

Vulnerability of Xylem Vessels to Cavitation in Sugar Maple. Scaling from Individual Vessels to Whole Branches¹

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The relation between xylem vessel age and vulnerability to cavitation of sugar maple (*Acer saccharum* Marsh.) was quantified by measuring the pressure required to force air across bordered pit membranes separating individual xylem vessels. We found that the bordered pit membranes of vessels located in current year xylem could withstand greater applied gas pressures (3.8 MPa) compared with bordered pit membranes in vessels located in older annular rings (2.0 MPa). A longitudinal transect along 6-year-old branches indicated that the pressure required to push gas across bordered pit membranes of current year xylem did not vary with distance from the growing tip. To understand the contribution of age-related changes in vulnerability to the overall resistance to cavitation, we combined data on the pressure thresholds of individual xylem vessels with measurements of the relative flow rate through each annual ring. The annual ring of the current year contributed only 16% of the total flow measured on 10-cm-long segments cut from 6-year-old branches, but it contributed more than 70% of the total flow when measured through 6-year-old branches to the point of leaf attachment. The vulnerability curve calculated using relative flow rates measured on branch segments were similar to vulnerability curves measured on 6-year-old branches (pressure that reduces hydraulic conductance by 50% = 1.6–2.4 MPa), whereas the vulnerability curve calculated using relative flow rates measured on 6-year-old branches were similar to ones measured on the extension growth of the current year (pressure that reduces hydraulic conductance by 50% = 3.8 MPa). These data suggest that, in sugar maple, the xylem of the current year can withstand larger xylem tensions than older wood and dominates water delivery to leaves.

The ability of water to withstand substantial negative pressures (Dixon and Joly, 1895; Briggs, 1950) allows plants to power the movement of water through the xylem by evaporation from leaf surfaces (Pickard, 1981). The negative pressures (tension) that these continuous columns of water can withstand before breaking (cavitation) is critical to the ability of a plant to tolerate periods of low water availability (Tyree and Sperry, 1989). The ability of xylem conduits to withstand tension-induced cavitation is typically inferred from “vulnerability curves” generated by measuring the change in the hydraulic capacity of xylem while subjecting a branch, stem, or root segment to some form of experimental stress, such as dehydration, centrifugation, and/or applied gas pressure (Tyree and Dixon, 1986; Sperry et al., 1988a, 1991; Cochard et al., 1992b, 2000; Jarbeau et al., 1995; Pockman et al., 1995; Alder et al., 1997; Kolb and Sperry, 1999b; Melcher et al., 2001). The pressure that

reduces hydraulic conductance by 50% (P_{50}) is generally used to characterize xylem vulnerability to tension-induced cavitation. A number of studies have demonstrated that P_{50} is related to both drought tolerance (Tyree et al., 1994; Jarbeau et al., 1995; Sperry, 1995) and to wood density (Hacke et al., 2000, 2001a).

Large variations in the shape and slope of vulnerability curves exist, both between species and from the same species growing in different environmental conditions (Sperry and Tyree, 1988, 1990; Magnani and Borghetti, 1995; Kolb and Sperry, 1999a; Melcher et al., 2001). Most striking is the difference between species with extremely steep vulnerability curves, such as petioles of *Alnus incana* (Hacke et al., 2001b) and *Juglans regia* (Cochard et al., 2000), and young stems of *Betula occidentalis* (Sperry and Pockman, 1993), *Populus fremontii*, *Salix gooddingii* (Pockman et al., 1995), and *Schefflera morototoni* (Tyree et al., 1991) in which the transition from fully conductive to a fully embolized state occurs over a small range in estimated tensions (approximately 1.0 MPa), versus those species, such as sugar maple (*Acer saccharum*; Sperry and Tyree, 1988), *Acer negundo* (Pockman et al., 1995), *Juniperus osteosperma* (Linton et al., 1998), Ponderosa pine (*Pinus ponderosa*; Hubbard et al., 2001), and *Rhizophora mangle* (Sperry et al., 1988b; Melcher et al., 2001), in which stem hydraulic capac-

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ity is gradually reduced as the tensions are increased. The shape of vulnerability curves has important implications for discussions of stomatal regulation of xylem tension (Sperry, 1986; Jones and Sutherland, 1991; Hacke and Sauter, 1995; Cochard et al., 2002). For example, a species that has a step-like vulnerability curve would require that its stomata must be extremely vigilant in preventing tensions from exceeding the threshold for cavitation, whereas in species with more sloping vulnerability curves, the degree of tolerance would be much greater (Tyree and Sperry, 1988). A number of simulation models have suggested that plants may optimize short-term performance by permitting some degree of xylem cavitation, although the degree to which this would be advantageous depends, in large part, upon the slope of the vulnerability curve (Jones and Sutherland, 1991; Comstock, 2000).

Despite the importance of vulnerability curves for our understanding of xylem transport and stomatal behavior, we know little of the underlying basis for the observed differences in the magnitudes and shapes of these curves. Because vulnerability curves are determined on the entire ensemble of conduits within a stem, the shape of the response results from the aggregate behavior of the collection of individual vessels. Whether variation in the pressures that result in cavitation (i.e. species with a gradual sloped vulnerability curve) reflects an intrinsic level of variation among vessels or is due to age-related changes in the vulnerability of older conduits is not known. Examination of SEM images and measurements of changes in air permeability through different aged annual rings of *Populus tremuloides* indicates that bordered pit membranes can deteriorate with age (Sperry et al., 1991). Here, we present results from experiments that measure the pressure threshold required to induce embolism at the level of individual vessels in branches of sugar maple. Our goal is to determine the relation between the cavitation thresholds of individual xylem vessels and the vulnerability curves measured on branch segments.

RESULTS

The pressure required to force gas across bordered pit membranes of individual xylem vessels varied along a chronological sequence (annual rings) in 6-year-old sugar maple branches (Fig. 1). Bordered pit membranes located in current year xylem were able to withstand significantly greater applied gas pressures compared with the amount of pressure required to force gas across bordered pit membranes located in all other annual rings (Fisher's pairwise comparison, $P < 0.05$). No differences were found in the pressure required to force gas across bordered pit membranes of current year xylem located in the extension growth of the current year (3.80 MPa \pm 0.45) compared with current year xylem located in older

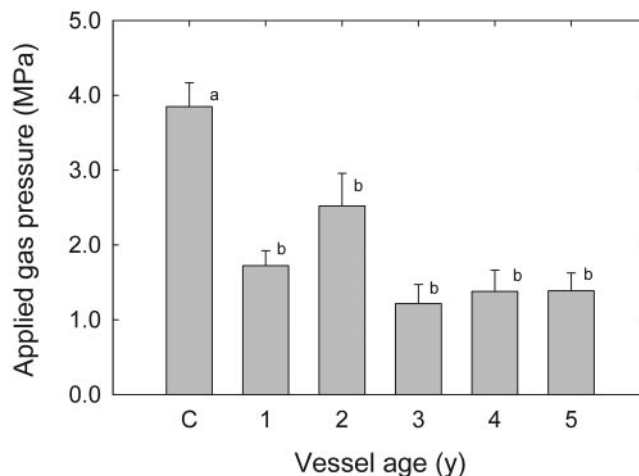


Figure 1. The pressure (mean \pm SE) required to push gas across bordered pit membranes of individual vessels located in annual rings of different ages. The current year xylem had significantly higher pressure thresholds compared with vessels in older growth rings. Sample size for individual vessel measurements located in annual rings from the current year through 6th year were 13, 9, 12, 5, 5, and 5, respectively. Each measurement was conducted on a new stem segment. Letters denote significant differences among means (Fisher's pairwise comparison, $P < 0.05$).

regions of the branch (1- through 6-year-old branches; 3.78 ± 0.55 MPa, ANOVA, $P = 0.95$).

The relative flow through each annual ring differed between the two measurement protocols (Fig. 2A). The first protocol (type I) followed the standard approach of measuring flow through relatively short (10-cm) branch segments. When measured this way, about 16% of the flow occurred within the growth ring of the current year. In contrast, when the relative contribution of each growth ring was measured using 6-year old branches that extended all the way to the growth of the current year (type II), about 70% of the total flow occurred within the growth ring of the current year. The flow rate through each annual ring, determined using the type I protocol (Fig. 1A, black bars), was correlated with the cross-sectional area of the ring (Fig. 2B, black bars). In contrast, the flow contribution of each annual ring determined from the type II measurement protocol (Fig. 2A, gray bars) was not dependent on xylem-conducting area (Fig. 2B, gray bars).

Vulnerability curves measured on current year versus 6-year-old branches differed markedly in both their shape and the pressure thresholds associated with a 50% loss in hydraulic conductance (Fig. 3). Current year branches (Fig. 3, black squares) had steep vulnerability curves and a P_{50} value of 3.8 MPa. In contrast, the vulnerability curve for 6-year-old branches had a more gradual slope and a P_{50} value of 1.6 MPa when measured by dehydration (Fig. 3, black circles) and approximately 2.4 MPa as determined by air injection (Fig. 3, black triangles). Higher apparent vulnerability in the dehydrated branches

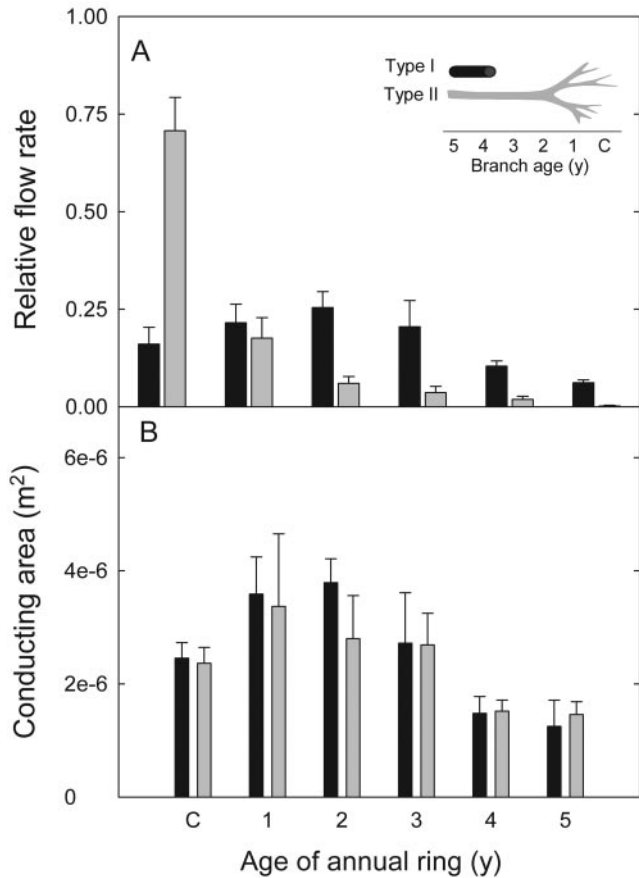


Figure 2. A, The relative flow rate (mean \pm SE) through annual rings of 6-year-old sugar maple branches. Type I (black bars), Relative flow rate through each annual ring determined on 10-cm-long branch segments ($n = 5$). Type II (gray bars), Relative flow rate through each annual ring using branches that extended from 6-year-old wood to the extension growth of the current year ($n = 5$). B, The cross sectional area (mean \pm SE) of each annual ring that corresponds to the relative flow rate data in A ($n = 5$ branch segments).

may be due to the high initial PLC, because branches were not artificially rehydrated before beginning the dehydration cycle.

Measurements of the pressure thresholds required to force gas across bordered pit membranes of individual xylem vessels of known age (Fig. 1) and the relative flow rate through the annual ring of each year (Fig. 2) were used to generate vulnerability curves (Fig. 3). When the relative flow rate through each annual ring was determined according to the standard protocol (type I), the calculated vulnerability curve (Fig. 3, gray circles) was similar to that measured on the 6-year-old branches (Fig. 3, black circles and triangles). However, when the relative flow rate through each annual ring was determined using branches (type II), rather than branch segments, the resulting vulnerability curve (Fig. 3, gray squares) agreed more closely with vulnerability curves measured on the extension growth of the current year (Fig. 3, black squares).

DISCUSSION

Deconstruction of the vulnerability curve of 6-year-old branches of sugar maple demonstrates two important trends as one moves radially across the xylem. The first is a significant increase in vulnerability (decrease in pressure threshold) with vessel age (Fig. 1), whereas the second is a decrease in the hydraulic connection to the leaves (Fig. 2). Whether this increase in vulnerability with vessel age is due to previous cavitation events (Hacke et al., 2001b), damage during winter months, or solely the passage of time is unknown. However, the fact that older regions of the xylem become increasingly less important in terms of their ability to supply water to the leaves suggests that there may be little selective pressure for maintaining the ability of pit membranes to withstand high tensions. Alternatively, having lower cavitation thresholds in older xylem could serve as a mechanism to release water from these older conduits to the more hydraulically active wood, thus allowing vessels in older annual rings to function in water storage (Sperry et al., 1991; Holbrook et al., 1995; Goldstein et al., 1998; Melcher et al., 2001).

The analysis presented here suggests that vulnerability curves measured on 6-year-old branch segments of sugar maple significantly underestimate the ability of the xylem to resist cavitation (Fig. 3). Vul-

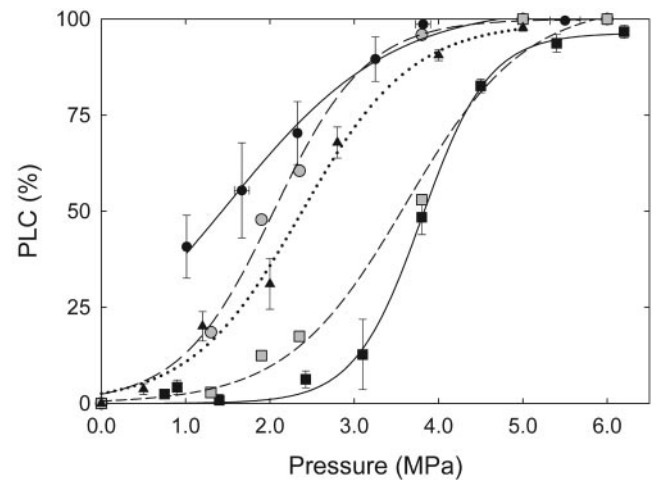


Figure 3. The percent loss in hydraulic conductivity (PLC) of 6-year-old stem segments subjected to varying levels of dehydration-induced tension (●, solid line) or air injection (▲, dotted line) and of current year extension growth subjected to dehydration (■, solid line). The gray symbols and dashed lines represent estimated PLC generated from single-vessel air injection pressure thresholds and flow rates through each annual ring determined using the two measurement protocols. Vulnerability curves estimated using relative flow rates of each annual ring determined on 10-cm-long stem segments (type I) are indicated by gray circles; vulnerability curves estimated using relative flow rates of each annual ring determined on 6-year-old branches (approximately 80-cm-long) that were continuous through to the extension growth of the current year (Type II) are indicated by gray squares. All lines were fitted using a three-parameter sigmoidal curve $y = a/(1 + e^{(x - x_0/b)})$; error bars represent SE.

nerability curves reconstructed from pressure thresholds of individual vessels as a function of age and the properly weighted hydraulic contribution of each annual ring (type II flow measurements) indicate that the functional water path is much more resistant to cavitation (Fig. 3). If our findings of age-related changes in both the vulnerability to cavitation and the hydraulic conductivity referenced to the points of leaf attachment hold true for other species, then we must re-evaluate previous studies that incorporate several years of xylem in their estimates of the tolerance to drought of a species. The real issue here is not that the vulnerability of individual xylem vessels changes with age, but that hydraulic importance of these older vessels may be overestimated when flow is measured through short segments. Although several studies have expressed caution when working with older branches of ring porous trees per the danger of inadvertently refilling cavitated vessels (Zimmermann, 1983; Sperry and Saliendra, 1994), the potential errors in estimating hydraulic conductance that we refer to here arise from a failure to take into account the degree to which older regions of the xylem are connected to the growth of the current year. Measurements of xylem hydraulic properties on whole shoots are immune from these potential problems and thus provide a useful reference point (Yang and Tyree, 1994; Kolb et al., 1996; Nardini et al., 2001).

The single-vessel technique holds great promise for studies of xylem hydraulic properties by providing a tool with which to examine portions of the plant that are too bulky or have vessels that are too long to be examined with other techniques. According to the "segmentation" hypothesis of Zimmermann (1978), the hydraulic architecture of trees results in cavitation being largely restricted to portions of the crown, such as leaves, that are most easily replaced, while protecting the more permanent stems and large branches. Zimmermann believed that this was due to the presence of hydraulic constrictions within the xylem (particularly at leaf insertions). However, cavitation could also be concentrated in distal regions if there were significant changes in cavitation thresholds along the transpiration path. Previous studies have shown significant variation in the cavitation threshold of different-aged branches (Sperry et al., 1991; Cochard et al., 1992a, 2002; Alder et al., 1996; Hacke and Sauter, 1996). Whether these differences were due to changes in xylem development or to aging of previously formed vessels are unknown. In this study, we found no evidence for changes in the vulnerability of current year xylem sampled along 6-year-old branches. However, further studies that extend these measurements to the whole plant are needed.

The microcapillary technique also provides the ability to probe the hydraulic properties of water-conducting versus cavitated conduits. Although ves-

sels were sampled at random in this study, without reference to their stress history, it is possible to identify functional vessels using dye before determining their cavitation thresholds. In this manner, one could determine whether the functional population of vessels differs from embolized conduits. In this study, the relatively small variation in the older growth rings does not suggest the existence of two populations of vessels (cavitated and fatigued versus functional and intact) within any one ring (Hacke et al., 2001b). However, further studies are needed to understand fully the observed variation in cavitation thresholds.

The findings presented here force us to incorporate an understanding of the way in which plants grow when making and interpreting measurements of xylem hydraulic properties. In particular, measurements of xylem parameters must be referenced to their effect on water supply to leaves, rather than solely to their effect on water flow across short segments. In the case of sugar maple, failure to take into account the ways in which the transport system is constructed by the plant leads to substantial errors in our assessment of its vulnerability to cavitation.

MATERIALS AND METHODS

Plant Material

For all measurements, large branches (approximately 2.0 m long) were collected during the growing season from the upper portions of the crowns (15–20 m high) of three mature sugar maple (*Acer saccharum* Marsh. var *Temple Upright*) trees growing at the Arnold Arboretum (Boston). Branches were collected in the mornings using an extendable boom lift and placed into plastic bags and transported back to the laboratory.

Pressure Thresholds of Individual Vessels

A total of 20 large branches that contained about 9 years of growth were collected from the upper portions of the crowns of three mature sugar maple trees. The excised branches were immediately placed in plastic bags to prevent excessive water loss during transport from the field to the laboratory. Small branch segments (5-cm-long) were then recut underwater from these branches, and the xylem cross-section was shaved clean with a fresh razor blade. Branch segments of varying age from current year to 6-year-old branches were attached to a micromanipulator, and the tip of a glass microcapillary was inserted into the open lumen of an individual xylem vessel using a 50× stereo microscope (SZ-STB2, Olympus, Tokyo) and a micromanipulator. After successful insertion, the microcapillary was rapidly fixed in place using low-viscosity, fast-setting cyanoacrylic glue (Loctite superbond 407). Glass microcapillary tubes were prepared by pulling them to a fine point using a horizontal pipette puller (Pul1 World Precision Instruments, Sarasota, FL). The glass tips were subsequently broken off such that the opening of the microcapillary tip ranged from 30 to 40 μm in diameter (the approximate diameter of sugar maple xylem vessels).

The pressure threshold required to force gas across intervessel-bordered pit fields was determined by attaching the glass microcapillary tube to a regulated pressure source. Nitrogen gas was applied to the system at a rate of 0.1 MPa min⁻¹, and the pressure required to force gas across bordered pit fields was determined when bubbles streamed from the downstream end of the vessel that was underwater and viewed with a 10× hand lens. Short (approximately 5-cm) branch segments were used for all the experiments because about 75% of the vessels in sugar maple are shorter than 3.0 cm (Zimmermann and Jeje, 1981). If gas streamed out of the xylem at low pressures (< 0.1 MPa), it was assumed that the vessel was continuous through the branch segment, and the measurement was discarded. All

measurements were made within 6 h of collection time. Sample size for vessels located in annual rings of the current year through 6th year were 13, 9, 12, 5, 5, 5, respectively.

Relative Hydraulic Capacity of Annual Rings

The relative flow rate through different-aged annual rings was determined using two protocols. For both protocols, flow rates were measured using an analytical balance (± 0.01 mg, model R160P, Sartorius, Gottingen, Germany) interfaced to a computer. The measurement solution was 10 mM KCl, and the pressure difference across the sample was 9.1 kPa. Solution flowed from a balance and through the branch where it flowed out of the cut ends. The entire sample was placed underwater during the flow measurement to reduce the effects of surface tension produced during the formation of water droplets on the downstream cut ends.

The flow rate through each annual growth ring was determined for both protocols by measuring the flow rate through the entire sample, followed by blocking the pith and the 6-year-old annual ring with fast-setting cyanoacrylic glue (Loctite superbond 407) and remeasuring the flow rate. This procedure was repeated until only the annual growth ring of the current year remained unglued. The relative flow rate through each annual growth ring was calculated by dividing the flow rate of each annual ring (determined by subtraction) by the total flow rate through that branch. A 50 \times stereo microscope (Olympus SZ-STB2) was used during the gluing procedure to make sure that only the target ring was blocked. To prevent xylem dehydration during the gluing process, the entire branch was placed in a container of water with only about 1 cm exposed to the air. Preliminary experiments in which the sequential gluing procedure was repeated three times on the same branch (removing the glued end each time) indicated that the actual application of the glue introduced little variation. Preliminary experiments using safranin to record the movement of water within the measured segment demonstrated that the perfusing solution did not travel through the portions of stems that were covered with glue.

The first protocol (type I) was conducted on 10-cm-long, 6-year-old branch segments. The second protocol (type II) was conducted on 6-year-old branches in which the distal ends had been severed just below the first leaf insertion. In this configuration, the perfusing solution enters the proximal (upstream) end of the branch and exits through the extension growth of the current year. This approach takes into account the fact that all flow through older portions of the branch must eventually be directed to the regions where the leaves are attached (i.e. the extension growth of the current year). The entire procedure was repeated on five branches for both protocols.

Vulnerability Curves by Dehydration

Embolism formation in the xylem of sugar maple was determined by measuring the loss of hydraulic conductance (kh) that occurred due to air blockage (Sperry et al., 1988a) as branches were subjected to different levels of dehydration. Sixty branches (approximately 2-m-long) were collected from three mature sugar maple trees and immediately placed in plastic bags that contained moist paper towels to prevent excessive transpirational water loss during transport from the field to the laboratory. Branches were not rehydrated before beginning the measurements, and maximum water potentials (Ψ) at the time of collection (7:30 AM) were approximately -0.8 MPa. The branches were removed from their bags and allowed to dry out while held at approximately 20°C and about 50% RH for varying intervals (0–5 d). At the end of each dehydration time interval, entire leafy branches were rebagged for about 10 h, to ensure equilibration of Ψ throughout the branch. Balancing pressures of a distal leafy twig were measured using a pressure chamber (PMS Instrument Co., Corvallis, OR). The chamber pressure was increased at a rate of 5 kPa s^{-1} , and the pressure at which water first appeared at the cut surface (viewed using a 30 \times stereo microscope) was recorded as the balancing pressure.

Paired measurements of leaf Ψ and percent loss of k_h were used to generate a vulnerability curve. At each dehydration interval, a 3-cm-long branch segment was excised under water and cleanly shaven at both ends with a fresh razor blade. The bark was carefully removed by hand, and the cut end was attached to the hydraulic manifold using soft fitting rubber tubing. Flow rates were measured using an analytical balance (± 0.01 mg, Sartorius model R160P) interfaced to a computer. The measurement solution was 10 mM KCl, and the delivery pressure was approximately 2.5 kPa.

Solution flowed from the balance and through the branch segment, which was kept underwater during the measurement. The difference in hydraulic pressure across the stem was determined by measuring the vertical distance between the water level on the balance and the water level in the container containing the branch segment. To accurately determine the height of the water column on the balance, the vertical distance between the top of the laboratory bench and the water meniscus of the water reservoir on the balance was measured using a previously calibrated pressure transducer (PX26-005GV, Omega Engineering Inc., Stamford, CT). Gas emboli were subsequently removed by applying a series of 5-min hydraulic pressure flushes (0.40 MPa) until measured values of k_h maximum remained constant between flushes (usually one to two flushes removed all emboli from these short segments). PLC was calculated as $(1 - [k_{h\text{ initial}}/k_{h\text{ maximum}}]) \times 100$.

Vulnerability Curves by Air Injection

Previous studies have demonstrated that air injection pressure thresholds and dehydration-induced embolism are well correlated (Cochard et al., 1992b; Sperry et al., 1996; Sperry and Ikeda, 1997). However, to check this, we constructed vulnerability curves using air injection on 6-year-old branches of sugar maple. Five branches sampled as described above were recut into 20-cm-long segments (approximately twice the length of the longest vessels [Zimmermann and Jeje, 1981; P. Melcher, unpublished data]) under tap water. Branch segments were recut with a razor blade and attached to a hydraulic apparatus that supplied a 10 mM KCl solution at constant pressure. Flow rate from the distal end of the branch segment was measured using an analytical balance (± 0.01 mg, Sartorius model R160P) interfaced to a computer. The PLC was determined following a slightly modified protocol described by Sperry et al. (1991). Initial (maximum) k_h of each branch segment was determined, after which the downstream end of the branch segment was placed into a pressure chamber (PMS Instrument Co.) and pressurized for 1 min. A short piece of tubing was attached to the end of the branch segment protruding from the pressure chamber such that the upstream end of the branch remained in contact with 10 mM KCl solution. After each pressurization, the upstream (proximal) end of the branch segment was re-attached to the hydraulic apparatus, and 1 min of 0.05-MPa suction was applied to the distal end before determining the hydraulic conductivity of the segment. Application of suction to the distal end was used to remove gas introduced into the ends of the segment during air injection. Preliminary experiments using low air injection pressures (approximately 0.1 MPa) indicated that this was necessary to prevent decreases in k_h due to gas entrapment in the ends of open vessels. The entire procedure was repeated for a series of increasing pressures, until the flow rate through the branch segment was reduced to a value near zero. The time needed to complete these measurements was approximately 5 h.

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