} \text{ m s}^{-1}$) and b was 1.24 ± 0.190 (1.05 to 1.50). For maple, coefficient a was $4.70 \times 10^{-4} \pm 3.13 \times 10^{-4}$ (2.01×10^{-4} to $9.76 \times 10^{-4} \text{ m s}^{-1}$) and b was 1.11 ± 0.060 (1.02 to 1.19). For both species, coefficient a was much higher than that of the standard Granier equation ($a = 1.19 \times 10^{-4} \text{ m s}^{-1}$), which would have significantly underestimated our sap velocities. The probable reason for this inconsistency is that the band of conducting tissue was substantially shorter than the length of the sensor probe (Clearwater et al. 1999). Our sap velocities are reported for tree-specific calibrations ($J = 5.79 \times 10^{-3} K^{1.38}$ for oak, $J = 5.50 \times 10^{-4} K^{1.02}$ for maple).

Sap flux patterns indicated more stable seasonal water uptake for the isohydric oak, as opposed to a general decline in the anisohydric maple (Figures 5b and 5c). The comparison in daily water uptake between the oak and maple was consistent with the branch- and leaf-specific conductivity measurements. Daily water transport was greater in maple on a basal area basis (total trunk cross section) than oak, despite its seasonal decline (Figure 5a); a difference that was consistent with maple's greater stem-area-specific conductivity at midday (Figure 1b). However, water transport on a leaf area basis was similar between the trees early in the season, and became greater in oak as the season progressed (Figure 5b). This is consistent with similar early-season leaf-specific conductivities between the species (Figure 2). As this pattern suggests, oak had a greater cross section of stem per leaf area than maple, both in the trees measured for sap flux and for the nearby population of juvenile trees used for the conductivity measurements.

If oak earlywood vessels embolize every day as xylem pressure drops, whereas those of maple do not, oak might show greater flux per root–crown water potential difference during the morning than in the afternoon relative to maple. However, hysteresis was similar in both trees (data not shown). The failure of the psychrometers to measure branch water potential reliably prevented a similar comparison above ground.

Discussion

The first question we posed was whether the xylem of ring-porous *Quercus gambelii* (oak) was truly as vulnerable to cavitation as indicated by its vulnerability curve based on the centrifugation method. The results suggest that the answer is yes. If the vulnerability curves are accurate, native embolism should be as high as predicted by the curve, or higher if previous embolism had not been reversed in situ. This was confirmed by repeated native embolism measurements, whether at midday or predawn, or early versus late in the season (Figures 1 and 3). Significant midday embolism of current-year earlywood was also indicated by dye perfusion data (Figure 3c) and balance pressure observation coupled with dye

perfusions (Figure 4). All lines of evidence point to many, if not most, of the current year earlywood vessels being embolized at midday. Native embolism in the diffuse-porous *Acer grandidentatum* (maple) was also consistent with its vulnerability curve, but it was negligible owing to the greater resistance of its vessels to cavitation.

Our results were obtained from 2–3- (mostly 2) year-old branches of vigorously growing juvenile oak shoots. In most ways they are consistent with our earlier work on *Q. gambelii* which was done with older branches (about 5–6 years) of mature trees a few km from the present study site (Sperry and Sullivan 1992, Sperry et al. 1994). These older branches showed high native PLC in summer compared with those of co-occurring diffuse-porous species, ranging from 40 to over 80 PLC over three years of monitoring. The high PLC was attributed to inflated maximum conductivities caused by the refilling of older winter-embolized earlywood during flushing. However, we now know that current-year earlywood can be extensively embolized (Figure 3c, 5c and 5d; Hacke et al. 2006, Li et al. 2008), so both factors likely contribute to high native PLC.

The vulnerability curve for the older *Q. gambelii* branches based on the dehydration method showed 100 PLC at about -4.5 MPa (Sperry and Sullivan 1992). We centrifuged our stems to only -1.5 MPa after which they were at about 90 PLC (Figure 1a). It is possible that the curve has a long tail indicating a population of highly resistant vessels including the latewood vessels. Balance-pressure patterns confirmed the functioning of these latewood vessels, though they were not stained reliably by our dye perfusion protocol. Our late-season vulnerability curve showed an increase in this population of resistant vessels and a correspondingly lower native PLC (Figure 3e; about 58 native PLC). The older stems studied previously (Sperry and Sullivan 1992) would have accumulated even more latewood, perhaps contributing to a more significant tail in their vulnerability curves. These surmises are consistent with the long-held notion that latewood serves as a high-safety but low-efficiency back-up system in ring-porous trees (Baas 1986, Sperry and Sullivan 1992, Woodcock 1994). However, we cannot be sure whether the shift to increased resistance that we saw in late summer was the result of production of more latewood or changing air-seeding thresholds across pit membranes in existing vessels.

Our second question was whether the highly vulnerable vessels of oak that are cavitated by normal midday stress were able to refill overnight. The results indicate that embolized vessels do refill overnight because native PLC, dye perfusions, and balance pressure observations showed that many more were functional at predawn than at midday (Figures 3 and 5). Vessel refilling occurred despite pressures in the transpiration stream apparently never rising above the predawn value, the least negative value of which averaged -0.34 ± 0.06 MPa (Figure 3a). This suggests that oak is able to divert water into embolized vessels and actively pressurize it to the near atmospheric threshold required for refilling, while neighboring functional vessels are under substantial negative pressure. The refilling threshold for air embolism is given by $-4T/D$, where

T is the surface tension of water (0.0728 Pa m at 20°C), and D is the diameter of the embolized vessel lumen. For earlywood oak vessels with a D averaging $70\ \mu\text{m}$ (Davis et al. 1999), xylem pressure must rise above -0.0042 MPa for refilling to proceed, far above the transpiration stream pressure indicated by the predawn measurements.

How refilling of this sort can happen is one of the great puzzles in whole-plant physiology. *Quercus gambelii* is another in a growing list of species that appears to show this behavior (Salleo et al. 1996, 2004, Tyree et al. 1999, Hacke and Sperry 2003, Bucci et al. 2003, Stiller et al. 2005). In a few cases, refilling has been associated with starch hydrolysis in adjacent tissues, suggesting the mobilization of osmoticum and the use of ATP (Bucci et al. 2003, Salleo et al. 2004). How these events result in the pressurization of water in the refilling vessel is unknown, although there are many models that await testing (Holbrook and Zwieniecki 1999, Hacke and Sperry 2003, Salleo et al. 2004). We tested for pressurized vessels during the night and detected no pressurization; however, we made the measurements too late in the season because we discovered that the refilling had also stopped (Figures 3d and 3e).

Our third question concerned the transport capacity of the contrasting anatomies studied. Transport capacity did not differ as much as predicted based on the difference between oak and maple in midday PLC (80 in oak versus less than 10 PLC in maple) (Figure 1a). Conductivity was lower in oak stems than in maple on a cross-sectional basis, but only by a factor of about 1.8 based on a comparison of the pooled midday means in Figure 1b, which is much less than the 24-fold difference between the percentage loss metrics shown in Figure 1a.

At the whole-tree scale, oak transported between one-half and one-third as much water per basal trunk area as maple over the 3-week measurement period (Figure 5c). So oak had about half the conductivity per stem area as maple, and also transported about half the volume of water. At the leaf-level, oak branches exhibited similar or greater transport capacity than maple, because oak had roughly a 1.8 times greater stem-cross sectional area per leaf area. This resulted in similar leaf-specific conductivities (Figure 2) and similar leaf-specific sap flow under low water stress at the beginning of the season between species (Figure 5b). As water potentials declined in maple during the drought (Figure 5a), water transport also declined (Figure 5b); consequently, oak transported more water per leaf area than maple over most of the 3-week measurement period.

The large diameter earlywood vessels in oak, though much more vulnerable than the narrow maple vessels, are apparently so much more efficient that transport capacity is still adequate even when most of the vessels are nonfunctional. A rough calculation explains why this is so. A single earlywood oak vessel of mean diameter $70\ \mu\text{m}$ carries as much water per unit time and pressure as 150 maple vessels of mean diameter $20\ \mu\text{m}$ (Davis et al. 1999); assuming an approximately constant proportionality of vessel conductivity to the Hagen-Poiseuille prediction for an ideal capillary (Wheeler et al. 2005). Maple, however, has about 10 times more vessels per xylem area than oak (445 versus $44\ \text{mm}^{-2}$; Hacke et al. 2006). The net result is

that roughly 90% of the xylem area in oak can be nonconducting and yet have the same water conducting capacity per area as in maple.

Our final question concerned the patterns of water status of the species. Although our sampling was limited by the number of psychrometers, the data indicated a qualitative difference in water status (Figure 5a). As expected, oak was almost perfectly isohydric despite 17 rainless days, and it was unresponsive to the single rain event, all suggesting a stable and deep water source. Maple, however, was markedly anisohydric. Its water status declined during the drought, and showed a pre-dawn response to the rain event—a pattern suggestive of greater reliance on shallow soil water. These observations tally with isotopic analysis of xylem sap from the same species in the same watershed (Philips and Ehleringer 1995, Williams and Ehleringer 2000).

What can we make of the adaptive significance of these contrasting patterns in anatomy and water relations? In the specific case of *Q. gambelii* and *A. grandidentatum*, the greater vulnerability of oak stem xylem is compatible with its more predictable isohydric water status and deep root system. Its isohydric behavior also facilitates diurnal refilling by returning to a similar baseline water potential overnight. Refilling increases conducting capacity in the morning, perhaps maximizing stomatal conductance and photosynthesis at a time of relatively low evaporation. Disadvantages include possibly slower growth as a result of lower transport capacity per basal area, and oak's limited capacity for establishment on dry slopes (Neilson and Wullstein 1985) because of its vulnerability to xylem cavitation. Oak's reliance on deep water places it in a different ecological niche to that of co-occurring maple, which shows an opportunistic use of shallower water, which requires an anisohydric water status and more cavitation-resistant xylem.

More observations are necessary to determine whether our results for oak and maple apply to ring- and diffuse-porous trees in general. There seems to be a characteristic ring-porous vulnerability curve with an initial steep loss of conductivity followed by a tail. This shape is also seen in stems of many vines and roots that contain large vessels (Sperry and Saliendra 1994, Tibbetts and Ewers 2000, Hacke et al. 2006). This type of curve, however, is highly sensitive to how maximum conductivity is determined. If the highly vulnerable and embolized vessels are not refilled beforehand, there will be little loss of conductivity until test pressures drop below the minimum field values. A shortcoming of the otherwise ingenious method of measuring conductivity while stems are centrifuged (Cochard 2002, Cochard et al. 2005) is that for ring-porous stems a true maximum conductivity cannot be obtained because even a slight negative pressure will induce significant embolism (Figure 1).

Occurrence of vulnerable ring-porous vessels seem to pair with a tendency for isohydry. Recent comparisons have shown that ring-porous trees tend to have a greater stomatal response to extreme vapor pressure deficits than diffuse-porous trees (Bovard et al. 2005, Bush et al. 2008). A deep-rooting habit typifies ring-porous species of *Quercus*, *Carya*, *Robinia*,

Gleditsia (Burns and Honkala 1990), and *Prosopis* (Nilsen et al. 1983). Whether other ring-porous species refill their vulnerable vessels daily is unknown, but refilling would be compatible with isohydry, and it may tend to offset the disadvantage of extensive midday cavitation. The more cavitation-resistant latewood may function to prevent complete transport failure in the event that severe drought requires a departure from strict isohydry and all of the earlywood vessels fail.

Although the earlywood of ring-porous xylem might generally be vulnerable, it is not the case that diffuse-porous xylem is uniformly resistant to cavitation. Diffuse-porous species range from highly resistant desert taxa to vulnerable riparian species, and at least some of the riparian species are isohydric (Saliendra et al. 1995, Pockman and Sperry 2000). However, most diffuse-porous vulnerability curves have a cavitation threshold above which there is little loss of conductivity, whereas cavitation in ring-porous xylem is significant as soon as pressures become negative.

Other sap-flux studies suggest that ring-porous trees transport less water per basal area than co-occurring diffuse-porous species. Three ring-porous species from the eastern deciduous forest of the USA transported on average less than half the water of two co-occurring diffuse-porous species on a basal area basis (Pataki and Oren 2003; cf. Bovard et al. 2005). A similar ratio was observed for trees in urban settings in Salt Lake City, Utah (Bush et al. 2008). Although these values were not based on a direct calibration as in our study, they are suggestive of a general trend. To the extent that whole-tree carbon gain is coupled to whole-tree water transport, ring-porous trees may have a tighter carbon budget.

How these putative hydraulic traits of ring-porous trees influence their life history and ecology is worthy of more investigation. It seems likely that ring-porosity is first and foremost an adaptation to winter freezing, with any consequences of this anatomy for growing season ecophysiology being secondary. Biogeographically, ring-porosity is primarily a north-temperate phenomenon that is associated with a winter-deciduous habit, and it has evolved multiple times (Wheeler and Baas 1993). Ring-porous trees package their water conducting capacity in a single row of vessels in each annual xylem ring. Although this single row of vessels is highly vulnerable to cavitation as a result of even a single freeze-thaw event, it is dependably replaced every year at bud break. This is an alternative to the diffuse-porous default of relying on the net carryover of functional vessels—the narrower ones not cavitared by freezing, cavitared ones that were refilled by root or stem pressures, and the gradual augmentation from current-year growth (Sperry et al. 1994). If adaptation to winter freezing was the primary driver for the evolution of large earlywood vessels, their vulnerability to growing season water stress may have been a consequence that perhaps led to secondary adaptations of isohydry, a deep rooting habit, and the capacity for vessel refilling.

As usual, although we may have answered the questions we posed, the results give rise to a new set of questions. Among them, questions about the mechanism of vessel refilling, whether our case-study is relevant to ring- versus diffuse-po-

rous trees in general, and what the results imply concerning the ecology and evolution of these two categories of trees.

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