Xylem Embolism in Response to Freeze-Thaw Cycles and Water Stress in Ring-Porous, Diffuse-Porous, and Conifer Species[1]

John S. Sperry* and June E. M. Sullivan

Department of Biology, University of Utah, Salt Lake City, Utah 84112

ABSTRACT

Vulnerability to xylem embolism by freeze-thaw cycles and water stress was quantified in ring-porous (*Quercus gambelii* Nutt.), diffuse-porous (*Populus tremuloides* Michx., *Betula occidentalis* Hook.), and conifer species (*Abies lasiocarpa* Nutt., *Juniperus scopulorum* Sarg.). Embolism was measured by its reduction of xylem hydraulic conductivity; it was induced by xylem tension (water-stress response) and by a tension plus a freeze-thaw cycle (freeze response). Conifers showed little (*Juniperus*) or no (*Abies*) freeze response even to repeated cycles. In contrast, *Quercus* embolized more than 90% by freezing at tensions below 0.2 MPa, whereas similar embolism without freezing required tensions above 4.5 MPa. Diffuse-porous trees (*Betula, Populus*) showed an intermediate freeze response. The magnitude of the freeze response was correlated with conduit volume but occurred at higher tensions than predicted from theory. Large early-wood vessels (2.8 × 10⁻⁹ m³) in oak were most vulnerable to embolism by freezing, small vessels in *Populus* and *Betula* were intermediate (approximately 7 × 10⁻¹¹ m³), and tracheids in conifers (about 3 × 10⁻¹³ m³) were most resistant. The same trend was found within a stem: embolism by freeze-thawing occurred preferentially in wider conduits. The water-stress response was not correlated with conduit volume; previous work indicates it is a function of interconduit pit membrane structure. Native embolism levels during winter corroborated laboratory results on freezing: *Quercus* embolized 95% with the first fall freeze, *Populus* and *Betula* showed gradual increases to more than 90% embolism by winter's end, and *Abies* remained below 30%.

Water in xylem conduits is usually under tension and vulnerable to cavitation—the breakage of the water column. The result of cavitation is an embolized conduit in which water has been displaced by water vapor and/or air. Embolism reduces the ability of xylem to conduct water and, if severe enough, will limit growth (13). In the present context, the terms cavitation and embolism are practically synonymous because of their causal association.

One cause of cavitation is high xylem tensions associated with water stress. The relationship between tension and cavitation has been documented for a number of species by measuring embolism in stems dehydrated to progressively higher tensions (see references in ref. 21). Species can vary considerably in their vulnerability to cavitation, with less vulnerable species tending to be more drought tolerant (21). The tension required to cause cavitation is a function of the permeability of the interconduit (intervessel and intertracheid) pits to an air-water interface. When air is pulled into functional conduits from neighboring embolized ones through shared pit membranes, it disrupts the cohesion of the water column causing cavitation (4, 17–19). This mechanism suggests that the size of the xylem conduit is not an important structural determinant of vulnerability to cavitation by water stress. This is contrary to the widely held assumption that larger conduits cavitate at lower tensions than smaller ones (1).

Cavitation is also caused by freezing and thawing of the xylem sap when it is under tension. Dissolved gases in the sap are insoluble in ice and freeze out as bubbles (7). If these bubbles are large enough when tension develops during thawing, they will grow and cause cavitation. The critical tension (T_{crit}, MPa) for bubble growth is an inverse function of bubble radius (r, m):

$$T_{crit} = 2\ t/r \qquad (1)$$

where t is the surface tension of water (7.28 × 10⁻⁸ MPa m at 0°C [8]). When cavitation is caused by freeze-thaw cycles, there is good reason to predict that larger volume conduits will be more vulnerable than small ones: the greater dissolved air content in larger conduits will freeze out to form larger bubbles that cause cavitation at lower tensions. Previous work supports the prediction of increasing vulnerability to cavitation by freeze-thaw events with increasing conduit dimensions (3, 5, 7), although much of this evidence is indirect and incomplete.

Seasonal studies indicate that most embolism in temperate woody plants occurs in response to freeze-thaw events during winter and during the growing season (14, 16). Adaptations minimizing and/or repairing this source of embolism may be important for the performance of these plants. To understand these adaptations, the vulnerability to cavitation by freeze-thaw events needs to be characterized and explained. There may be significant trade-offs that come with minimizing cavitation; for example, if small conduits are less vulnerable to cavitation during freeze-thaw cycles, they will probably also be less efficient in conduction and may support lower transpiration rates.

The purpose of the present study was to compare the embolism response to xylem tension with and without freeze-

thaw cycles in ring-porous, diffuse-porous, and coniferous trees. Embolism was measured in response to both water-stress and freeze-thaw treatments. By studying species representing a wide range of xylem structure, we could evaluate the relationship between cavitation vulnerability and conduit size for both treatments, as well as possible trade-offs between vulnerability and conducting efficiency. We also measured naturally occurring embolism during the winter months for comparison with the laboratory vulnerability data.

MATERIALS AND METHODS

Plant Material

Tree species native to the Wasatch Mountains of northern Utah were selected for the project: *Quercus gambelii* is ring porous, *Populus tremuloides* and *Betula occidentalis* are diffuse porous, *Abies lasiocarpa* and *Juniperus scopulorum* are conifers. Branches were collected, wrapped in plastic bags, and transported to the laboratory for embolism measurements (see below). *Populus* and *Abies* were collected at about 2825 m near Brighton, UT; *Quercus* and *Betula* were collected at about 1370 m in the Red Butte Canyon research area near the University of Utah in Salt Lake City; *Juniperus* were collected at about 2133 m near Parley's summit east of Salt Lake City.

Measurement of Embolism

Embolism was quantified by how much it reduced the hydraulic conductivity in the xylem. Hydraulic conductivity was defined as mass flow rate of solution through a stem segment (kilograms per second) divided by the pressure gradient along the segment (megapascals per meter). It was measured on branch segments between 4 and 15 cm long and 0.5 to 1 cm in diameter cut from larger branches collected from the field. Segments were cut under water to avoid causing additional embolism. Segments were located far enough from the original branch base to avoid including conduits embolized by cutting the branch in air during collection; this distance was at least as long as the longest conduits (Fig. 2). Segments were shaved on both ends with a razor blade and fitted to a tubing apparatus filled with filtered (0.22 μm) 10 mM oxalic acid solution (to inhibit microbial growth). Hydraulic conductivity of each segment was measured using a gravity-induced positive pressure gradient (about 70 kPa m^{-1}) across the segment. The method and apparatus is described in detail elsewhere (15).

In *Quercus*, it was important to keep the magnitude of the gradient below 40 kPa m^{-1} because higher gradients reversed embolism during the course of a measurement by flushing gas bubbles out of the vessels that were continuous through the segments. This was only a problem with *Quercus* because of its exceptionally wide and long vessels (Figs. 1 and 2).

Two methods were used to estimate the reduction in hydraulic conductivity due to embolism. For the single-segment method, embolism was measured by the percentage the initial conductivity of a segment was below its maximum value obtained after repeated "flushes" of oxalic acid solution through the stem under approximately 175 kPa of pressure that refilled any embolized vessels (15).

For the paired-segment method (17, 19), conductivity was
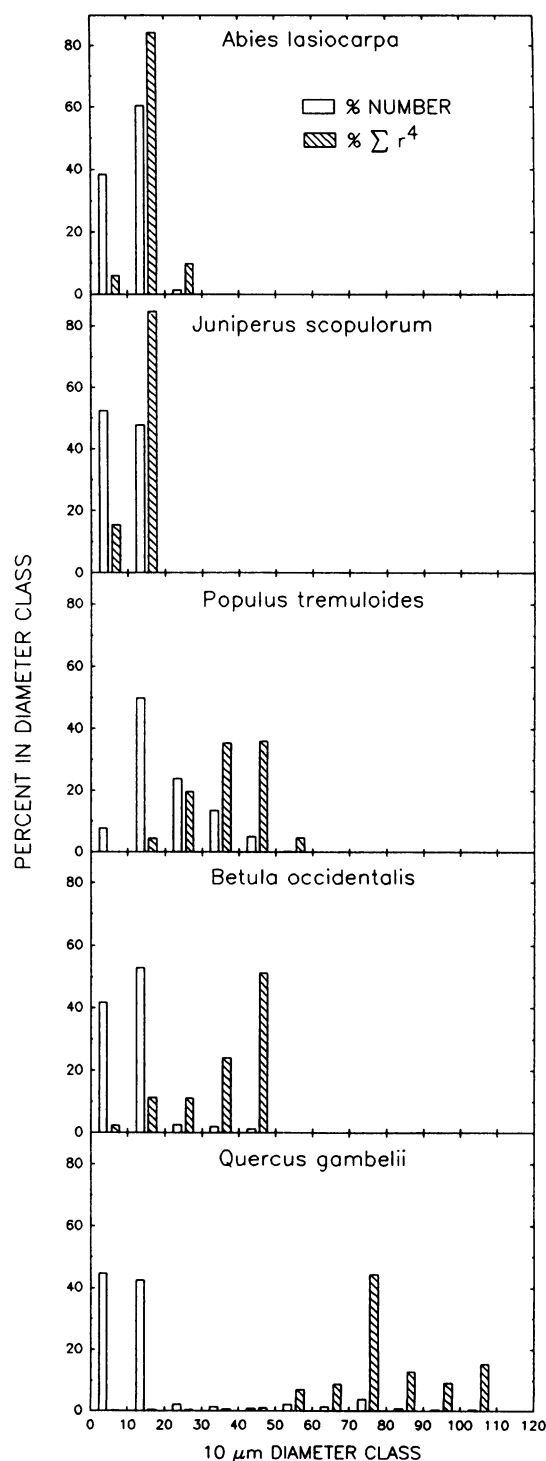
Figure 1. Percentage of conduits in 10-μm diameter classes. Open bars show percentages on the basis of total conduit number. Hatched bars show percentages on the basis of contribution to sum of all conduit radii to the fourth power (% Σr^4): this reflects the relative hydraulic importance of each diameter class as estimated from the Poisueille equation. The disproportionate conductance in wider conduits is especially pronounced in vessel-bearing species, ring-porous *Quercus* in particular.
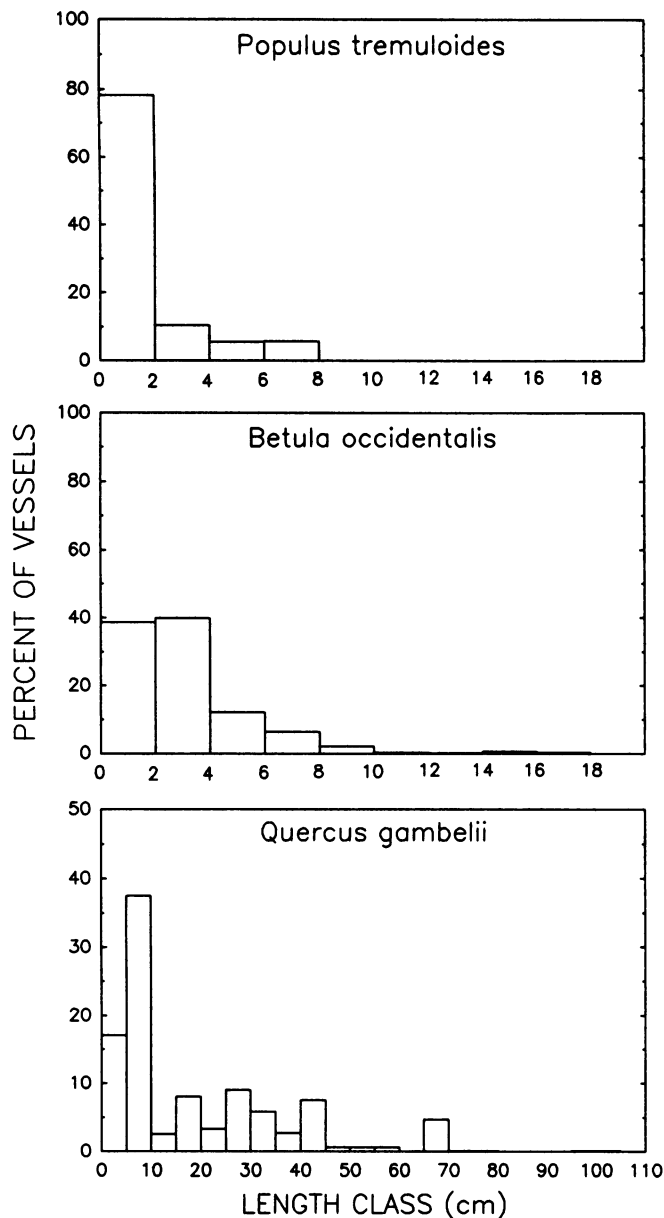
Figure 2. Percentage of vessels in length classes for each species; results are pooled from five branches per species. Branch diameter was between 0.4 and 1.5 cm. *Populus* and *Betula* length classes are 2 cm; *Quercus* length classes are 5 cm.

compared in two segments cut from the same branch, one before and one after an embolism-inducing treatment involving drying the stem in air to induce xylem tension (see below). The percentage of embolism was calculated by the percentage the conductivity of the treated segment was below that of the control segment. This method required no systematic difference between the conductivities of the paired segments in the absence of treatment-induced cavitation. For this reason, paired segments were located in a length of stem free of side branches. In addition, after the control segment was removed, the treated segment had to be far enough from the branch end to avoid conduits embolized at the cut end as the branch was dehydrated during the treatment. These require-

ments limited the paired-segment method to species with conduits shorter than about 5 cm (conifers and *Populus*; Fig. 2).

Both the paired- and single-segment techniques gave a percentage of embolism value: the single-segment method yielded the total embolism in a stem, whereas the paired-segment protocol only measured the embolism induced by a treatment. The paired-segment method was used whenever possible for constructing vulnerability curves (e.g. *Populus* and conifers) because it avoided the time-consuming flushing procedure required of the single-segment technique.

Vulnerability to Water-Stress-Induced Embolism

This was quantified by measuring the embolism induced by dehydrating stems to a known xylem tension measured with the pressure bomb or stem psychrometer (Dixon Instruments, Ontario, Canada). Pressure equilibration was promoted by enclosing the dehydrated branch in a plastic bag for at least 30 min before measuring. The embolism induced by the dehydration was measured according to either the paired-segment (*Populus*, conifers) or single-segment (*Quercus*, *Betula*) method.

Vulnerability to Freeze-Thaw-Induced Embolism

This protocol was identical with the water-stress measurements except that a freeze-thaw cycle was added following dehydration and equilibration of branches at a known xylem tension. Dehydrated branches were put in a plastic bag and frozen in a $-20°C$ freezer, usually overnight. Freezing exotherms measured by inserting thermocouples in the xylem confirmed apoplastic freezing for all tensions used in the experiments. Maximum xylem tensions lowered the freezing point to a minimum of $-10°C$ degrees; this was most noticeable in vessel-bearing species. Frozen branches were thawed at approximately 20°C in their bag until reaching ambient temperature; this required a maximum of 90 min. After the branches were thawed, the xylem tension was sometimes remeasured and the conductivity segment was measured as required by either the paired- or single-segment protocol.

With the exception of one *Populus* experiment, vessel-bearing species used for freezing experiments were collected during frost-free weather so that conduits would not already be embolized by freezing. The conifer species were found not to embolize by freezing (at least under natural conditions; see "Results") and were collected for experiments throughout the year.

Measurement of Native Embolism Levels

We also measured the accumulation of embolism in each species during the winter months when the xylem sap was subjected to repeated freezing and thawing under natural conditions. At approximately 5-week intervals from fall (August in *Populus*) to early spring, every species except *J. scopulorum* was measured for embolism using the single-segment method. Data for *Quercus* and *Abies* were from the winter of 1989 to 1990; the remaining species were measured in the winter of 1990 to 1991, with some *Betula* measure-

ments made in the fall of 1991. The protocol was similar for each species. Branches were cut from three trees and brought back to the laboratory in plastic bags. Trees were from the same area, and no tree was sampled more than once. Any foliage present was misted with water before bagging. Five conductivity segments, 10 cm long, were prepared from each tree, making 15 segments per collection date per species. Embolism was measured as described for the single-segment method above.

The especially long vessels in *Quercus* (some more than 1 m; Fig. 2) required special collecting procedures. Branches brought in from the field were 2 to 3 m so that conductivity segments (cut under water) could be located at least 1.8 m distant from the original cut end of the branch. To test whether this excluded significant numbers of severed and embolized conduits from the conductivity segment, some branches were cut from the tree under water using water-tight collars made from funnels. Embolism percentages levels in these segments ($x = 74 \pm 10.1$, $n = 7$) were not statistically different from those collected in the normal fashion ($x = 82 \pm 10.3$, $n = 8$).

Xylem tensions were also monitored in the field with the pressure bomb if foliage was present. In the absence of foliage, branches were collected, bagged, and measured with stem psychrometers in the laboratory. Winter tensions were normally measured on twigs collected in an unfrozen condition.

## Determination of Conduit Volume

Conduit volumes were estimated from length and diameter assuming a cylindrical shape; they are overestimates to the extent that conduits taper at their ends. Conduit volume was measured because it should be directly proportional to the dissolved gas content and, hence, bubble size in frozen conduits. Dimensions were weighted according to the contribution of conduits to total hydraulic conductance (see below) to be consistent with the hydraulic basis of the embolism measurements. The difference between nonweighted and hydraulically weighted volume is considerable because of the Poisueille relation between tube diameter and conductance (Fig. 1, compare open versus hatched histograms).

Diameter was calculated as the equivalent circle diameter for transverse lumen areas. At least 30 conduits were measured in the 1990 growth ring from 10 trees of each species (300 measurements per species). Branches were of thickness and age comparable to those used in embolism measurements. The average diameter of conduits in the classes contributing more than 20% to the total hydraulic conductance (Fig. 1, hatched histogram) was used as the hydraulically weighted average for the volume calculation.

Vessel lengths were measured using the paint-infiltration method of Zimmermann and Jeje (22). At least five branches per species were measured; branches had ages and diameters typical of those used in the embolism measurements. Results of the five stems were pooled and are shown in Figure 2. To estimate the length corresponding to the hydraulically weighted diameter, we assumed that diameter was proportional to length as previously demonstrated (6, 23). We chose the length as the midpoint of the length class occupying the
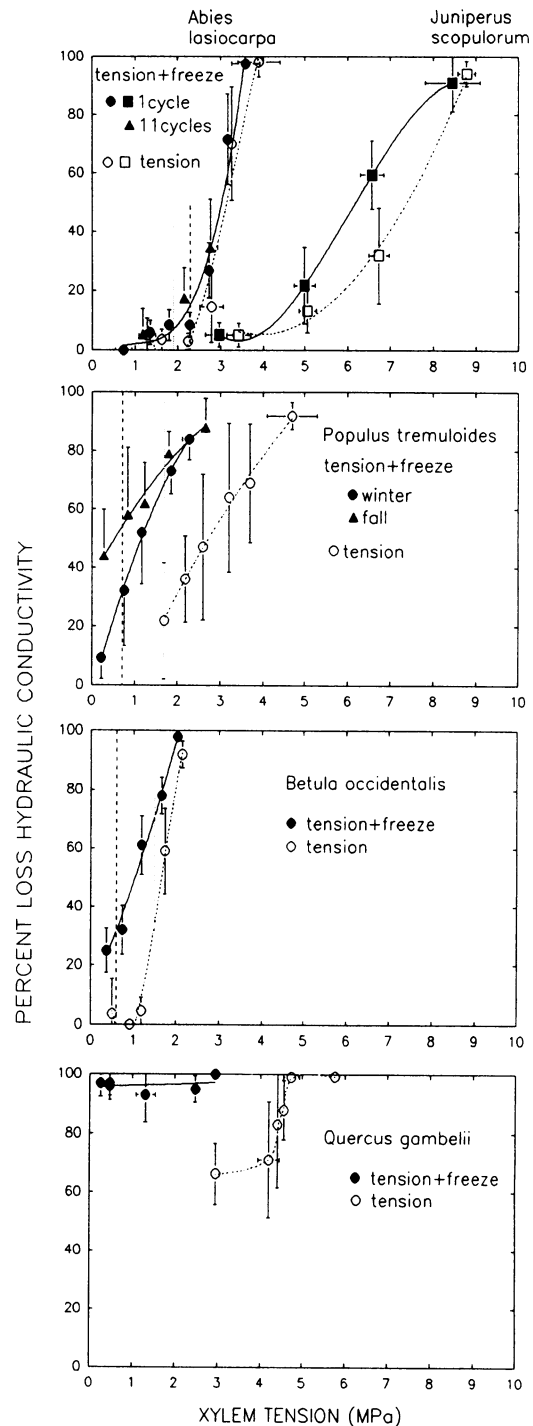
Figure 3. Percentage of loss in hydraulic conductivity versus xylem tension for each species. Solid symbols and lines show response to tension with a single freeze-thaw cycle (tension + freeze); solid triangles in *Abies* curve represent 11 cycles. Open symbols and dotted lines show the response to tension without a freeze-thaw cycle (tension). Error bars are 95% confidence limits ($n \geq 5$). Dashed vertical lines indicate maximum observed winter tensions; dotted vertical lines indicate maximum observed growing-season tensions. *Populus* and conifers were measured using the paired-segment method; *Betula* and *Quercus* were measured with the single-segment technique.
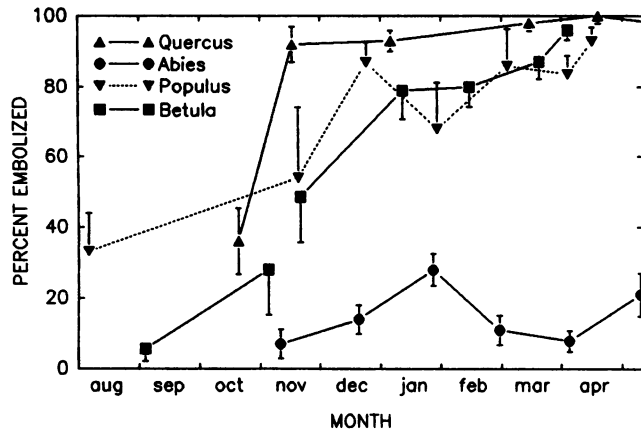
Figure 4. Native embolism levels from fall to spring as measured by the single-segment method. Quercus and Abies data were from the winter of 1989 to 1990; Populus and Betula data were from the winter of 1990 to 1991, except Betula data for September and early November, which were from the fall of 1991. Error bars are 95% confidence limits (n = 15).

corresponding position in the length distribution as the hydraulic diameter average occupied in the diameter distribution (Fig. 1, "% number").

Tracheid lengths for A. lasiocarpa and J. scopulorum were taken from published values for congeners of similar habitat: A. balsamea, 3.33 ± 0.43 mm; J. osteosperma, 1.18 ± 0.29 mm (9). Owing to considerably lower variation in lengths of tracheids as opposed to vessels, mean tracheid length was used in the volume calculations.

In addition to comparing conduit size and vulnerability across species, we also investigated whether freeze-thaw-induced embolism occurred preferentially in the larger diameter conduits of single stems as predicted. These experiments were done on Betula branches frozen and thawed at tensions below those causing embolism by tension alone (i.e. lower than 1.5 MPa; Fig. 3, Betula). Dye perfusions (see below) were done to determine which vessels were functional; dye-stained vessels were assumed to be functional; nonstained vessels were assumed to be embolized. Measurements were made on complete sectors (radial wedges) of the most recent growth ring; sectors contained at least 400 vessels.

Comparison of Vulnerability with Conducting Efficiency

An additional objective was to evaluate any trade-offs between vulnerability to cavitation and the conducting efficiency of the xylem. Conducting efficiency was quantified by measuring the hydraulic conductivity per transverse-sectional area of functional xylem; this is referred to as "specific conductivity." Functional versus nonfunctional xylem conduits were identified by perfusing stems with 0.1% (w/v) safranan dye filtered to 0.22 μm. Perfusions were made at the same pressure gradient used in the hydraulic conductivity measurements (between 30 and 70 kPa m⁻¹), except negative rather than positive pressure was used to minimize lateral flow of dye from conduits (preliminary experiments showed

positive versus negative gradients did not influence conductivity measurements, probably because conductance to lateral flow is quantitatively insignificant compared with longitudinal flow). Stems were perfused for standard times determined by control experiments in which nonembolized segments were perfused until the narrowest conduits (lowest velocity) were stained.

RESULTS

All species except A. lasiocarpa responded differently to the freeze-thaw and water-stress treatments (Fig. 3), suggesting that the two treatments induce cavitation by different mechanisms. There was also no correlation between vulnerability to the two different treatments. For example, Quercus was the second least vulnerable species to water stress after Juniperus, and yet it was by far the most vulnerable to the freeze-thaw treatment.

The water-stress experiments (Fig. 3, dotted lines, open symbols) showed that tensions causing significant embolism levels (loss of hydraulic conductivity) were only slightly higher than maximum tensions measured in the field during the growing season (Fig. 3, compare vertical dotted lines for each species with water-stress curves; no field tension values for Juniperus). This is consistent with all other species studied in this regard and suggests that vulnerability to cavitation by water stress sets real limits to the xylem tensions developed in trees during the growing season (21).

Comparison of water-stress and freeze-thaw results (Fig. 3, dotted versus solid lines, respectively) showed that vessel-bearing species (Quercus, Populus, Betula) were more vulnerable to cavitation by freezing than by water stress. In each species, the xylem embolized at lower tensions when subject to a freeze-thaw cycle than when subject to tension alone. This was most pronounced in Quercus, in which embolism was approximately 100% at the lowest tensions used in the freezing treatment (0.2 MPa); tension alone induced similar embolism levels at values greater than 4.5 MPa. The Quercus water-stress curve did not decrease below 66% embolism because this was the native value at the field site at the time of the experiment, and the single-segment method was used, which measured total embolism in the stem. Dye perfusions indicated that this relatively high native value was largely due to the previous year's embolized vessels that were not irreversibly plugged (e.g. by tyloses).

In Populus, the freezing response was similar in shape to the water-stress response but with similar embolism levels occurring at 1 to 2 MPa lower tensions in frozen stems versus water-stressed ones. The fall freeze-thaw curve for Populus showed a more sensitive response than the winter curve, probably because in winter a substantial number of vessels were already embolized by freeze-thaw events (Fig. 4). Betula had a freezing response similar to that of Populus, although it was much more vulnerable to water stress. Like Quercus, Betula was measured using the single-segment method, but the native embolism level was not significantly different from zero. This was evident from the water-stress curve, which decreased to zero at the lowest tensions (Fig. 3; Betula, dotted lines, open symbols).

The conifers were not as vulnerable to cavitation by freez-

ing as the vessel-bearing species. In *A. lasiocarpa*, the freeze-thaw results were identical with the water-stress results, suggesting that there was no embolism caused by the freeze-thaw cycle. This was true even when stems were frozen and thawed 11 times instead of once (Fig. 3; *Abies*, compare solid triangles with solid circles).

Juniperus was much more resistant to embolism by water stress than *Abies* (Fig. 3; *Juniperus*) and showed a significant response to freezing when mean embolism between 6 and 8 MPa was compared between treatments (*t* test, P = 0.05). However, the difference between the freeze-thaw curve and the water-stress curve was much less than for vessel-bearing species, and there was no embolism by freezing at tensions below what initiated embolism by tension alone.

Cavitation by freeze-thaw cycles would be expected to rehydrate the stem by releasing water held in conduits, especially when transpiration was minimized during the thaw as in our experiments. When tensions before and after freezing were compared for initial tensions below those causing embolism by water stress, there was no significant difference for *Abies* (0.092 ± 0.30 MPa difference, n = 30, NS at P = 0.05) as expected, given the absence of detectable cavitation (Fig. 3; *Abies*). There was a significant rehydration of 0.68 ± 0.34 MPa for *Betula* and *Populus* (n = 6, P = 0.05), consistent with cavitation in these species (Fig. 3; *Betula, Populus*). The presence of transpiration during the thaw would probably increase the freeze-thaw response in vessel-bearing species by reducing this rehydration.

The greater vulnerability of vessel-bearing species than conifers to freezing-induced cavitation was consistent with observed trends in native embolism levels during winter. The vessel-bearing species (*Quercus, Populus, Betula*) all exceeded 90% embolism by late winter, whereas the one conifer measured (*Abies*) never exceeded 30% (Fig. 4). Not surprisingly given its freeze-thaw response, *Quercus* showed the most abrupt increase in embolism during the winter, increasing from 34 to 92% after the first freeze of the season (−6°C) as measured by a weather station within 1 km of the site. Embolism accumulated more gradually in *Populus* and *Betula*, not reaching 90% until late March and early April.

Freeze-thaw cycles were the most likely cause of the embolism in the vessel-bearing species as depicted in Figure 4, not only because of the correlation between freezing and embolism in *Quercus* but also because xylem tensions during the winter in *Betula* and *Populus* never exceeded 1.0 MPa and thus were not great enough themselves to induce embolism based on the water-stress vulnerability curves (Fig. 3; *Betula, Populus*, compare vertical dashed line with water-stress curves). The moderate embolism seen in *Abies* could have been caused by water stress, however, because we measured maximum xylem tensions during winter of 2.3 MPa, which is near the range of tensions causing embolism in the laboratory (Fig. 3; *Abies*, compare vertical dashed line with water-stress curve). In the growing season, tensions were not found in excess of 1.9 MPa (Fig. 3; *Abies*, vertical dotted line). It is unclear what caused the apparent reversal in embolism in *Abies* from January to March; no positive pressures were detected in this species.

We evaluated the proportionality of cavitation vulnerability to conduit volume using the data in Figure 3. We used the

tension required to induce a 50% reduction in hydraulic conductivity for both the water-stress and freeze-thaw curves as a quantitative "index" of vulnerability and plotted this versus average conduit volume for each species (Fig. 5). To be strictly comparable between paired-segment (*Populus* and conifer species) and single-segment methods (*Betula* and *Quercus*), the index must refer to a 50% reduction from the native embolism level. This was always zero for the paired method, which only measured treatment-induced embolism, but it was not necessarily zero for the single-segment technique, which measured total embolism. In *Quercus*, the native embolism value was 66%, and the appropriate index tension for water stress corresponded with 83% embolism, i.e. the midpoint between the native value of embolism and 100%. For the *Quercus* freeze-thaw curve, the value was taken as the lowest tension used in the experiment (0.2 MPa) because no tension used gave significantly less than 100% embolism. There is no freezing vulnerability for *Abies* because no freezing response was detected (Fig. 3; *Abies*).

There was a negative correlation between conduit volume and tension at 50% embolism for freezing-induced embolism (Fig. 5, solid circles). In other words, species with larger volume conduits were more vulnerable (embolized at lower tensions) to cavitation by freeze-thaw cycles than species with smaller volume conduits. No correlation was found between volume and tension at 50% embolism for water-stress-induced embolism (Fig. 5, open circles), meaning that vulnerability to embolism caused by water stress was independent of the conduit volume.
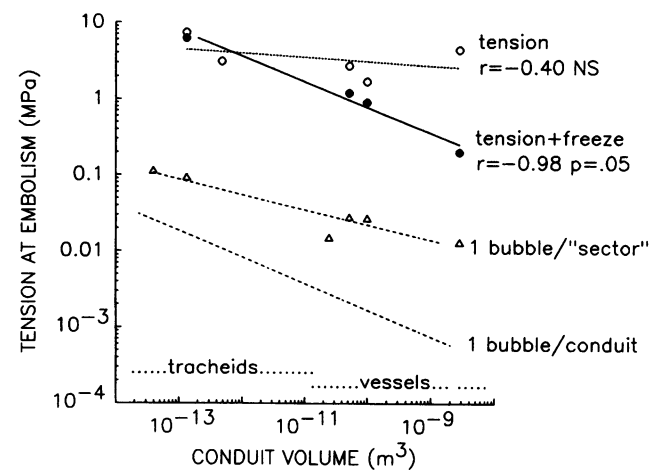
Figure 5. Log-log plot of conduit volume versus tension at the time of embolism; range of volumes for tracheids and vessels indicated along the abscissa. Circles represent tension at 50% embolism obtained from data in Figure 3: open circles and dotted line are for water stress (tension); closed circles and solid line are for freeze-thaw treatment (tension + freeze). Correlation coefficient (r) is indicated followed by NS if not significant (P > 0.05) or the probability (P) if significant. Dashed line with no symbols represents theoretical response to freezing for a single bubble per conduit (one bubble/conduit). Dashed line with open triangles represents predicted freeze-thaw response for one bubble freezing out per "sector," where a sector is a length of conduit as long as it is wide (one bubble/"sector"): data points represent the study species, plus one for a relatively large *S. sempervirens* tracheid (9).
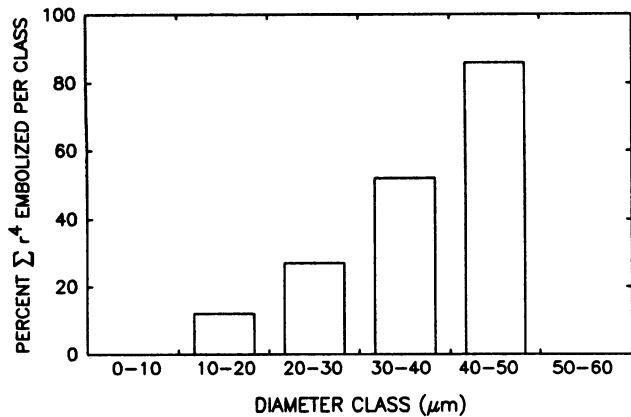
Figure 6. Percentage of conduits in each size class that were nonfunctional (not dye stained) following a freeze-thaw cycle in *B. occidentalis*. Percentages were based on the sum of conduit radii to the fourth power (% Σr^4) for each diameter class. Data were from a branch frozen at a xylem tension of 0.55 MPa and showing an average of 25% embolism on thawing. The larger the diameter class, the greater the fraction of embolized conduits.

The dashed line in Figure 5 shows the theoretical relationship between conduit volume and tension required to cause embolism by a freeze-thaw cycle, assuming (a) air-saturated water at 0°C and atmospheric pressure, (b) all dissolved air freezing out as a single bubble, (c) no dissolving of air before tensions redevelop after thawing, and (d) the cavitation tension obeys Equation 1. Predicted tensions were approximately proportional to the inverse cube root of conduit volume (slope of log-log plot = −0.33) because the radius of a bubble frozen out would be proportional to the cube root of volume and cavitation tension is inversely proportional to bubble radius (Equation). The slight deviation from this proportionality (actual theoretical slope is −0.35) is due to compression of the bubble by surface tension, which increases with decreasing bubble size.

Although the slopes of theoretical versus actual tensions at freezing-induced cavitation are very similar (−0.35 versus −0.34), the actual tensions required for 50% cavitation at a given average conduit volume were more than 2 orders of magnitude higher than what was predicted to embolize a conduit of the same volume (Fig. 5, compare tension + freeze line with dashed line). Assuming this theory is correct would mean that 50% of the hydraulic conductance was carried by conduits small enough to avoid embolism. Solving the theoretical relationship (tension = 5.5 × 10⁻⁷ volume⁻⁰·³⁵) for this volume at the observed embolizing tension indicates it would have to be 8 orders of magnitude smaller than the hydraulic average. This unlikely circumstance was not observed (Fig. 1, hatched histogram), indicating that the theory was inadequate. Possible reasons for this are evaluated in "Discussion."

The tendency for larger conduits to embolize at lower tensions when frozen and thawed than smaller conduits was also observed in single stems. In these experiments, *Betula* stems were frozen and thawed at tensions below what caused embolism by water stress. Analysis of dye perfusions showed that larger diameter vessels were more embolized than narrow ones by the treatment (Fig. 6).

There was a significant negative correlation between specific conductivity and tension at 50% embolism by freezing (Fig. 7, solid circles). This means there was a significant trade-off between conducting efficiency (specific conductivity) and vulnerability to embolism caused by freezing: the higher the conducting efficiency, the lower the tension required to induce embolism under freezing and thawing conditions. There was no evidence for a trade-off between conducting efficiency and vulnerability to embolism caused by water stress (Fig. 7, open circles).

DISCUSSION

Laboratory and field results were consistent with the hypothesis that freeze-thaw-induced embolism occurs at lower tensions for larger conduits than for smaller ones (Fig. 5, solid circles). Conduit volume, however, was not important in determining the tension causing embolism by water stress alone (Fig. 5, open circles). This is in agreement with recent evidence implicating the pit membrane as the structural basis for water-stress vulnerability (4, 17, 19). The often repeated assumption that larger conduits are more vulnerable to cavitation (1) was only true of the response to freeze-thaw events. The only instance in which water-stress vulnerability has been empirically shown to increase with conduit diameter was within single stems, and it was because the larger conduits had more permeable pit membranes than the smaller ones (19).

The fact that the different mechanisms of cavitation by freeze-thaw cycles and water stress are dependent on different structural features has important consequences for how the safety of the xylem from cavitation influences its hydraulic efficiency. There was no necessary trade-off between the two where safety from water stress was concerned (Fig. 7, open circles). Any influence of pit membrane structure on hydraulic conductance was apparently negligible compared
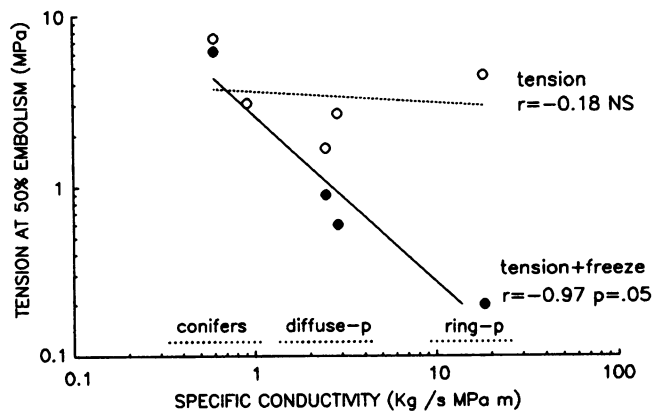
Figure 7. Log-log plot of specific conductivity versus tension at 50% embolism from water-stress (tension) and freeze-thaw cycles (tension + freeze). There is no significant correlation for the tension data (NS, P > 0.05) but a significant negative correlation for the tension + freeze response (P = 0.05). Ranges of specific conductivities are shown for conifers, diffuse-porous (diffuse-p) species, and ring-porous (ring-p) species (ref. 16, and J. Sperry, unpublished data).

to the importance of conduit volume. If conduit volume could be held constant, as is approximately the case for temperate conifers, decreasing permeability of pits to air may translate into decreasing hydraulic conductance (19). In contrast to water-stress vulnerability, there was a significant trade-off between hydraulic conductance and safety from freeze-thaw-induced embolism (Fig. 7, closed circles); this was undoubtedly because of the importance of conduit diameter for hydraulic conductance.

Despite their disadvantage in reducing hydraulic efficiency, small-volume conduits are clearly adaptive for minimizing embolism by freeze-thaw cycles. This may explain the trend for decreasing conduit sizes with increasing latitude and altitude (1), including the rarity of woody vines at high latitudes (6). Small-volume conduits would be especially critical for evergreen temperate trees exhibiting gas exchange and thus high xylem tensions on an opportunistic basis year-round. The dominance of conifers in cold climates is certainly consistent with their having tracheids. Small-volume conduits would be less critical for deciduous trees if mechanisms exist for reestablishing sufficient conduction before the growing season. This could occur by new growth (e.g. ring-porous species) and/or by refilling mechanisms (many diffuse-porous species [14, 16]). Each of the species in this project exhibited mechanisms for embolism recovery that are currently being investigated. These are essential because *Quercus*, *Betula*, and *Populus* were all more than 95% embolized in early spring (Fig. 6).

Our results predict that, although conifers are more resistant to embolism by freezing and thawing than vessel-bearing species, as a group they are not immune to it. The *Juniperus* results suggest that tracheids will cavitate in response to freezing if the xylem can sustain tensions high enough without cavitating first by water stress. It would be useful to find a conifer even more resistant to water-stress-induced cavitation than *J. scopulorum*: if tracheid volumes are similar, we would expect its freezing response to be similar to that in *J. scopulorum*. In addition, our empirical relationship between volume and vulnerability (Fig. 5; solid circles) predicts that larger volume tracheids (e.g. 1×10^{-11} m^3; *Sequoia sempervirens*) will develop 50% embolism at tensions of 1.7 MPa. Tracheid volume may be an important factor determining range limits of some conifers. These predictions must be tested to further evaluate our interpretation of conduit volume being the main structural variable determining vulnerability.

Although the observed proportionality of vulnerability to volume was very close to what was expected from theory, actual tensions were much higher than predicted on the basis of a single bubble freezing out per conduit (Fig. 5, compare solid and dashed lines). This is essentially the same problem raised by previous studies showing an apparent lack of freeze-thaw-induced cavitation in conifers (7, 12, 20). Here it arises from a directly measured cavitation response in both tracheid- and vessel-bearing trees.

Apparently, assumptions behind the predicted tensions are incorrect. There is no evidence that the xylem water would not be fully saturated with air (7). It is likely, however, that many small bubbles would freeze out rather than a single large one as assumed. When this was modeled for one bubble

freezing out per unit length equal to the conduit diameter (Fig. 5, open triangles, one bubble/"sector"), the tensions were still at least 1 order of magnitude below observed values. Furthermore, the few observations of bubbles in frozen conduits have been made for conifer tracheids in which even the smallest bubbles (diameter = 0.7 μm) were big enough to cause cavitation at tensions as low as 0.42 MPa and were present in 86% of ice-filled tracheids (20); our results show no cavitation in tracheids at these tensions (Fig. 3).

Not all the air dissolved in the xylem sap may freeze out as bubbles if it can diffuse ahead of the ice into adjacent air spaces (2). This is predicted to occur only at slow freezing rates (less than 2.5 μm s^{-1} [11]) and only if freezing proceeds toward a water-air boundary (a previously embolized conduit, for example). However, freezing seems to occur centripetally within a conduit, as indicated by bubbles occurring in lumen centers (10, 20); therefore, escape of air by this mechanism may be unlikely regardless of the freezing rate.

Perhaps the least valid assumption in the calculation is that no air redissolves during thawing when tensions may be momentarily reduced. Dissolving would be favored by a steep concentration gradient created by the recently degassed water and by elevated xylem pressures arising from expansion of water during freezing (approximately 8%). The pressure increase will be greatest for rapid freezing rates and high resistance to water flow away from advancing ice. This flow could occur through both apoplastic and symplastic routes to nonfreezing locales (roots or protoplasts) or into previously embolized trachieds or fibers. Hammel (7) proposed that the torus isolates water in freezing tracheids, creating high pressures, but this is unlikely because most pits are not aspirated in frozen conifer xylem (10). However, elevated pressures in freezing stems have been recorded in the field for *P. tremuloides* and *B. occidentalis* (J. Sperry, unpublished data) and in the laboratory for conifers (11). As long as transpiration is minimal, these pressures will not decrease to the prefreezing value until thawing is complete, allowing time for bubbles to dissolve completely or partially.

Another factor promoting redissolving of air would be rehydration due to cavitation in small numbers of conduits having the largest bubbles. This could reduce xylem tension in remaining conduits enough to prevent cavitation and allow their bubbles to dissolve. This was Sucoff's proposal (20) to explain minimal cavitation in conifers by freeze-thaw cycles. The degree of rehydration (with no transpiration) depends on the volume of water released, the pressure-volume relations of the symplast, and the kinetics of equilibration with the symplast. If this was happening, we would expect to see a rehydration following the freeze-thaw cycle and a greater embolism response to multiple cycles at a given tension than to a single cycle. Although this was not true for *Abies* and, thus, does not completely explain the conifer response, we did see significant rehydration for *Populus* and *Betula*. In addition, our field results showed a gradual increase in embolism during the winter for *Populus* and *Betula*, suggesting that multiple freeze-thaw cycles did increase the embolism response at a given range of tensions in these species.

It is likely that all of these scenarios play some role in reconciling the predicted versus observed cavitation tensions. All could preserve the proportionality with volume while

increasing the actual embolism-inducing tensions. These factors suggest that embolism formation will be dependent on rates of freezing and thawing as well as the direction of ice propagation in stems; we plan to test this in the future.

Although details linking theory and observation remain unclear, our findings indicate a preliminary quantitative relationship among conduit volume, vulnerability to cavitation by freeze-thaw events, and hydraulic efficiency. Conduit volume and conducting efficiency are independent of vulnerability to cavitation by water stress. The degree to which these characteristics of the xylem influence whole-plant stress responses and tolerances to drought and freezing depends in part on how closely leaf physiology is coupled to xylem transport capability. Modeling studies show a very close link (21), and experimental evaluation is underway.

ACKNOWLEDGMENTS

LITERATURE CITED

1. Baas P (1983) Ecological patterns in xylem anatomy. In TJ Givnish, ed, On the Economy of Plant Form and Function. Cambridge University Press, Cambridge, UK, pp 327–352
2. Carte AE (1961) Air bubbles in ice. Proc Phys Soc 77: 757–768
3. Cochard H, Tyree MT (1990) Xylem dysfunction in Quercus: vessel sizes, tyloses, cavitation and seasonal changes in embolism. Tree Physiol 6: 393–407
4. Crombie DS, Hipkins MF, Milburn JA (1985) Gas penetration of pit membranes in the xylem of Rhododendron as the cause of acoustically detectable sap cavitation. Aust J Plant Physiol 12: 445–453
5. Ewers FW (1985) Xylem structure and water conduction in conifer trees, dicot trees, and lianas. IAWA Bull 6: 309–317
6. Ewers FW, Fisher JB, Chiu ST (1990) A survey of vessel dimensions in stems of tropical lianas and other growth forms. Oecologia 84: 544–552
7. Hammel HT (1967) Freezing of xylem sap without cavitation. Plant Physiol 42: 55–66
8. Oertli JJ (1971) The stability of water under tension in the xylem. Z Pflanzenphysiol 65: 195–209
9. Panshin AJ, de Zeeuw C (1970) Textbook of Wood Technology, Vol 1. McGraw Hill, New York
10. Robson DJ, McHardy WJ, Petty JA (1988) Freezing in conifer xylem. II. Pit aspiration and bubble formation. J Exp Bot 39: 1617–1621
11. Robson DJ, Petty JA (1987) Freezing in conifer xylem. I. Pressure changes and growth velocity of ice. J Exp Bot 38: 1901–1908
12. Robson DJ, Petty JA (1991) A proposed mechanism of freezing and thawing in conifer xylem. In A Raschi, M Borghetti, J Grace, eds, Water Transport in Plants Under Climatic Stress. Cambridge University Press, Cambridge, U.K. (in press)
13. Schultze HR, Matthews MA (1988) Resistance to water transport in shoots of Vitis vinifera L.: relation to growth at low water potential. Plant Physiol 88: 718–724
14. Sperry JS (1991) Winter xylem embolism and spring recovery in Betula cordifolia, Fagus grandifolia, Abies balsamea, and Picea rubens. In A Raschi, M Borghetti, J Grace, eds, Water Transport in Plants Under Climatic Stress. Cambridge University Press, Cambridge, U.K. (in press)
15. Sperry JS, Donnelly JR, Tyree MT (1987) A method for measuring hydraulic conductivity and embolism in xylem. Plant Cell Environ 11: 35–40
16. Sperry JS, Donnelly JR, Tyree MT (1988) Seasonal occurrence of xylem embolism in sugar maple (Acer saccharum). Am J Bot 75: 1212–1218
17. Sperry JS, Perry AH, Sullivan JEM (1991) Pit membrane degradation and air-embolism formation in ageing xylem vessels of Populus tremuloides Michx. J Exp Bot 42: 1399–1406
18. Sperry JS, Tyree MT (1988) Mechanism of water stress-induced xylem embolism. Plant Physiol 88: 581–587
19. Sperry JS, Tyree MT (1990) Water-stress-induced xylem embolism in three species of conifers. Plant Cell Environ 13: 427–436
20. Sucoff E (1969) Freezing of conifer xylem sap and the cohesion-tension theory. Physiol Plant 22: 424–431
21. Tyree MT, Sperry JS (1989) Vulnerability of xylem to cavitation and embolism. Annu Rev Plant Phys Mol Biol 40: 19–38
22. Zimmermann MH, Jeje AA (1981) Vessel-length distribution in some American woody plants. Can J Bot 59: 1882–1892
23. Zimmermann MH, Potter D (1982) Vessel-length distribution in branches, stem and roots of Acer rubrum L. IAWA Bull 3: 103–109