

The spatial pattern of air seeding thresholds in mature sugar maple trees

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ABSTRACT

Air seeding threshold (P_a) of xylem vessels from current year growth rings were measured along the vertical axis of mature sugar maple trees (*Acer saccharum* Marsh.), with sampling points in primary leaf veins, petioles, 1-, 3-, and 7-year-old branches, large branches, the trunk and roots. The air seeding threshold was taken as the pressure required to force nitrogen gas through intervessel pit membranes. Although all measurements were made on wood produced in the same year, P_a varied between different regions of *A. saccharum*, with distal organs such as leaves and petioles having lower P_a than basal regions. Mean (SE) P_a ranged from 1.0 (\pm 0.1) MPa in primary leaf veins to 4.8 (\pm 0.1) MPa in the main trunk. Roots exhibited a P_a of 2.8 (\pm 0.2) MPa, lower than all other regions of the tree except leaf veins and petioles. Mean xylem vessel diameter increased basipetally, with the widest vessels occurring in the trunk and roots. Within the shoot, wider vessels had greater air seeding thresholds, contrasting with trends previously reported. However, further experimentation revealed that differences in P_a between regions of the stem were driven by the presence of primary xylem conduits, rather than differences in vessel diameter. In 1-year-old branches, P_a was significantly lower in primary xylem vessels than in adjacent secondary xylem vessels. This explained the lower values of P_a measured in petioles and leaf veins, which possessed a greater ratio of primary xylem to secondary xylem than other regions. The difference in P_a between primary and secondary xylem was attributed to the greater area of primary cell wall (pit membrane) exposed in primary xylem conduits with helical or annular thickening.

Key-words: cavitation; primary xylem; secondary xylem; segmentation.

INTRODUCTION

The negative hydrostatic pressures needed to transport water through the xylem at rates sufficient to meet the

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transpirational demand of sunlit leaves makes this essential transport pathway vulnerable to the formation of air emboli. One strategy for dealing with the dangers inherent in relying on a metastable state is to develop physiological and structural features to minimize the deleterious effects of cavitation. Zimmermann (1983) proposed that the hydraulic architecture of plants should be structured in such a way that cavitation occurred primarily in leaves and small branches, a concept he called 'segmentation.' According to the segmentation hypothesis, distal organs, which are easily replaceable, (e.g. terminal twigs and leaves) will be preferentially sacrificed to protect the main stem and older branches in which a greater investment has been made. Localizing cavitation to distal organs can have additional benefits in times of water stress. Because cavitation in distal regions often leads to dieback of small branches (Sperry & Pockman 1993; Rood *et al.* 2000), the resulting reduction in total leaf area will lead to lower xylem tensions within the remaining portions of the plant (Tyree & Sperry 1988).

The restriction of air emboli to more distal regions can result from natural gradients in water potential alone. Because water movement through the xylem depends on a gradient in pressure between the roots and the leaves, the xylem water potential (and hydrostatic pressure) must be most negative at the leaves and least negative at the roots for water to be transported to the leaf surface (Dixon & Joly 1895). Thus, because xylem tension is greater in distal branches and leaves, if all else is equal, cavitation is more likely to occur there than in the trunk and roots. Zimmermann (1983) believed that these natural gradients in water potential were accentuated by the existence of substantial hydraulic resistances at branch junctions and leaf insertions, leading to distinct decreases in xylem pressures between each 'segment' of the flow path. In this way, cavitation could be confined to well-defined morphological regions.

Localization of embolism may also be effected through differences in the vulnerability to cavitation of xylem tissue in different regions of the plant. Water-stress-induced cavitation is thought to be caused by the penetration of gas through intervessel pit membranes, a process known as air seeding (Zimmermann 1983). If pit membranes in one

region of the plant are less resistant to gas penetration, embolism will occur at lower xylem tensions than in other regions of the plant. In some species, distal organs such as petioles and leaves are more vulnerable to cavitation than branches (Tyree *et al.* 1993; Tsuda & Tyree 1997; Salleo *et al.* 2000). However, other studies have shown the opposite trend, with distal organs less vulnerable to cavitation than branches (Sperry & Saliendra 1994; Hacke & Sauter 1996). Numerous studies have demonstrated that root xylem is more vulnerable to cavitation than stem xylem (Alder, Sperry & Pockman 1996; Kavanagh *et al.* 1999; Kolb & Sperry 1999).

The basis of such differences in vulnerability to cavitation is poorly understood. Although vessel diameter is only weakly correlated with vulnerability to water-stress-induced cavitation at the interspecific level (Tyree & Zimmermann 2002), a number of studies have shown that when comparisons are made within a single plant, wide xylem vessels are more vulnerable to water-stress-induced cavitation than narrow vessels (Lo Gullo & Salleo 1991; Hargrave *et al.* 1994; Sperry & Saliendra 1994; Lo Gullo *et al.* 1995). There are two commonly proposed explanations for why a correlation between vessel diameter and vulnerability to cavitation should hold. First, there may be a developmental relationship between pit membrane porosity and vessel size, such that larger vessels have more porous pit membranes. Second, it may relate to the fact that wider vessels are usually longer and will therefore have a greater total area of pit membrane connection to other vessels (Zimmermann & Jeje 1981). Because vessel diameter typically increases as one moves from the leaves to the roots, a pattern thought to result from the effects of auxin, a mechanistic link between conduit size and vulnerability would result in patterns consistent with the segmentation hypothesis (Aloni & Zimmermann 1983; Roberts, Gahan & Aloni 1988).

Previous studies investigating spatial partitioning of vulnerability to cavitation in trees have suffered from two major limitations. First, measurements on the trunks or main branches of mature trees have not been included, and second, the age of xylem tissue among regions of the tree has not been controlled for. The first deficiency has developed primarily because standard techniques do not allow for measurement of large branches or trunks. In conifer species, measurements of this nature have been successfully carried out by excising small blocks of sapwood from large trunks (Domec & Gartner 2001, 2002). However, this has not been attempted with hardwood species, which have xylem conduits of much greater length. The microcapillary technique described by Zwieniecki, Melcher & Holbrook (2001) enables characteristics of individual xylem vessels from older regions of a mature tree to be studied. With this approach it is also possible to restrict measurements to xylem vessels from a particular year of growth. This is important because past studies have shown that vulnerability to cavitation may increase sharply in older wood (Sperry, Perry & Sullivan 1991; Melcher, Zwieniecki & Holbrook 2003).

In this study we examined air seeding threshold (P_a) along the vertical axis of mature sugar maple (*Acer saccharum* Marsh.) trees. The air-seeding threshold represents the pressure difference at which gas will penetrate pit membranes; a lower P_a indicates a greater vulnerability to water-stress-induced cavitation because less tension is required in the xylem sap for air seeding to occur. Because xylem in the current year growth ring of *A. saccharum* accounts for a high proportion ($\approx 70\%$) of the total flow when the pathway to the leaves is included (Melcher *et al.* 2003) and the fact that this is the only age class that can be compared along the entire axis of the tree (i.e. from leaf veins to roots), we examined P_a in current year xylem vessels in different regions of the tree. We hypothesized that differences in P_a of current year xylem vessels would depend on the region of the tree and specifically that distal regions would be more vulnerable to cavitation than the trunk.

METHODS AND MATERIALS

Plant material

Branches and wood sections were collected from mature (15–20 m height) trees of *Acer saccharum* Marsh. at the Harvard Forest (Petersham, MA, USA) during June, July and August of 2003 and July of 2004. A canopy lift (Scanlift SL240, Joensuu, Finland) was used to access branches from the top of the tree. Sections were collected from the trunk and large branches using a mallet and chisel. Once cut, branches or sections of wood were sealed in plastic bags with moist paper towels for transport back to the laboratory, and then stored in a refrigerator. All samples were collected between 0800 and 1100 h on clear days.

Air seeding threshold

All measurements of air seeding threshold were made on xylem vessels produced in the current year, with regions of the tree measured including primary leaf veins, petioles, 1-, 3-, and 7-year-old branches, large branches, the main trunk, and roots. With the exception of the roots, all measurements were made on two mature trees (15–20 m). Because root excavation was not permitted in the same trees from which stem samples were collected, root xylem samples (5–10 mm in diameter) were collected from another tree growing at Harvard Forest.

Measurements on all regions except leaves and petioles were made on individual xylem vessels, following the protocol of Zwieniecki *et al.* (2001). Glass microcapillary tubes were pulled using a micropipette puller (Pull1; World Precision Instruments, Sarasota, FL, USA). The microcapillary tips were broken using a razor blade to an opening of approximately 30–50 microns in diameter, the approximate diameter of xylem vessels in *A. saccharum* (Melcher *et al.* 2003). Microcapillaries were inserted into xylem vessels under a stereo dissector microscope (Wild Heerbrugg, Heerbrugg, Switzerland) using a micro manipulator. Ves-

sels were selected on the basis of size and location, with preference for the oldest vessels of the current year xylem, those nearest the previous year's ring, and those with the largest diameter at the cut surface of the branch. As such, sampling was restricted to the wider earlywood xylem vessels, although the difference in diameter between earlywood and latewood vessels is not as great in *A. saccharum*, which is diffuse porous, as in ring porous trees. In *A. saccharum* approximately 75% of xylem vessels will terminate in a stem segment 5 cm in length (Zimmerman & Jeje 1981). Thus, segments 6–10 cm long were used to ensure that almost all vessels contained an endwall. Once inserted, the microcapillary was glued in place using cyanoacrylic glue (Loctite 409 Instant Adhesive; Henkel Loctite Corp., Rocky Hill, CT, USA) and an accelerator (Loctite 712 Accelerator). Compressed nitrogen gas was applied to the capillary tube and xylem vessel using a modified pressure chamber (Plant Moisture Stress, Corvallis, OR, USA) while the segment was held under water. The air seeding threshold at the junction between two xylem vessels was taken as the positive pressure at which a stream of bubbles became visible emerging from the end of the segment. This pressure is equal, but opposite in sign, to the negative pressure in xylem sap required to draw a bubble through an intervessel pit membrane pore. If the vessel was open at both ends, air emerged from the end of the segment at a very low pressure (≈ 0.1 MPa) and the measurement was discarded.

Petioles and primary leaf vein vessels were too small to be measured using the microcapillary technique. Measurements on petioles and leaf veins were instead carried out using a pressure chamber to supply pressurized gas to all open vessels at the cut surface of the tissue. Single leaves were collected and stored with moist paper towelling in plastic bags during transport to the laboratory. The cut end of the petiole was inserted into the pressure chamber and the primary veins of the leaf were cut under water. The petiole was then pressurized while the cut veins were held underwater. The air seeding threshold was recorded for the first stream of bubbles emerging from a primary vein. For petioles, small sections of current year shoots with no through vessels to petioles were placed into the pressure chamber. Petioles attached to the branch section emerging from the pressure chamber were cut underwater at the base of the lamina and the pressure was increased until a stream of bubbles was observed coming from one of the petioles.

The sample sizes for each region of the tree are as follows: primary leaf veins = 9, petioles = 14, 1-year branches = 19, 3-year branches = 12, 7-year branches = 11, large branches = 4, trunk = 5, roots = 7. Initial analysis of the data revealed no significant variation between the trees and thus measurements of air seeding threshold were pooled for all trees.

Further measurements were made in July of 2004 to differentiate between P_a of primary and secondary xylem vessels in 1-year-old branches. The air seeding threshold was measured on individual vessels as described above, except that measurements on primary xylem vessels, which form

clusters of large vessels around the pith, were distinguished from secondary vessels.

Anatomical measurements

Measurements of xylem vessel diameter were made on sections cut from the segments used for single vessel measurements. Wood sections were cut using a sliding microtome and stained with 0.01% toluidine blue. Sections were observed using brightfield optics. Images collected with an AxioCam camera (Zeiss, Jena, Germany) were then analysed using NIH image (NIH, Bethesda, MD, USA). The lumen area of each xylem vessel was measured and converted to a diameter from the equivalent circular area. At least 200 current year xylem vessels were measured for each age region examined.

RESULTS

Air seeding thresholds of current year xylem vessels were lower in distal regions: gas penetrated the intervessel pit membranes at lower pressure differences in leaf veins, petioles, 1-year-old branches and the roots than in older branches and the trunk (Fig. 1). Mean P_a was significantly lower ($P < 0.05$, Tukey HSD) in petioles and primary leaf veins than in all other parts of the stem, and the P_a of 1-year-old branches was significantly lower than in all older regions of the stem, except large branches (Fig. 1). There was no significant difference in P_a between regions of the stem older than 1-year-old branches. Mean P_a in the roots was lower than in all regions of the stem except for the petioles and leaf veins.

Measurements of P_a in 1-year-old branches made in 2003 exhibited a bimodal distribution. Longitudinal sections of xylem tissue in leaf veins, petioles and 1-year-old branches revealed a high proportion of primary xylem vessels with extensible helical or annular secondary wall thickenings in these regions (Fig. 2). To investigate the basis of the bimodal distribution, further measurements were done on 1-year-old branches in 2004 to differentiate between primary and secondary xylem conduits. Primary xylem vessels in 1-year-old branches of *A. saccharum* had a mean P_a of 2.6 (± 0.3) MPa, significantly lower ($P < 0.0001$, *T*-test) than adjacent secondary xylem vessels, 5.2 (± 0.1) MPa. Note that the mean P_a given for 1-year-old branches in Fig. 1 includes both primary and secondary conduits sampled in 2003.

Mean P_a increased with mean vessel diameter within the shoot (Fig. 3). However, this relationship is complicated by the differences in P_a of primary and secondary xylem in 1-year-old branches. If only measurements of secondary xylem vessels are considered, then there is no relationship between mean vessel diameter and P_a within the shoot; there was no significant difference ($P > 0.05$, Tukey HSD) between P_a of secondary xylem vessels within the shoot as mean vessel diameter increased. The root xylem differed from that of the shoot, having the highest mean vessel diameter and a lower P_a than other regions possessing secondary xylem vessels.

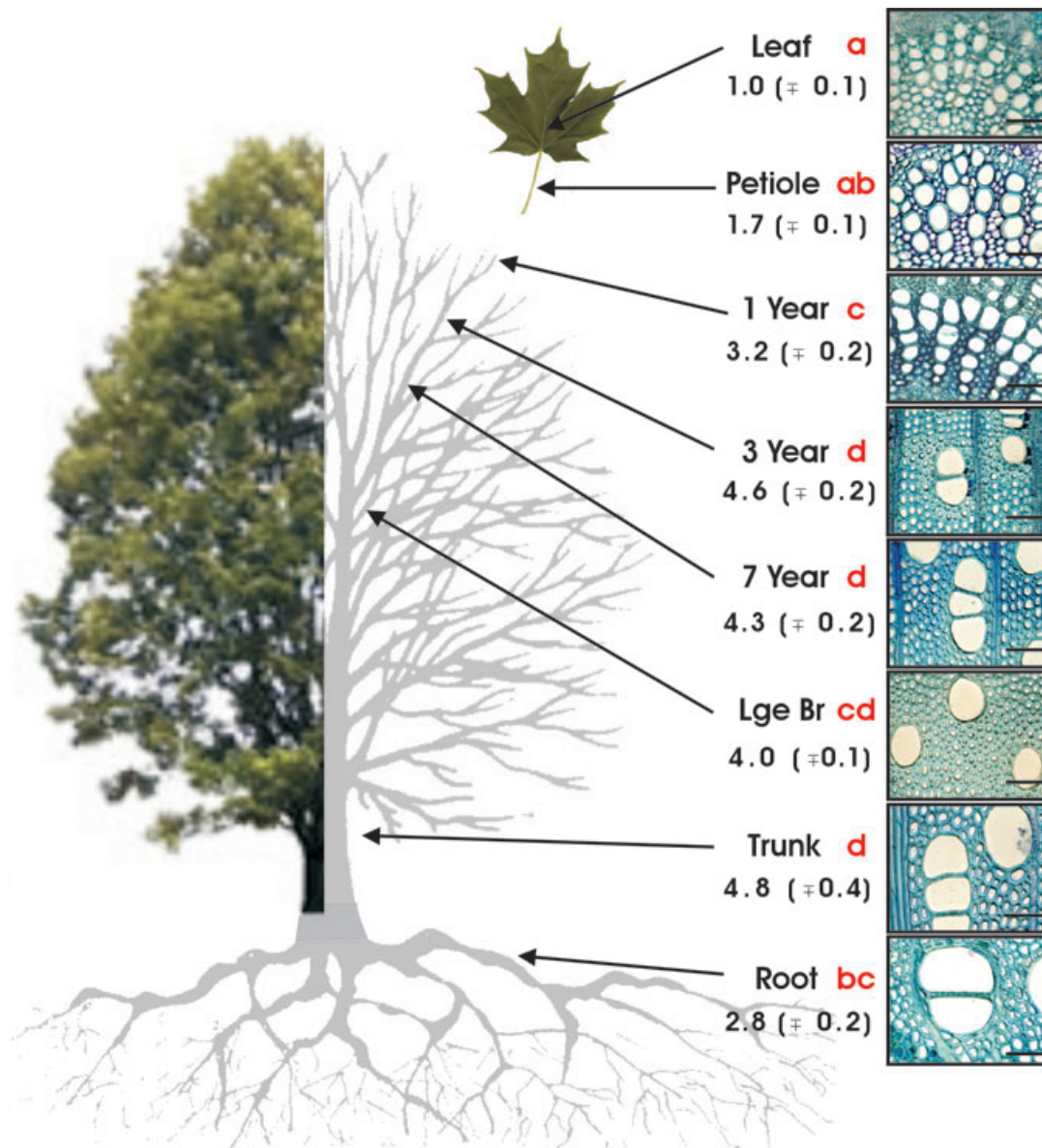


Figure 1. Mean air seeding threshold (P_a , MPa) in current year xylem from different regions of mature sugar maple (*Acer saccharum*) trees. Measurements in petioles and primary leaf veins represent the P_a of multiple vessels. In all other regions, measurements were made on individual xylem vessels. Letters following measurements indicate homogenous groups ($P < 0.05$, Tukey HSD unequal n). Images show transverse sections of xylem tissue stained with 0.01% toluidine blue. All images were taken at 400 \times magnification and scale bars = 50 μ m. For measurements of P_a , the numbers of samples for each region are as follows: leaf veins, $n = 9$; petioles, $n = 14$; 1 years, $n = 19$; 3 years, $n = 12$; 7 years, $n = 11$; large branches, $n = 4$; trunk, $n = 5$; roots, $n = 7$.

DISCUSSION

Spatial variation in air seeding threshold

Primary leaf veins, petioles and 1-year-old branches had significantly lower P_a than more basal regions of the tree, a pattern consistent with the segmentation hypothesis (Zimmermann 1983). The P_a of current year xylem in 1-year-old branches, 3.2 (± 0.2) MPa, was lower than that reported by Melcher *et al.* (2003) for the same region, 3.8 (± 0.4) MPa. The difference is explained by measurements made in 2004, which indicated that the lower P_a of 1-year-old branches, petioles and leaf veins was caused by the presence of pri-

mary xylem vessels with only partial secondary wall thickening. Measurements on 1-year-old branches made by Melcher *et al.* (2003) excluded the primary xylem.

A number of studies in which vulnerability to cavitation was assessed by measuring the percentage loss of hydraulic conductivity (PLC) report patterns similar to those found here (Tyree *et al.* 1993; Tsuda & Tyree 1997). For example, Tyree *et al.* (1993) demonstrated that petioles were more vulnerable to cavitation than branches in *Juglans regia* and consequently, that prior to leaf shedding, petioles suffered 87% loss of conductivity whereas branches suffered only 14% loss of conductivity. Similarly, Tsuda & Tyree (1997)

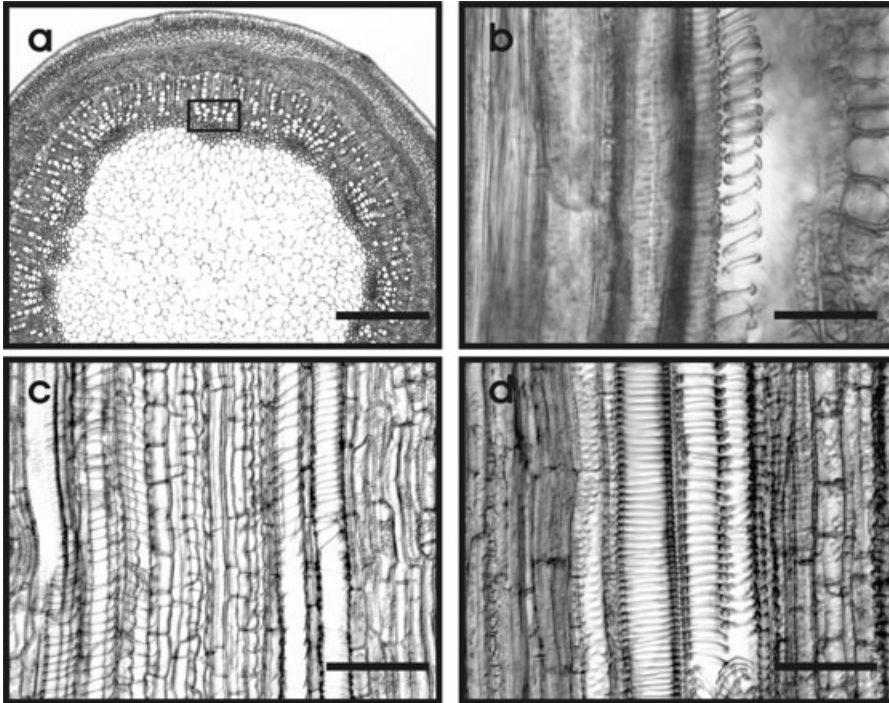


Figure 2. Light micrographs of xylem tissue in current year growth of *Acer saccharum*. (a) TS of 1-year-old branch with groups of primary xylem conduits clustered around the pith (one group identified in box), 40× magnification, scale bar = 500 μm. (b) LS of primary conduit with helical thickening in 1-year-old branch, 400× magnification, scale bar = 50 μm. (c) LS showing primary xylem conduits in petioles 400× magnification, scale bar = 50 μm. (d) LS showing primary conduits in primary leaf veins, 400× magnification, scale bar = 50 μm.

showed that petioles of *Acer saccharinum* lost 50% of hydraulic conductivity at -0.5 MPa, whereas in branches, a 50% loss of hydraulic conductivity occurred at roughly -1.7 MPa. Other studies, however, have reported the opposite trend (Sperry & Saliendra 1994; Hacke & Sauter 1996). Sperry & Saliendra (1994) observed that petioles and twigs were less vulnerable to cavitation than the main stem in *Betula occidentalis*, although loss of conductivity in petioles

was estimated from a correlation between vessel diameter and cavitation threshold rather than measured directly. Differences in patterns of vulnerability reported by Sperry & Saliendra (1994) and those observed in this study may simply reflect interspecific variation. However, it may also relate to differences in methodology. In the present study, the use of the single vessel technique allowed for measurement of P_a in current year xylem vessels, whereas the tech-

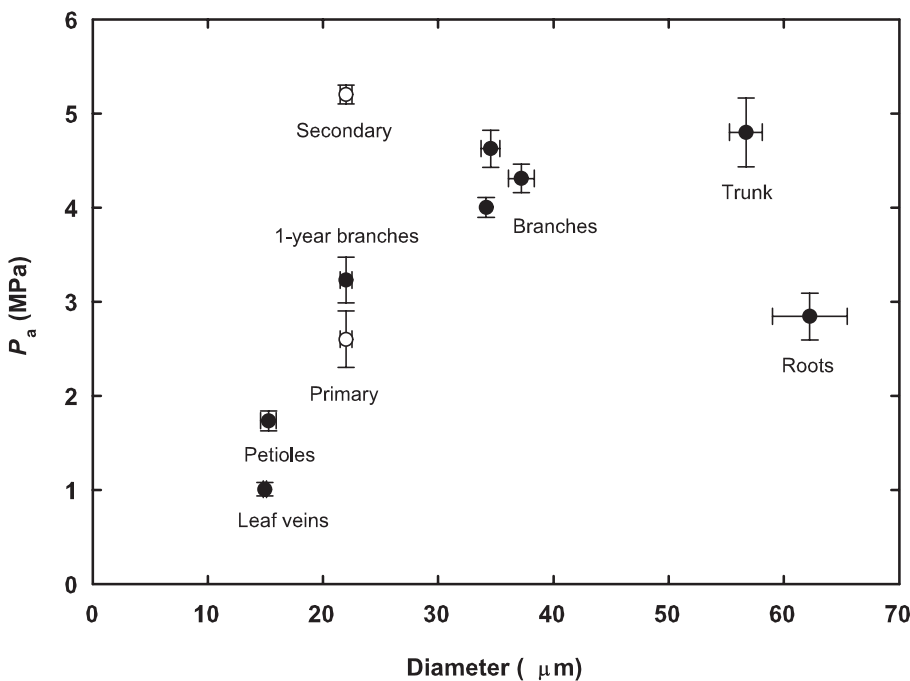


Figure 3. Mean air seeding threshold (P_a , MPa) plotted against mean xylem vessel diameter (μm) for different regions of mature sugar maple (*Acer saccharum*) trees. Measurements in petioles and primary leaf veins were made on multiple vessels; in all other regions, measurements were made on individual xylem vessels. Solid symbols show measurements made in 2003 on different regions of the tree. Hollow symbols show measurements made in 2004 on primary and secondary xylem of 1-year-old branches. Error bars show one standard error.

niques used for measuring PLC typically sample across multiple growth rings. Given evidence from other studies suggesting that air seeding thresholds decline with age (Sperry *et al.* 1991; Melcher *et al.* 2003), it is possible that the pattern of increased vulnerability in the main stem reflects the inclusion of xylem vessels from multiple growth rings in the measurement.

Differences in the methods used to measure P_a for petioles and primary leaf veins versus the more woody portions of the plant mean that comparisons between these regions must be made with care. The pressure chamber technique used for petioles and leaf veins should measure the minimum P_a of all conduits within the measured segment. In contrast, the microcapillary technique will yield a value close to the average P_a . Because vulnerability curves are typically quite steep in leaf tissue, the tension at which cavitation is initiated often does not differ substantially from the average P_a (Brodribb & Holbrook 2003). Although the P_a of 1.0 MPa for primary leaf veins appears low, past studies indicate that stomatal closure is initiated close to leaf water potentials of -1.0 MPa in *A. saccharum*, with the turgor loss point estimated to be -1.3 MPa (Yang & Tyree 1993; Sack *et al.* 2003). This is consistent with the notion that stomatal closure may be linked to cavitation in leaf veins and often precedes substantial loss of hydraulic conductivity (Brodribb & Holbrook 2003). Minimum values of P_a for older growth regions were above those recorded for petioles and leaf veins, indicating that the average P_a of petioles and leaf veins is likely to be lower than in branches and the trunk. This agrees with the results of Salleo *et al.* (2000, 2001) and Brodribb & Holbrook (2003), which showed that cavitation was initiated at higher xylem tensions in stems than in leaves.

The advantage that plants gain by having lower cavitation resistances in more distal regions lies in the protection of organs which represent a greater investment in metabolic energy, such as the trunk. In conditions of drought-stress the leaf water potential will decrease as evaporative flux increases. A model presented by Tyree & Sperry (1988) showed that 'runaway' embolism has the potential to reduce leaf area by up to 60% in *Acer saccharum*, but that this loss of leaf area would result in decreased water-stress in surviving branches because of the reduced transpirational demand. Thus, the loss of leaves and small twigs is predicted to improve the water status of larger branches and the trunk. In reality, excessive transpiration is usually limited by stomatal closure before catastrophic embolism can occur (Salleo *et al.* 2000, 2001). However, the lower P_a in leaf veins and petioles of *A. saccharum* may act to initiate stomatal closure before substantial cavitation can occur in branches and the trunk (Brodribb & Holbrook 2003).

Xylem anatomy and air seeding threshold

Within the shoot, a positive trend was observed between mean vessel diameter and P_a . However, measurements in 2004 indicated that the lower P_a of 1-year-old branches was caused by the presence of primary xylem conduits. In 1-

year-old branches, petioles and leaf veins a large proportion of the xylem tissue is generated by extension growth and thus many of the xylem conduits have only partial secondary wall thickening. Primary xylem represents a continuum of secondary wall thickening, progressing from the first formed conduits (protoxylem), which have highly extensible helical or annular thickening, to later formed primary xylem (metaxylem), possessing scalariform or pitted secondary walls (Esau 1977). Vessels of the secondary xylem are produced after extension growth has been completed and always have pitted secondary walls.

The results of experiments differentiating between P_a of primary and secondary xylem conduits show that there was not a simple relationship between vessel diameter and P_a , but rather suggest developmental effects relating to the wall structure of the xylem conduits. Indeed, the P_a of secondary vessels in 1-year-old branches was not lower than P_a of older branches and the trunk despite having a smaller mean diameter. Consistent with data reported by Melcher *et al.* (2003), there was little difference in the P_a of current year xylem vessels from the stem when only measurements on secondary vessels were considered. It is important to note that in the present study only earlywood vessels were examined within the secondary xylem. Previous studies have demonstrated that within one growth ring, wide earlywood vessels are more vulnerable to cavitation than narrow latewood vessels (Lo Gullo & Salleo 1993; Lo Gullo *et al.* 1995). This should not be confused with the comparisons of cavitation threshold made in our study, which were primarily between different age regions of the tree.

Although root xylem vessels had pitted secondary walls they had lower P_a than other regions in which only secondary xylem vessels were measured. This finding is consistent with many other studies which have demonstrated that for a given species, xylem vessels in roots are more vulnerable to cavitation than those found in stems and branches (e.g. Alder *et al.* 1996; Kavanagh *et al.* 1999; Kolb & Sperry 1999). According to the air seeding hypothesis, differences in vulnerability to cavitation are related to the porosity of intervessel pit membranes (Zimmermann 1983). Higher vulnerability to cavitation in roots of *Acer grandidentatum* was associated with greater porosity of pit membranes in root xylem compared with stem xylem (Alder *et al.* 1996). Whether a difference in the structure of root and stem pit membranes exists in *A. saccharum* remains to be verified.

The question remains as to why primary xylem conduits had lower P_a than secondary xylem vessels. The air seeding hypothesis dictates that more vulnerable conduits should have wider pit membrane pores. The results of Choat *et al.* (2003) indicate that the distribution of pore sizes in pit membranes of hardwood trees may vary between membranes such that rare large pores responsible for air seeding may only occur in a few of the thousands of pit membranes connecting two vessels. Conduits with partial secondary thickening have a greater area of primary cell wall (pit membrane) exposed than conduits with pitted secondary walls. If the statistical probability of a large pore occurring between two vessels increases with the total

exposed area of primary cell wall (pit membrane), then primary xylem conduits should be significantly more vulnerable to cavitation than vessels with pitted secondary walls. It is also possible that the lack of support from secondary walls would make pit membranes in primary xylem conduits more prone to stretching or rupture. Deflection and stretching of pit membranes in response to large pressure differences that develop between embolized and functional conduits has recently been shown to influence P_a in angiosperm tree species by altering membrane porosity (Choat *et al.* 2004).

In *Acer rubrum* seedlings, primary conduits within the stem appear to lack the ability to refill following cavitation (unpublished results, Zwieniecki and Holbrook). It is possible that these conduits are active in water transport only up to the time xylem tensions sufficient to cause cavitation are first encountered in the growing season, at which point they become permanently gas filled and all further water transport requirements are fulfilled by secondary xylem vessels. Thus, differences in vulnerability to cavitation between different age regions of the plant may be reduced later in the growing season. In leaf veins and petioles, which have a relatively high proportion of primary xylem, the continued function of primary xylem conduits may be of greater importance, but it appears that plants have substantial ability to repair cavitated conduits in these organs (Zwieniecki *et al.* 2000; Bucci *et al.* 2003; Brodribb & Holbrook 2004). The contribution of primary xylem to flow and differences in P_a between primary and secondary xylem should be carefully considered when generating vulnerability curves in tissue from the current year of growth.

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REFERENCES

- Alder N.N., Sperry J.S. & Pockman W.T. (1996) Root and stem xylem embolism, stomatal conductance, and leaf turgor in *Acer grandidentatum* populations along a soil moisture gradient. *Oecologia* **105**, 293–301.
- Aloni R. & Zimmermann M.H. (1983) The control of vessel size and density along the plant axis – a new hypothesis. *Differentiation* **24**, 203–208.
- Brodribb T.J. & Holbrook N.M. (2003) Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiology* **132**, 2166–2173.
- Brodribb T.J. & Holbrook N.M. (2004) Diurnal depression of leaf hydraulic conductance in a tropical tree species. *Plant, Cell and Environment* **27**, 820–827.
- Bucci S.J., Scholz F.G., Goldstein G., Meinzer F.C. & Sternberg L.D.L. (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 and Environment* **26**, 1633–1645.
- Choat B., Ball M., Luly J. & Holtum J. (2003) Pit membrane porosity and water stress-induced cavitation in four co-existing dry rainforest tree species. *Plant Physiology* **131**, 41–48.
- Choat B., Jansen S., Zwieniecki M.A., Smets E. & Holbrook N.M. (2004) Changes in pit membrane porosity due to deflection and stretching: the role of vested pits. *Journal of Experimental Botany* **55**, 1569–1575.
- Dixon H.H. & Joly J. (1895) On the ascent of sap. *Philosophical Transactions of the Royal Society of London B* **186**, 563–576.
- Domec J.C. & Gartner B.L. (2002) Age- and position-related changes in hydraulic versus mechanical dysfunction of xylem: inferring the design criteria for Douglas-fir wood structure. *Tree Physiology* **22**, 91–104.
- Domec J.C. & Gartner B.L. (2001) Cavitation and water storage capacity in bole xylem segments of mature and young Douglas-fir trees. *Trees-Structure and Function* **15**, 204–214.
- Esau K. (1977) *Anatomy of Seed Plants*. Wiley, New York.
- Hacke U. & Sauter J.J. (1996) Drought-induced xylem dysfunction in petioles, branches, and roots of *Populus balsamifera* L and *Alnus glutinosa* (L) Gaertn. *Plant Physiology* **111**, 413–417.
- Hargrave K.R., Kolb K.J., Ewers F.W. & Davis S.D. (1994) Conduit diameter and drought-induced embolism in *Salvia mellifera* Greene (Labiatae). *New Phytologist* **126**, 695–705.
- Kavanagh K.L., Bond B.J., Aitken S.N., Gartner B.L. & Knowe S. (1999) Shoot and root vulnerability to xylem cavitation in four populations of Douglas-fir seedlings. *Tree Physiology* **19**, 31–37.
- Kolb K.J. & Sperry J.S. (1999) Transport constraints on water use by the Great Basin shrub, *Artemisia tridentata*. *Plant, Cell and Environment* **22**, 925–935.
- Lo Gullo M.A. & Salleo S. (1991) Three different methods for measuring xylem cavitation and embolism – a comparison. *Annals of Botany* **67**, 417–424.
- Lo Gullo M.A. & Salleo S. (1993) Different vulnerabilities of *Quercus Ilex* L to freeze-induced and summer drought-induced xylem embolism – an ecological interpretation. *Plant, Cell and Environment* **16**, 511–519.
- Lo Gullo M.A., Salleo S., Piaceri E.C. & Rosso R. (1995) Relations between vulnerability to xylem embolism and xylem conduit dimensions in young trees of *Quercus cerris*. *Plant, Cell and Environment* **18**, 661–669.
- Melcher P.J., Zwieniecki M.A. & Holbrook N.M. (2003) Vulnerability of xylem vessels to cavitation in sugar maple. Scaling from individual vessels to whole branches. *Plant Physiology* **131**, 1775–1780.
- Roberts N.W., Gahan P.B. & Aloni R. (1988) *Vascular Differentiation and Plant Growth Regulators*. Springer-Verlag, New York, USA.
- Rood S.B., Patino S., Coombs K. & Tyree M.T. (2000) Branch sacrifice: cavitation-associated drought adaptation of riparian cottonwoods. *Trees – Structure and Function* **14**, 248–257.
- Sack L., Cowan P.D., Jaikumar N. & Holbrook N.M. (2003) The ‘hydrology’ of leaves: co-ordination of structure and function in temperate woody species. *Plant, Cell and Environment* **26**, 1343–1356.
- Salleo S., Lo Gullo M.A., Raimondo F. & Nardini A. (2001) Vulnerability to cavitation of leaf minor veins: any impact on leaf gas exchange? *Plant, Cell and Environment* **24**, 851–859.
- Salleo S., Nardini A., Pitt F. & Lo Gullo M.A. (2000) Xylem cavitation and hydraulic control of stomatal conductance in Laurel (*Laurus nobilis* L.). *Plant, Cell and Environment* **23**, 71–79.
- Sperry J.S. & Pockman W.T. (1993) Limitation of transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*. *Plant, Cell and Environment* **16**, 279–287.

- Sperry J.S. & Saliendra N.Z. (1994) Intra-plant and inter-plant variation in xylem cavitation in *Betula occidentalis*. *Plant, Cell and Environment* **17**, 1233–1241.
- Sperry J.S., Perry A.H. & Sullivan J.E.M. (1991) Pit membrane degradation and air-embolism formation in aging xylem vessels of *Populus tremuloides* Michx. *Journal of Experimental Botany* **42**, 1399–1406.
- Tsuda M. & Tyree M.T. (1997) Whole-plant hydraulic resistance and vulnerability segmentation in *Acer saccharinum*. *Tree Physiology* **17**, 351–357.
- Tyree M.T. & Sperry J.S. (1988) Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water-stress – answers from a model. *Plant Physiology* **88**, 574–580.
- Tyree M.T. & Zimmermann M.H. (2002) *Xylem Structure and the Ascent of Sap*. Springer-Verlag, New York, USA.
- Tyree M.T., Cochard H., Cruiziat P., Sinclair B. & Ameglio T. (1993) Drought-induced leaf shedding in walnut – evidence for vulnerability segmentation. *Plant, Cell and Environment* **16**, 879–882.
- Yang S.D. & Tyree M.T. (1993) Hydraulic resistance in *Acer saccharum* shoots and its influence on leaf water potential and transpiration. *Tree Physiology* **12**, 231–242.
- Zimmermann M.H. (1983) *Xylem Structure and the Ascent of Sap*. Springer-Verlag, New York, USA.
- Zimmermann M.H. & Jeje A.A. (1981) Vessel length distribution in stems of some American woody plants. *Canadian Journal of Botany – Revue Canadienne de Botanique* **59**, 1882–1892.
- Zwieniecki M.A., Hutyra L., Thompson M.V. & Holbrook N.M. (2000) Dynamic changes in petiole specific conductivity in red maple (*Acer rubrum* L.), tulip tree (*Liriodendron tulipifera* L.) and northern fox grape (*Vitis labrusca* L.). *Plant, Cell and Environment* **23**, 407–414.
- Zwieniecki M.A., Melcher P.J. & Holbrook N.M. (2001) Hydraulic properties of individual xylem vessels of *Fraxinus americana*. *Journal of Experimental Botany* **52**, 257–264.

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