

THE RELATIONSHIP BETWEEN XYLEM CONDUIT DIAMETER AND CAVITATION CAUSED BY FREEZING¹

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The centrifuge method for measuring the resistance of xylem to cavitation by water stress was modified to also account for any additional cavitation that might occur from a freeze-thaw cycle. A strong correlation was found between cavitation by freezing and mean conduit diameter. On the one extreme, a tracheid-bearing conifer and diffuse-porous angiosperms with small-diameter vessels (mean diameter <30 μm) showed no freezing-induced cavitation under modest water stress (xylem pressure = -0.5 MPa), whereas species with larger diameter vessels (mean >40 μm) were nearly completely cavitared under the same conditions. Species with intermediate mean diameters (30–40 μm) showed partial cavitation by freezing. These results are consistent with a critical diameter of 44 μm at or above which cavitation would occur by a freeze-thaw cycle at -0.5 MPa. As expected, vulnerability to cavitation by freezing was correlated with the hydraulic conductivity per stem transverse area. The results confirm and extend previous reports that small-diameter conduits are relatively resistant to cavitation by freezing. It appears that the centrifuge method, modified to include freeze-thaw cycles, may be useful in separating the interactive effects of xylem pressure and freezing on cavitation.

Key words: cavitation; embolism; freezing stress; hydraulic conductivity; water transport; wood anatomy; xylem structure and function.

Woody plants of temperate climates must survive sub-freezing temperature. This stress is especially problematic if freezing occurs during the growing season when the plant is physiologically active. While supercooling can be an important freezing avoidance mechanism in some circumstances (Rada et al., 1987), freezing generally occurs in the extracellular space (apoplast) within a few degrees below 0°C . Apoplastic freezing can disrupt symplastic function by causing severe cellular dehydration and physical damage, and adaptations that minimize these problems have been the subject of considerable study (Sakai and Larcher, 1987). Apoplastic freezing can also disrupt the vital process of xylem transport (Zimmermann, 1983). Adaptation to this latter stress is the subject of this paper.

The problem begins with dissolved gases in the xylem sap. Freezing the sap forces these gases out of solution to form bubbles in the ice. On thawing, these bubbles can either dissolve back into the xylem sap or they can grow to obstruct the entire xylem conduit, a process called "cavitation." Cavitation is followed by embolism, wherein the largely vapor-filled conduit becomes filled with air at atmospheric pressure as gases diffuse from surrounding tissue. Whether a bubble dissolves or nucleates, cavitation depends on its internal pressure (P_b), which is a function of its radius of curvature (R), the surface tension of the xylem sap (T), and the xylem sap pressure (P_x ; all pressures relative to atmospheric):

$$P_b = 2T/R + P_x \text{ (for } P_b \geq \text{ pure vacuum).} \quad (1)$$

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The bubble will consist at least in part of water vapor, so P_b must at a minimum exceed the vapor pressure of water (P_{wv}) for the bubble to dissolve (how much greater depends on the air content of the bubble and the surrounding sap; Yang and Tyree, 1992). Therefore, cavitation will unequivocally occur for $P_x \leq P_{wv} - (2T/R)$, where P_x is the xylem pressure following the thaw.

It follows that susceptibility to freezing-induced cavitation should increase for more negative P_x and larger R . The magnitude of P_x depends in part on the transpiration rate and soil water potential during the freeze-thaw event. The R will depend at least in part on the size of the xylem conduit. The larger the conduit, the more air will be frozen out of solution and the larger the bubbles should be in the frozen conduit. Considerable evidence confirms that larger xylem vessels are more vulnerable to cavitation by freezing than smaller vessels and tracheids (Hammel, 1967; Sucoff, 1969; Ewers, 1985; Sperry and Sullivan, 1992; LoGullo and Salleo, 1993; Hacke and Sauter, 1996; Langan, Ewers, and Davis, 1997). Conifers actually suffer no freezing-induced cavitation unless P_x becomes very negative (i.e., < -5 MPa) during the freeze (Sperry and Sullivan, 1992). This presumably lengthens their growing season, allowing them to retain evergreen foliage and continue gas exchange despite experiencing frequent frosts.

Why do not all temperate plants have small enough vessels or tracheids so they can avoid the problem of cavitation by freezing? One obvious reason is that smaller conduits are less efficient in water transport and would tend to support lower rates of gas exchange for a given stem diameter. Rather than avoid freezing-induced cavitation with a concomitant loss in conducting efficiency, many plants cavitate extensively during freezing weather. These plants tend to be winter deciduous and restore transport after freezing by growing new conduits or refilling cavitared ones by increasing P_x to near or above

Table 1. Study species and sites. Species are listed in order of ascending mean conduit diameter.

| Species | Abb./Site | Family/growth form | Phenology | Wood type |
|---------------------------------------|-----------|------------------------|-----------|----------------|
| <i>Abies lasiocarpa</i> (Hook.) Nutt. | Al/G | Pinaceae, tree | evergreen | coniferous |
| <i>Euonymus kiautschovicus</i> Loes. | Ek/U | Celastraceae, shrub | evergreen | diffuse-porous |
| <i>Acer grandidentatum</i> Nutt. | Ag/R | Aceraceae, tree | deciduous | diffuse-porous |
| <i>Prunus virginiana</i> L. | Pv/R | Rosaceae, tree | deciduous | diffuse-porous |
| <i>Alnus incana</i> (L.) Moench | Ai/R | Betulaceae, small tree | deciduous | diffuse-porous |
| <i>Betula occidentalis</i> Hook. | Bo/R | Betulaceae, tree | deciduous | diffuse-porous |
| <i>Acer negundo</i> L. | An/U | Aceraceae, tree | deciduous | diffuse-porous |
| <i>Cornus sericea</i> L. | Cs/R | Cornaceae, shrub | deciduous | diffuse-porous |
| <i>Hedera helix</i> L. | Hh/U | Araliaceae, vine | evergreen | vine |
| <i>Rhus aromatica</i> Ait. | Ra/U | Anacardiaceae, shrub | deciduous | ring-porous |
| <i>Eleagnus angustifolia</i> L. | Ea/U | Eleagnaceae, tree | deciduous | ring-porous |
| <i>Quercus gambelii</i> Nutt. | Qg/U | Fagaceae, tree | deciduous | ring-porous |

Note: U = University of Utah campus in Salt Lake City, Utah; R = Red Butte Canyon Natural Area near Salt Lake City, Utah (~ 111°47' W, 40°47' N; elevation 1770 m); G = Guardsman's pass near Brighton Utah (~ 111°35' W, 40°36' N elevation 2825 m). Abbreviations (Abb.) are those used in Fig. 2.

atmospheric via root pressure and other means (Ewers, 1985; Sperry and Sullivan, 1992; Sperry et al., 1994; Hacke and Sauter, 1996). A cost of the deciduous "strategy" is a shortened growing season, with the later bud-break in the spring occurring in the progressively larger vesseled species (Wang, Ives, and Lechowicz, 1992).

The susceptibility to cavitation by freezing provides a hypothetical link between xylem structure, vegetative phenology, plant distribution with respect to frost, gas exchange capability, and refilling capability. Unfortunately, our ability to test these hypotheses has been limited by the laborious procedures required to quantify the resistance of xylem to freezing-induced cavitation. The purpose of this paper is to introduce a simple procedure based on the centrifugal force technique for measuring water-stress induced cavitation (Pockman, Sperry, and O'Leary, 1995; Alder et al., 1997). We use this method to develop an empirical relationship between conduit diameter and resistance to cavitation by a freeze-thaw event.

MATERIALS AND METHODS

Plant material—Branches were collected from plants growing on the campus of the University of Utah in Salt Lake City or neighboring natural areas (Table 1). Material was collected from fall through early summer of 1996–1997. Twelve species were collected (Table 1). Branches were cut from plants, sealed in plastic bags, and brought to the laboratory. Segments 260 mm long and ~7–12 mm diameter were cut from the branches under water in the laboratory. Segments were either unbranched or had only minor side branches and were relatively straight so they could fit in a custom-designed centrifuge rotor.

Cavitation and hydraulic conductivity measurements—To standardize between segments of differing conductivity, we expressed the loss of conductivity from cavitation as a percentage decrease from the maximum value. We accomplished this by measuring the maximum hydraulic conductivity of stem segments before applying stress, then immediately after a stress treatment (negative pressure plus a freeze-thaw event) we measured hydraulic conductivity a second time.

Hydraulic conductivity was calculated as the flow rate of measuring fluid through the stem per pressure gradient. Branch segments were mounted in a tubing apparatus, and flow rate was measured gravimetrically under a hydraulic pressure head. In coniferous and diffuse-porous trees we used pressure heads of ~5–10 kPa. In ring-porous trees, these pressures were sufficient to displace air from gas-filled vessels that were

longer than the segment, causing immediate refilling. For segments of these species we used pressures below 2.5 kPa, which avoided the refilling problem.

To improve the accuracy of the conductivity measurement, we determined the flow rate in the absence of an applied pressure head before and after measuring the pressure-induced flow for each segment. The zero-pressure "background" flow was averaged and subtracted from the pressurized flow to obtain the net flow under pressure, which was used in the conductivity calculation. We used purified and filtered (0.22 μm) water as our measuring solution. In earlier work we have used various acids to inhibit microbial growth in the tubing. We now use frequent bleaching of the apparatus to control microbial growth.

For the non-ring-porous species, we flushed the stems with water at ~50 kPa pressure for ~30 min to reverse any native embolism and insure that the initial conductivity measurement reflected the normal maximum conductivity of the segment. We did not flush ring-porous trees because that caused refilling of multiple years of earlywood vessels that would never be functional in situ (cf. Sperry, Perry, and Sullivan, 1991; Sperry and Sullivan, 1992; Sperry et al., 1994). To avoid inducing embolism while harvesting branches from the long-vesseled ring-porous species, we cut them from the parent plant under water using a split funnel sealed around the stem. Ring-porous species were collected in early summer when their native conductivity would be near their maximum value.

For each segment we estimated the "specific conductivity" of the branch from the maximum (flushed) conductivity divided by the stem transverse area. Ring-porous material was flushed for these measurements. Stem area was estimated from stem diameter and included pith and bark tissue. A more accurate determination of specific conductivity could be obtained by staining the sapwood, allowing a precise measurement of the sapwood transverse area.

Centrifuge experiments—Segments were mounted in custom-designed centrifuge rotors designed to fit either IEC or Sorvall centrifuges (IEC model B20A, Needham Heights, Massachusetts; Sorvall Model RC-5C, Kendro Laboratory Products, Newton, Connecticut). Plans for these rotors are available from the second author on request. The center of each segment was held at the axis of rotation, and the ends were immersed in water during rotation. The negative pressure in the xylem is a function of the segment length and the angular velocity of the rotor (Alder et al., 1997).

In control experiments, segments were held at a constant negative pressure (constant angular velocity) for ~5 min in the centrifuge, removed, and the loss of conductivity caused by the pressure was determined. Previous work has shown that the cavitation response is saturated by 2 min of spinning at a given pressure and does not change after 4 h (Pockman, Sperry, and O'Leary, 1995; Alder et al., 1997). In

some experiments, single segments were respun to allow a stepwise increase in negative pressures so that an entire “water stress vulnerability curve” was obtained, which showed the complete relationship between xylem pressure and loss of conductivity for that segment. The water stress vulnerability curve for *Abies lasiocarpa* was taken from Alder et al. (1997) for material collected at the same site (Table 1) and measured by similar methods.

In freezing experiments, segments were held at negative pressure for 3 h while undergoing a controlled freeze–thaw cycle. As in controls, the cavitation caused by the combined negative pressure and freeze–thaw treatment was estimated from the loss of hydraulic conductivity. Unlike controls, vulnerability curves were not made by reusing the same segments and spinning to progressively more negative pressures. Instead, separate segments were used at each test pressure. This was done to avoid the possibility that a freeze–thaw cycle would alter the subsequent vulnerability of the segments to cavitation by water stress. Centrifuge temperatures were regulated in part by using the temperature controls of the centrifuge itself. In addition, the centrifuge chamber was lined with copper tubing through which methanol was circulated from an external temperature bath (model 1157, VWR Scientific; model Lt-50, Neslab Instrument Inc., Portsmouth, New Hampshire). Vulnerabilities of control (nonfrozen) and frozen stems were compared with a Student’s *t* test at each pressure.

Preliminary experiments monitored the rate of cooling and warming, minimum temperature, and freezing temperature in spinning stems during the 3-h freeze–thaw protocol. We used a battery-operated device to log temperature in the stem xylem at the center of rotation (HOBO HTEA-37+46, Onset Computer Corp., Pocasset, Massachusetts). We settled on a protocol that gave a 0.25°C/min cooling rate to a minimum temperature of –12°C to –15°C. Exotherms were observed between –2°C and –6°C. Thawing occurred at 0.45°C/min. In this protocol, the centrifuge chamber and nonspinning rotor was precooled to ~4°C while the circulating bath temperature was set to –4°C. Stems were inserted and allowed to equilibrate for 10 min. The rotor was spun at the desired angular velocity, while the temperature on the circulating bath was set to –20°C for 1 h and 30 min. To warm the stems, the centrifuge and bath were set to 10°C.

Xylem conduit diameter—For each species, six of the segments used in the cavitation experiments were also used for measurements of conduit diameter. A transverse section was cut at the midpoint of the segment, and all the conduits were measured in at least three radial sectors of the most recent growth ring. Sectors were located randomly around the stem circumference. Diameter measurements were made by tracing the inner perimeter of the conduit lumen using a camera lucida and bit pad arrangement (Microplan II, Donsanto, Inc., Natuck, Massachusetts). Diameters were calculated for the circle of the same area as the conduit lumen. Means for each segment were calculated (*N* > 100 conduits), and a grand mean was determined for each species (*N* = 6 segment means).

We also calculated the mean conduit diameter on the basis of each conduit’s contribution to hydraulic conductivity. According to the Hagen–Poiseuille relationship, a conduit’s hydraulic conductivity is proportional to its diameter to the fourth power. Therefore, we took each conduit diameter (*d*) and multiplied it by its diameter to the fourth power (*d*⁴) to represent the conduit in proportion to its contribution to conductivity. The mean of this hydraulically weighted diameter distribution was the sum of all conduit diameters ($\sum d^5$) divided by the total number of conduits ($\sum d^4$; Sperry et al., 1994).

RESULTS

Freeze–thaw and control vulnerability curves were determined for three species of contrasting phenology and xylem conduit diameter (Fig. 1): a conifer (*Abies lasiocarpa*, tracheid diameter = 11.8 ± 1.48 μm; mean ± SE,

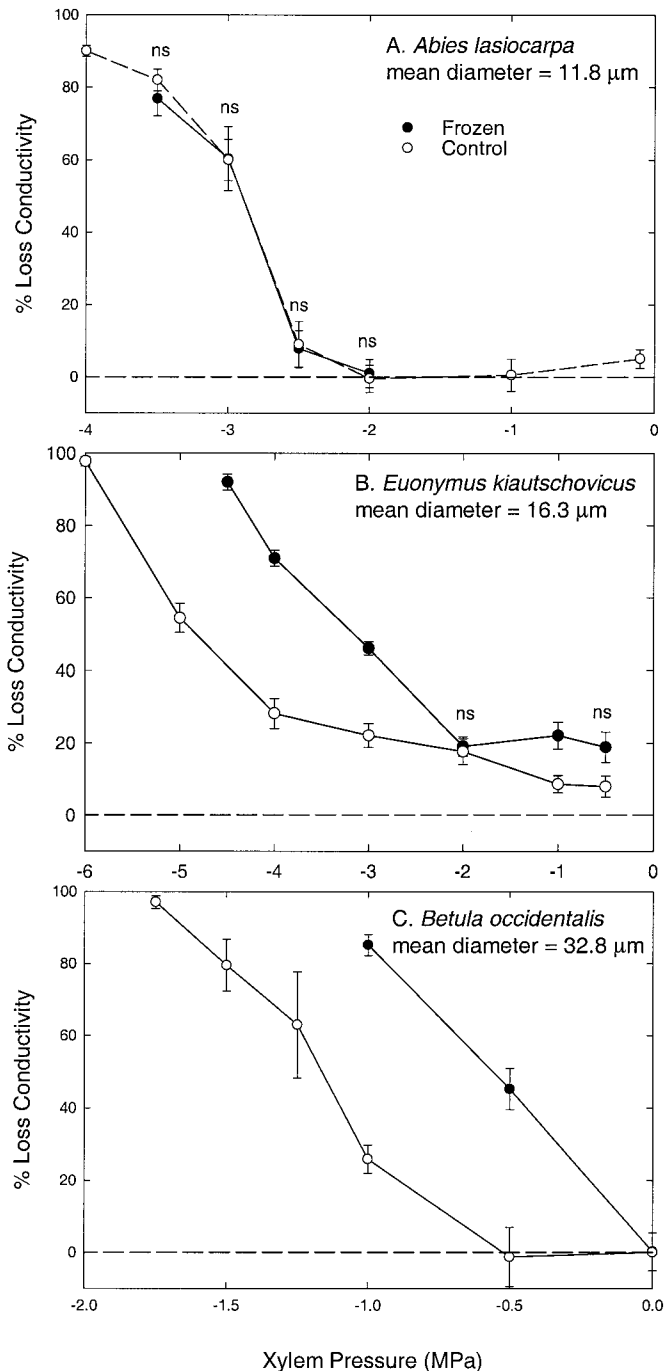


Fig. 1. Vulnerability curves showing percentage loss of conductivity for frozen (solid symbols) and control (open symbols) stems vs. xylem pressure for (A) *Abies lasiocarpa*, (B) *Euonymus kiautschovicus* and (C) *Betula occidentalis*. Mean conduit diameter is indicated. Error bars are SE, *N* = 8 except for frozen *Abies lasiocarpa* where *N* = 4. Note difference of scale on xylem pressure axes. The “ns” indicates no difference between control and frozen stems based on a *t* test at 0.05 significance level.

N = 6), an evergreen angiosperm (*Euonymus kiautschovicus*, vessel diameter 16.3 ± 1.02 μm), and a diffuse-porous deciduous angiosperm (*Betula occidentalis*, vessel diameter 32.8 ± 1.55 μm). Results indicated increasing vulnerability to cavitation by freezing with increasing

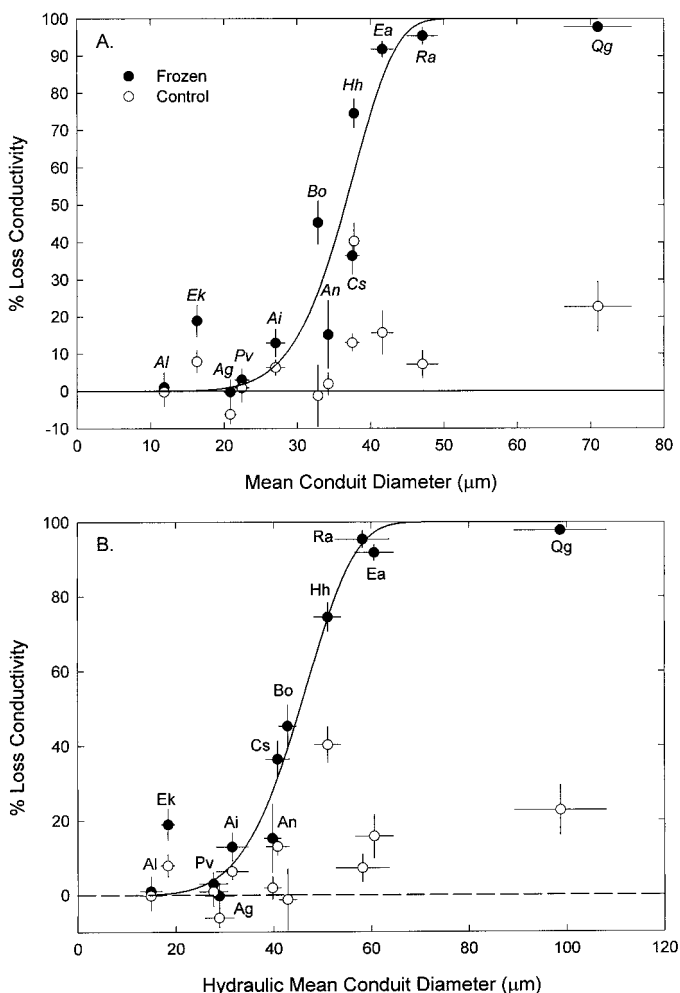


Fig. 2. Percentage loss of conductivity for frozen (solid circle) and control (open circle) stems vs. (A) mean conduit diameter and vs. (B) mean hydraulic diameter. Error bars are SE for $N = 6-8$. Curve through frozen data points is a Weibull function fit for visualizing the trend. Letters refer to species abbreviations as listed in Table 1.

conduit diameter. Freezing caused no additional loss of conductivity relative to water stress controls in *Abies lasiocarpa* over the entire range of pressures (Fig. 1A). In *Euonymus kiautschovicus*, freezing did not cause a significant increase in loss of conductivity by freezing at -0.5 or -2 MPa but a slight increase at -1.0 MPa. Only at pressures of -3 MPa and below did freezing cause a large loss of conductivity relative to controls (Fig. 1B). In *Betula occidentalis*, freezing increased the loss of conductivity over controls at every pressure examined below 0 MPa (Fig. 1C).

To conduct the survey of the 12 species in Table 1, we compared the loss of conductivity in control vs. frozen stems at a single test pressure. We chose -0.5 MPa as the test pressure because this was previously determined for some of the species examined to be a conservative estimate for the negative pressure that xylem would be experiencing during a freeze-thaw cycle in nature (Sperry and Sullivan, 1992; Sperry et al., 1994). Nonfrozen controls showed an average of 9.2% loss of conductivity at -0.5 MPa with no trend related to conduit diameter (Fig.

2, open circles). Frozen stems showed a sharp rise in loss of conductivity from an average of 7.2% in species with conduits narrower than $30 \mu\text{m}$ to 95% for species with conduit diameters above $40 \mu\text{m}$ (Fig. 2A, solid circles). Results using the hydraulically weighted conduit diameter were qualitatively similar, but shifted to a larger diameter range and with a less abrupt rise in the loss of conductivity with diameter (Fig. 2B).

Equation 1 predicts that all conduits over a given "Cavitation diameter" will cavitate during the freeze-thaw cycle, assuming that the bubble radius R is proportional to conduit diameter. By first approximation the cavitation diameter should be near $45 \mu\text{m}$ because according to Fig. 2B this is the mean hydraulic diameter associated with 50% loss of conductivity. At this point, half of the vessels (= half of the conductivity according to the weighted diameter distribution) are above the cavitation diameter (= hydraulic mean) and are nonfunctional. This assumes that the conduit diameter distribution is symmetric (mean = median).

A more precise estimate of the cavitation diameter was obtained from the original conduit diameter distributions. If we assume that the conductivity of the segment is proportional to the sum of the conduit diameters raised to the fourth power, we can calculate the percentage loss of conductivity associated with any arbitrarily chosen cavitation diameter (d_c) as:

$$\% \text{ loss of conductivity} = 100 (1 - \frac{\sum (d \geq d_c)^4}{\sum d^4}) \quad (2)$$

where $\sum (d \geq d_c)^4$ is the sum of diameters greater than or equal to the cavitation diameter raised to the fourth power, and $\sum d^4$ is the sum of all the diameters raised to the fourth power. The predicted cavitation diameter is the one given the best fit to the empirical data in Fig. 2A. As seen in Fig. 3, the best fit ($r^2 = 0.96$) was achieved for a cavitation diameter of $44 \mu\text{m}$. This analysis assumed that the only cause of a loss of conductivity in the experiment was cavitation by freezing (i.e., no cavitation caused by the -0.5 MPa pressure in the absence of a freeze-thaw cycle). The data point showing the largest deviation from prediction was *Euonymus kiautschovicus* where the measured percentage loss of conductivity was greater than the predicted value of 0%, perhaps owing to limited cavitation by water stress at the -0.5 MPa pressure.

The specific conductivity (maximum conductivity divided by stem transverse area) of the 12 species showed a positive correlation with their vulnerability to cavitation by freezing as represented by the percentage loss of conductivity by freezing at -0.5 MPa (Fig. 4).

DISCUSSION

The results indicated an abrupt $30\text{-}\mu\text{m}$ threshold in mean conduit diameter above which plants were extremely sensitive to cavitation by freezing even for a modest xylem pressure of -0.5 MPa (Fig. 2A). A plant with a mean conduit diameter over $40 \mu\text{m}$ would be $<95\%$ cavitated after a single freeze-thaw cycle. In terms of the hydraulically weighted conduit distributions, the corresponding mean threshold diameter was $35 \mu\text{m}$, with near total cavitation for means above $55 \mu\text{m}$ (Fig. 2B). These results were consistent with a predicted cavitation diam-

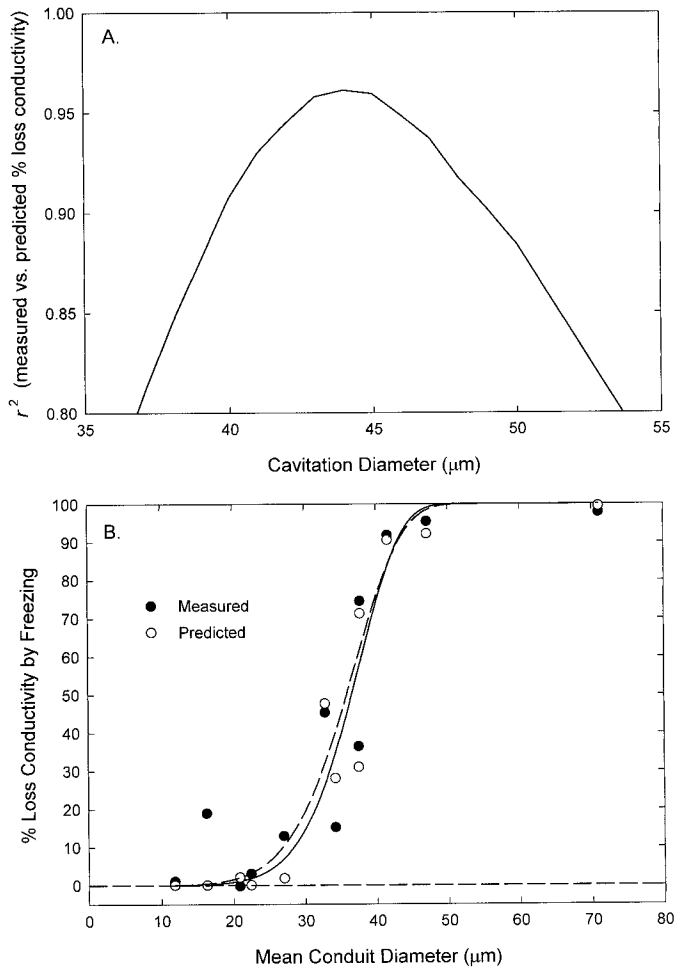


Fig. 3. (A) The r^2 for the correlation between predicted and measured loss of conductivity by freezing for different cavitation diameters. All vessels with diameters equal to or greater than the cavitation diameter were assumed to cavitate during a freeze-thaw cycle at -0.5 MPa. Maximum correlation ($r^2 = 0.96$) was associated with a cavitation diameter of $44 \mu\text{m}$. (B) The measured (solid circles and line) or predicted (open circles, dashed line) percentage loss of conductivity by freezing vs. mean conduit diameter. Predicted values were based on a cavitation diameter of $44 \mu\text{m}$. Line through the data is a Weibull function fit for visualizing the trend (solid line, measured data; dashed line, predicted values).

eter of $44 \mu\text{m}$ for xylem conduits undergoing a freeze-thaw cycle at $P_x = -0.5$ MPa (Eq. 2). All conduits greater than or equal to this diameter would be expected to cavitate, while all less than this diameter would remain functional. From Eq. 1, the bubble radius (R) nucleating cavitation in vessels $\geq 44 \mu\text{m}$ for $P_x = -0.5$ MPa would be $R \geq 0.36 \mu\text{m}$.

A priori, we expected the centrifuge method to overestimate the cavitation associated with a freeze-thaw cycle compared to experiments employing more natural conditions. In a non-transpiring intact plant or branch, the pressure in the liquid phase should increase while ice is forming because of the expansion of ice (Robson and Petty, 1987). If this increased pressure is also present during the thawing phase, bubbles could dissolve more rapidly than otherwise and be smaller (and more resistant to cavitation) when pressures dropped as ice completely

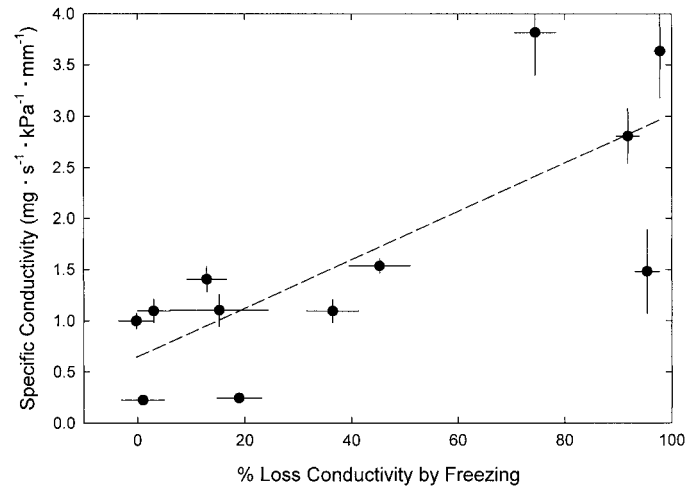


Fig. 4. Specific conductivity (maximum conductivity per stem transverse area) vs. percentage loss of conductivity by freezing for the species in Fig. 2. The $r^2 = 0.61$ ($P < 0.01$). Error bars are standard errors for $N = 6-8$.

melted. In contrast, in the centrifuge experiments the liquid water should remain under a constant negative pressure during the freezing and thawing process, which would favor bubble expansion and cavitation.

In fact, the centrifuge results were very similar to results obtained by freezing and thawing branches under more natural conditions. We purposely chose three species examined earlier by Sperry and Sullivan (1992) for the sake of comparison. Our results showing no freezing-induced cavitation in the conifer *Abies lasiocarpa* (Fig. 1A), intermediate cavitation in *Betula occidentalis* (Fig. 1C), and nearly complete cavitation in *Quercus gambelii* (Fig. 2) are nearly identical to their findings (their Fig. 3). Our results are also consistent with those of Langan, Ewers, and Davis (1997) on two chaparral shrubs. They reported $\sim 90\%$ loss of conductivity during gradual freeze-thaw cycles in *Rhus laurina* at approximately -0.5 MPa. The mean vessel diameter in this species was $40 \mu\text{m}$ (maximum = $80 \mu\text{m}$). In contrast, the smaller vesseled *Ceanothus megacarpus* (mean 21 , maximum $42 \mu\text{m}$) showed $\sim 35\%$ loss of conductivity while gradually frozen and thawed at approximately -1.2 MPa. This percentage would probably have been lower at the pressure of -0.5 MPa employed in our survey. Our prediction of $44 \mu\text{m}$ as the critical conduit diameter causing cavitation by freezing is also similar to Ewers' estimate of $49 \mu\text{m}$ based on observation of bubble formation in xylem conduits (Ewers, 1985).

Sperry and Sullivan (1992) argued that the determining factor for bubble size in thawing conduits was conduit volume rather than conduit diameter, because it is the volume of water that determines the volume of air frozen out of solution, and thus the size of the bubbles. However, if the freezing occurs in a centripetal direction from the conduit wall, the air will diffuse towards the center of the lumen and freeze out in a long narrow and possibly interrupted cylinder, similar to what has been observed in frozen conifer tracheids (Sucoff, 1969; Robson, McHardy, and Petty, 1988). On thawing, as these long bubbles round up, their radius of curvature could be rel-

atively independent of conduit length, at least for conduits longer than some minimum length. Whether volume or diameter is more important to freezing vulnerability could be resolved, perhaps, by repeating our experiment on tracheid-bearing species with significant numbers of tracheids over 44 μm in diameter. If diameter is the primary determining factor, they should fall on the trend line shown in Fig. 2. If volume is more important, they should show less cavitation than predicted from Fig. 2.

With the exception of *Hedera helix*, the other two evergreen species in our survey (Table 1) had sufficiently narrow conduits to avoid freezing-induced cavitation. *Hedera helix*, as a vine, presents an interesting situation. Although it had relatively large vessels, averaging 37 μm in diameter, they were not large enough to cause complete failure of water transport as has been reported for other temperate deciduous vines (Sperry et al., 1987). We would expect this species to either refill its cavitated vessels prior to the growing season, as in some other vines, or to simply replace them with new large-diameter conduits as in ring-porous trees (Hacke and Sauter, 1996). The three species with ring-porous wood exhibited severe loss of conductivity by freezing. They are all winter-deciduous species and tend to be the latest to leaf out in the spring (Sperry and Sullivan, 1992). Among the diffuse-porous species, the earliest to leaf out is *Prunus virginiana* (J. S. Sperry, personal observation). Consistent with this are its relatively narrow conduits and minimal loss of conductivity from freezing. Many of the larger diameter diffuse-porous species are known to exhibit root pressure in the spring, which in some cases has also been shown to refill cavitated conduits (*Alnus incana*, *Cornus sericea*, *Betula occidentalis*; Sperry and Sullivan, 1992; Sperry et al., 1994; J. S. Sperry, personal observation).

The centrifugal method shows promise for refining our understanding of freezing-induced cavitation in xylem. We now can put a provisional limit on the conduit diameter above which cavitation by freezing should occur under most field conditions. We predict that temperate-zone species with significant numbers of conduits over 44 μm have evolved mechanisms for coping with a potentially extensive loss of water transport capability caused by freezing events. These mechanisms would include timing of vegetative phenology and radial xylem growth, refilling capability, and protection of surfaces against water loss. Plants having all conduits narrower than 44 μm would not be constrained by freezing-induced cavitation (whether conifers or angiosperms), but would have less efficient xylem for conducting water during the growing season (Fig. 4). The centrifugal method should also help resolve effects of freezing and thawing rates (Langan, Ewers, and Davis, 1997), minimum freezing temperature (LoGullo and Salleo, 1993; Pockman and Sperry, 1997), multiple freeze-thaw cycles (Sperry et al., 1994; Utsumi et al., 1998) and possibly refilling capability.

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