

Tracheid diameter is the key trait determining the extent of freezing-induced embolism in conifers

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Summary We tested the hypotheses that freezing-induced embolism is related to conduit diameter, and that conifers and angiosperms with conduits of equivalent diameter will exhibit similar losses of hydraulic conductivity in response to freezing. We surveyed the freeze–thaw response of conifers with a broad range of tracheid diameters by subjecting wood segments (root, stem and trunk wood) to a freeze–thaw cycle at -0.5 MPa in a centrifuge. Embolism increased as mean tracheid diameter exceeded $30\ \mu\text{m}$. Tracheids with a critical diameter greater than $43\ \mu\text{m}$ were calculated to embolize in response to freezing and thawing at a xylem pressure of -0.5 MPa. To confirm that freezing-induced embolism is a function of conduit air content, we air-saturated stems of *Abies lasiocarpa* (Hook.) Nutt. (mean conduit diameter $13.7 \pm 0.7\ \mu\text{m}$) by pressurizing them 1 to 60 times above atmospheric pressure, prior to freezing and thawing. The air saturation method simulated the effect of increased tracheid size because the degree of super-saturation is proportional to a tracheid volume holding an equivalent amount of dissolved air at ambient pressure. Embolism increased when the dissolved air content was equivalent to a mean tracheid diameter of $30\ \mu\text{m}$ at ambient air pressure. Our centrifuge and air-saturation data show that conifers are as vulnerable to freeze–thaw embolism as angiosperms with equal conduit diameter. We suggest that the hydraulic conductivity of conifer wood is maximized by increasing tracheid diameters in locations where freezing is rare. Conversely, the narrowing of tracheid diameters protects against freezing-induced embolism in cold climates.

Keywords: cavitation, freeze–thaw, hydraulic conductivity, temperature.

Introduction

The freezing of wood forces air from the xylem sap and produces bubbles in frozen conduit lumens (Sucoff 1969, Robson et al. 1988). During the thaw, these bubbles may nucleate cavitation and ultimately block sap flow (Sperry and Sullivan 1992, Sperry 1993). Both field and laboratory studies have shown that, in contrast to woody angiosperms, conifers exhibit

little, if any, loss of water transport capacity following a freeze–thaw cycle (Hammel 1967, Sucoff 1969, Sperry et al. 1994, Hacke and Sauter 1996, Davis et al. 1999). The apparent resistance of conifers to freezing-induced hydraulic failure has no satisfactory explanation, although it has frequently been discussed (Tyree et al. 1994, Lambers et al. 1998, Sperry and Robson 2001). In this study, we explored the freeze–thaw response of conifers and reevaluated the conclusion that tracheid-based xylem is inherently more resistant to freezing-induced cavitation than vessel-based angiosperm wood.

To explain the immunity of conifers to freeze–thaw embolism, two theories have been proposed (Hammel 1967 and Sucoff 1969). Hammel (1967) hypothesized that the torus-margo structure of the pit membrane was crucial to the prevention of embolism. The freezing and simultaneous expansion of water in the lumen would cause the pressure in the tracheid to rise, forcing the pit membrane to deflect, thus effectively sealing the tracheid from its neighbors. During thawing, the increased pressure in the tracheid acts to compress the bubbles before an embolism occurs. However, Robson et al. (1988) observed both appressed and non-appressed pit membranes in frozen conifer wood, so Hammel's conjecture remains equivocal. Alternatively, Sucoff (1969) postulated that, during the thaw, tracheids with the largest bubbles embolize and thus release water to neighboring conduits. This rehydration strategy supposedly prevents the considerable losses in water transport commonly detected in angiosperms. However, Sucoff's idea is inconsistent with the observation that repeated freeze–thaw cycles do not result in any loss of water transport in conifer wood as would be expected if a portion of the tracheid population is embolized with each cycle (Sperry and Sullivan 1992).

More recent work has focused on the role of conduit dimensions in bubble formation. Ring- and diffuse-porous woods suffer large losses in water transport following a freeze–thaw cycle, presumably because their large vessels hold a greater amount of sap, and thus a higher quantity of dissolved gases that can freeze out compared with small conifer tracheids (Ewers 1985, Sperry and Sullivan 1992, Hacke and Sauter 1996, Feild and Brodribb 2001). Minute air bubbles formed in smaller conduits can redissolve in sap at more negative xylem

pressures (P_x) than larger bubbles, thus preventing embolism (Yang and Tyree 1992). This is shown explicitly in Equation 1:

$$P_x^* = P_b^* - 2T/r_b \quad (1)$$

where T is the surface tension of water (0.0728 Pa m at 20 °C), r_b is the bubble radius, P_b^* is the minimum internal pressure of the bubble required for its collapse and P_x^* is the corresponding critical cavitation pressure, below which the bubble will expand. Because the bubble will contain a mixture of air and water vapor, P_b^* must at least exceed the saturated vapor pressure of water (−0.1 MPa at 20 °C at sea level) for it to condense (Sperry and Robson 2001). Large conduits may be more vulnerable to freezing-induced embolism because they produce bubbles with larger r_b that expand at less negative P_x .

Whether conduit volume or diameter is more important in determining r_b depends on the xylem sap freezing process. If the air in the sap coalesces into a single or a few large bubbles, then r_b will be proportional to conduit volume. However, if the sap freezes centripetally such that a longitudinal file of bubbles is produced, then r_b will be a function of conduit diameter. Sucoff's (1969) and Robson et al.'s (1988) direct observations of a central series of bubbles in frozen tracheids support the latter hypothesis.

If conduit diameter is the determinant of the freeze–thaw response, then conifers should be just as vulnerable to freezing embolism as angiosperms, provided the diameter range of tracheids and vessels is comparable. This implies that conifers resist freezing-induced cavitation not because they are short, have low volume or are anatomically unique, but simply because they are narrow. Davis et al. (1999) alluded to this idea when they demonstrated that woody species with a mean conduit span below 30 μm experienced little embolism following a freeze–thaw event at a P_x of −0.5 MPa. In their experiments, all species with mean vessel diameters above 30 μm exhibited considerable losses of hydraulic conductivity. Whether gymnosperm wood will show a similar response is unknown, because previous experiments testing the freezing response of conifers used species with mean tracheid diameters much smaller than 30 μm where little embolism would be expected (Sperry and Sullivan 1992, Sperry et al. 1994, Feild and Brodribb 2001). Many conifers have tracheids above this 30 μm diameter threshold (Panshin and de Zeeuw 1980). Will they be just as susceptible to freezing-induced embolism as angiosperms with similarly sized vessels?

We answered this question with two experimental approaches. We used the centrifuge method of Davis et al. (1999) to survey the freeze–thaw response of several gymnosperm species with mean tracheid diameters ranging from 11 to 41 μm . We complemented this approach with experiments on one species of *Abies* where variation in tracheid size was simulated by changing the air content of the xylem sap prior to freezing and thawing.

Materials and methods

Plant material

Table 1 lists the study species and collection sites. For each species, a minimum of six root, stem or trunk segments was analyzed. Roots and branches ranging from 5 to 16 mm in diameter, corresponding to 1 to 12 years in age, were cut from adult trees, wrapped in a paper towel, double-bagged in plastic to prevent water stress, and transported to the laboratory. The segments were straight and no less than 20 cm in length. Material was collected at all times of the year, between 1999 and 2001, with the roots being removed from the top 30 cm of well-hydrated soil. Trunk wood was also analyzed in one species, *Pinus contorta* Dougl. ex Loud. Trunk wood samples were collected from two felled trees, approximately 30 cm in diameter at the base and about 20 to 30 years of age. The trunk was cut into ~40-cm-long segments, which were moistened, double-bagged and transported to the laboratory where the wood was stored at 4 °C for no longer than 7 days before use.

Hydraulic conductivity measurements

Hydraulic conductivity (K) was measured in the laboratory with the apparatus described by Sperry (1993) and calculated as the flow rate of water for a given pressure gradient, through a segment of known length.

To prepare wood samples for K measurements, segments were recut under water to a length of 142 mm to remove any emboli caused during collection, and the ends shaved with a razor blade. The bark was left intact on all stems and roots with the exception of *Abies concolor* (Gord. & Glend.) Lindl. where about 1 cm of bark was removed from the terminal ends of the root segments to prevent the accumulation of excess resin and mucilage. To prepare *P. contorta* trunk wood for conductivity measurements, trunk segments were chopped into smaller sections (with the grain) and whittled longitudinally into cylinders measuring 142 mm in length and 8–10 mm in diameter, thus including four to five of the most recent growth rings. The wood was kept wet at all times.

Segments were mounted on the tubing apparatus where they were continually immersed in water, and K was measured gravimetrically under a pressure head of 4 to 5 kPa, using distilled and filtered (0.22 μm) water. We determined the flow into the segments without a pressure head before and after each gravimetric flow measurement. These “background” flows were averaged and subtracted from the pressure-induced flow in order to improve accuracy (Davis et al. 1999).

Xylem specific conductivity (K_s) was calculated by dividing K by the xylem and pith area, with the latter contributing less than 4% to the overall area. The xylem area in *Taxodium distichum* (L.) material was obtained by initially perfusing the segment with a 0.1% saffranin stain for 24 h, sectioning the perfused wood, and then measuring the area of the stained growth rings with the aid of a dissecting scope.

Centrifuge experiments

Freeze–thaw experiments were performed under a mild tension of −0.5 MPa based on the centrifuge method of Davis et

Table 1. Study species, collection sites and tracheid diameters ($\mu\text{m} \pm \text{SE}$) of examined gymnosperm material. Species names are listed in alphabetical order.

Species/family	Collection site ¹	Organ	Mean tracheid diameter ²	Maximum tracheid diameter ³
<i>Abies concolor</i> (Gord. & Glend.) Lindl./Pinaceae	Uinta, UT	Root	30 \pm 1.2	55 \pm 2.1
<i>Abies lasiocarpa</i> (Hook.) Nutt./ Pinaceae	Brighton, UT	Root	25 \pm 1.2	48 \pm 1.9
<i>Ginkgo biloba</i> L./Gingkoaceae	Univ. Utah, UT	Root	26 \pm 1.8	46 \pm 3.8
		Branch	16 \pm 2.7	28 \pm 2.1
<i>Juniperus scopulorum</i> Sarg./ Cupressaceae	Uinta, UT	Root	22 \pm 0.8	38 \pm 0.9
		Branch	11 \pm 0.3	19 \pm 0.3
<i>Pinus contorta</i> Dougl. ex Loudon/ Pinaceae	Uinta, UT	Root	29 \pm 0.8	54 \pm 1.2
		Branch	14 \pm 0.6	23 \pm 1.4
		Trunk	27 \pm 0.4	52 \pm 0.8
<i>Sequoia sempervirens</i> (D. Don) Endl./Cupressaceae	Sonoma, CA	Branch (large)	26 \pm 1.2	48 \pm 2.8
		Branch (small)	20 \pm 0.8	42 \pm 2.5
<i>Taxodium distichum</i> (L.)/ Cupressaceae	Durham, NC	Branch	40 \pm 2.7	76 \pm 7.6
		Root	41 \pm 1.3	70 \pm 1.9

¹ Uinta, UT = Uinta/Wasatch-Cache National Forest, 2500 m (40°3' N, 111°37' W); Brighton, UT = Guardsman's Pass near Brighton, 2825 m (40°46' N, 111°35' W); Univ. Utah, UT = University of Utah campus, 1440 m (40°47' N, 111°47' W); Sonoma, CA = 355 m (38°53' N, 123°25' W); and Durham, NC = Lost Shoe Swamp near Durham, North Carolina, 130 m (35°52' N, 79°59' W).

² Mean tracheid diameters were calculated from a minimum of 150 tracheids per sample (see Materials and methods); $n \geq 6$.

³ Mean maximum tracheid diameters were calculated from the largest tracheid in each sample analyzed; $n \geq 6$.

al. (1999) whereby P_x was a function of segment length and the angular velocity of the rotor (Alder et al. 1997). We chose -0.5 MPa as a conservative estimate for the in vivo P_x , which may be considerably more negative during a natural freeze-thaw cycle (Sperry et al. 1994, Davis et al. 1999). A Sorvall RC-5C centrifuge (Kendro Laboratory Products, Newton, CT) was fitted with a centrifuge rotor custom-designed to hold three segments. The rotor was surrounded by a copper coil (1.3 cm diameter) attached to a temperature-controlled bath (Model 1157, VWR Scientific, West Chester, PA) that regulated the temperature of the heat transfer fluid (Type XLT, Polyscience, Niles, IL) circulating in the coil.

Each stem or root segment was subjected to three K measurements prior to the freeze-thaw cycle in the centrifuge. Initially, we measured the field, or 'native' conductivity (K_{native}) of the material. Maximum hydraulic conductivity, K_{max} , was then determined by flushing the segments with purified water at a maximum pressure of 27 kPa for 15 min to remove the native emboli. Percent loss of conductivity (PLC), quantifying the native embolism, was calculated as the percentage decrease from K_{max} such that $\text{PLC}_{\text{native}} = (1 - K_{\text{native}}/K_{\text{max}})100$. To evaluate the embolism caused by a freeze-thaw cycle at -0.5 MPa, we first measured the conductivity at this tension following a 3-min centrifugation at -0.5 MPa at 20 °C ($K_{-0.5}$). The $\text{PLC}_{-0.5}$ was calculated as $(1 - K_{-0.5}/K_{\text{max}})100$. The PLC due to freezing and thawing (PLC_{FT}) at -0.5 MPa was related to this value such that $\text{PLC}_{\text{FT}} = (1 - K_{\text{FT}}/K_{-0.5})100$, where K_{FT} represents the sample conductivity following a freeze-thaw cycle.

The freezing experiments determined the PLC_{FT} following a 3-h freeze-thaw cycle at a P_x of -0.5 MPa as described by Davis et al. (1999). At the start of the experiment, the centrifuge was cooled to -3 °C, with the bath set to -20 °C. Centrifuge temperatures were reduced by 3 °C every 18 min to a minimum of -15 °C for the first 90 min of the treatment. Segments were thawed during the remaining 90 min, after which the centrifuge and the bath reached a minimum of $+10$ °C. The cooling rate was 0.2 °C min^{-1} , such that the minimum temperatures in the segment reached -10 to -14 °C as measured by a temperature logger (HOBO, Onset Computer, Pocasset, MA). Stems were warmed at a rate of about 0.2 °C min^{-1} . To determine if any PLC occurred in the absence of a freeze-thaw cycle, we measured K as described above, with the exception that the 3-h centrifugation treatment at -0.5 MPa was conducted at room temperature.

The effect of minimum freezing temperature on PLC_{FT} was determined with stems of *Ginkgo biloba* L. and roots of *P. contorta*. The above protocol was followed except that the minimum temperatures during the freeze-thaw cycle were altered to 0, -5 and -10 °C in addition to the -15 °C standard.

To test whether the conductivity of segments whittled from trunk wood could be measured in the same manner as that of stems with bark, stems of *P. contorta*, about 1 cm in diameter, were divided into two groups: those that were stripped of bark and those left intact. Both groups were then subject to measurements of K_{native} , K_{max} , $K_{-0.5}$ and K_{FT} as described above. We observed no difference in the PLCs caused by the control and freeze-thaw treatments between debarked and original stems,

so the wood cores were handled no differently than the root and stem tissues.

Air saturation experiments

We pressurized the stems of *Abies lasiocarpa* (Hook.) Nutt. with air to test the hypothesis that PLC_{FT} is a function of dissolved air content in xylem sap. This method simulated the effect of increased conduit size because the degree of supersaturation (applied air pressure relative to ambient) is proportional to a conduit volume holding an equivalent amount of dissolved air at ambient pressure.

Abies lasiocarpa branches measuring about 1.5 cm in diameter were cut, double-bagged and transported to the laboratory. The branches had at least a 10-cm length free of side branches and several small side shoots for measurements of P_x . The P_x of each branch was measured with a pressure chamber (PMS Systems, Corvallis, OR), and if found to be more negative than -2 MPa, the branch was not used.

Two 5-cm-long segments (*A* and *B*) separated by 1 cm were located on the basal end of a stem that was free of side branches. The *A* segment was the more basal of the two and was cut from the branch under water for K_{native} measurements. The *A* segment was at least 1 cm from the original cut end of the branch to avoid air entry during harvest. The rest of the branch, including the *B* segment (now 1 cm from the branch end and protected from air entry), was placed completely inside a long pressure chamber and pressurized for 16 h to ensure full super-saturation of the xylem sap. While still under pressure, the chamber with the branch inside was frozen to -15 °C for 3 h. The branch was removed from the chamber and thawed in a plastic bag for 1 h at room temperature. The *B* segment was cut from the thawed branch under water for K_{FT} measurement. The PLC_{FT} was calculated as $(1 - K_{FT}/K_{native})100$. Control experiments were identical to this protocol except that there was no freeze-thaw treatment.

Tracheid diameter measurements

Hand-cut, transverse sections of each wood segment used in the freeze-thaw experiments were stained in Toluidene Blue for 3 min, rinsed in distilled water and mounted in glycerin on a glass slide. The sections were photographed at 200 \times magnification with a Nikon CoolPix digital camera mounted on an Olympus BH-5 microscope (Scientific Instruments, Salt Lake City, UT), and the perimeter of the tracheid lumen was manually traced using image analysis software (ImagePro, Media Cybernetics, Carlsbad, CA). To ensure a representative population of cell diameters, we measured three to four files of tracheids within a sector defined by two rays crossing all growth rings. The mean cell diameter was calculated from a minimum of 150 tracheids.

Results

Mean tracheid diameters of our study species ranged from 11 ± 0.3 μ m for *Juniperus scopulorum* Sarg. stems to 41 ± 1.3 μ m for *Taxodium distichum* roots, corresponding to a nearly fourfold variation in tracheid span (Figure 1, Table 1).

On average, roots had larger tracheid diameters than stems, as shown for the wood of *J. scopulorum* and *P. contorta* (Figure 2).

The PLC_{native} for all stem, root and trunk material was less than 4% (data not shown). This low value of native embolism is not surprising because specimens were collected from sites that were well-hydrated at all times of the year, and at least 30 cm of snow insulated the wet soil, thus preventing roots from freezing during the winter. The $PLC_{-0.5}$ values were between 0 and 10% (data not shown), indicating little cavitation by water stress at -0.5 MPa.

The PLC_{FT} values varied from 0% in the branches of *J. scopulorum* to 64% in the roots of *T. distichum* (Figure 3), indicating that, even among tracheid-bearing species, there is a broad range in embolism response to freezing. Control experiments revealed no increases in PLC attributable to a 3-h centrifugation at -0.5 MPa (data not shown). There was a significant correlation ($r^2 = 0.81$) between species' mean conduit diameter and their PLC_{FT} , such that species with broader tracheids suffered greater freezing-induced embolism than species with narrow tracheids. The shape of this freeze-thaw response closely corresponded to the sigmoidal relationship derived primarily from angiosperm species by Davis et al. (1999; also shown in Figure 3, grey open symbols), with a similar 30 μ m mean diameter threshold for embolism.

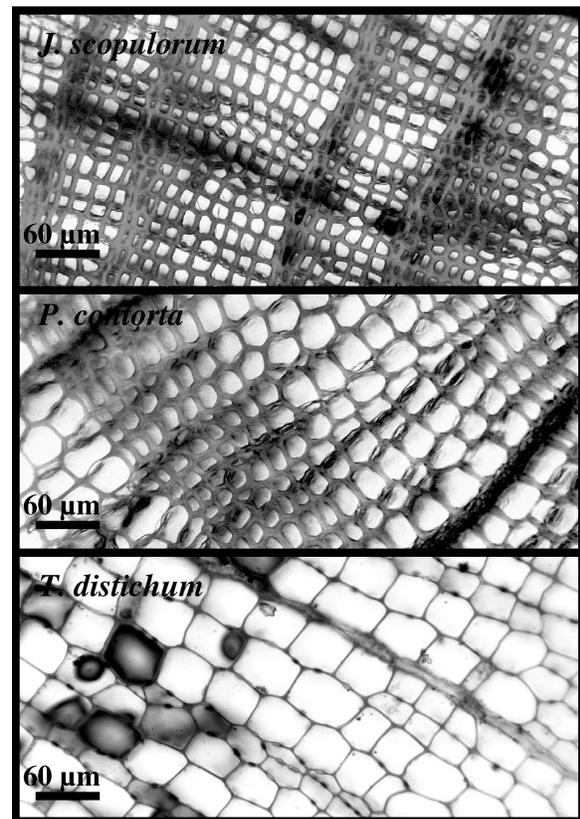


Figure 1. Photographs of transverse sections of *Juniperus scopulorum* branch xylem, and the root xylem of *Pinus contorta* and *Taxodium distichum* at 200 \times (light microscope).

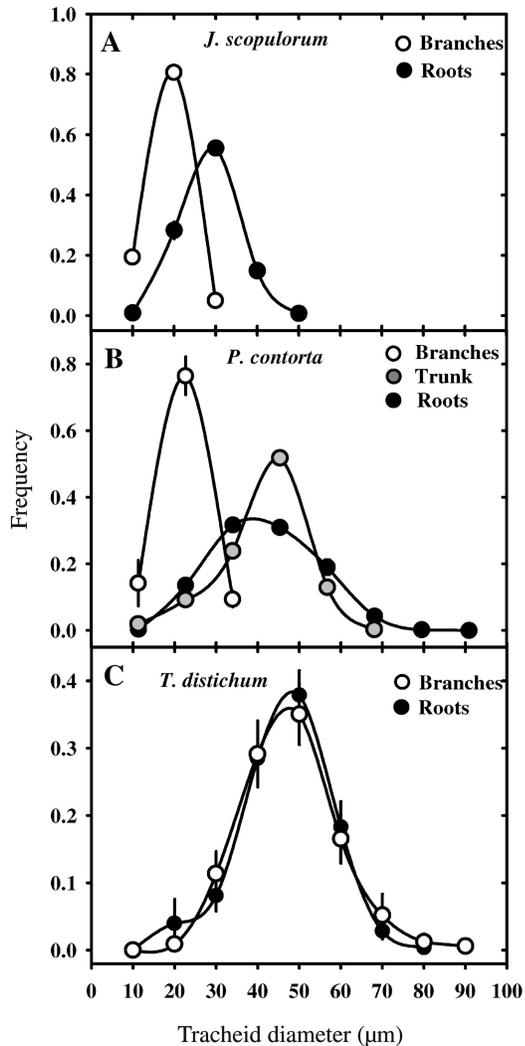


Figure 2. Frequency distribution of tracheid diameters in the root and branch wood of *Juniperus scopulorum* (A), in the root, branch and trunk wood of *Pinus contorta* (B), and in the root and branch wood of *Taxodium distichum* (C) ($n \geq 6$; error bars may be smaller than symbols).

If Equation 1 correctly describes the freeze–thaw response, and r_b increases with tracheid diameter, there will be a critical conduit diameter (d_c) above which tracheids will embolize at a P_x of -0.5 MPa. According to the Hagen–Poiseuille relationship, the conductivity of a stem is proportional to the sum of its conduit diameters raised to the fourth power (Tyree et al. 1994). Hence, the PLC_{FT} may be predicted ($PLC_{predicted}$) according to:

$$PLC_{predicted} = 100(1 - (\Sigma d \geq d_c^4 / \Sigma d^4)) \quad (2)$$

where d is measured tracheid diameter. The d_c that best agreed with measured PLC_{FT} was $43 \mu\text{m}$ ($r^2 = 0.42$).

Supersaturating *A. lasiocarpa* stems with varying degrees of air pressure prior to freezing and thawing produced PLC_{FT}

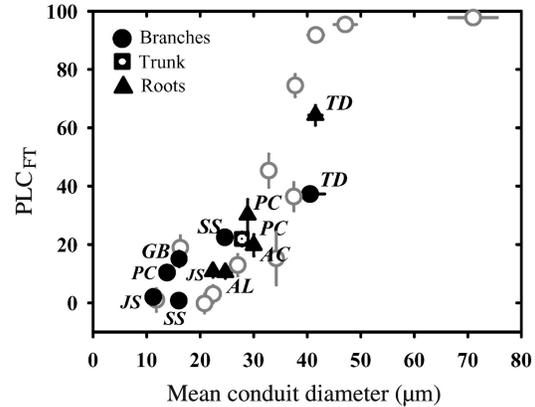


Figure 3. Percent loss of conductivity due to freezing and thawing (PLC_{FT}) as a function of conduit diameter in conifer root, trunk and branch material (solid symbols) ($n \geq 6$; error bars may be smaller than symbols). These data are superimposed on the freeze–thaw response of the mostly angiosperm stem material of Davis et al. (1999; grey open symbols). Abbreviations: AC = *Abies concolor*; AL = *Abies lasiocarpa*; GB = *Ginkgo biloba*; JS = *Juniperus scopulorum*; PC = *Pinus contorta*; SS = *Sequoia sempervirens*; and TD = *Taxodium distichum*.

trends similar to those in Figure 3. Stems frozen and thawed at ambient pressure had a PLC_{FT} lower than 20%, an expected result given their mean tracheid diameter of $13.7 \mu\text{m}$. Increasing air saturation during the freeze–thaw cycle caused an increase in PLC_{FT} up to nearly 100% for stems pressurized above $20\times$ ambient (Figure 4, solid symbols, upper x-axis). Unfrozen but air-saturated control stems exhibited a PLC below 40% (Figure 4, grey symbols). The lower x-axis in Figure 4 shows the corresponding increase in tracheid diameter required to hold the same amount of air per unit length at ambient pressure, assuming a constant tracheid shape. Essentially the same pattern is seen as in Figure 3 where significant PLC_{FT} occurs for a mean tracheid diameter over $30 \mu\text{m}$, and 100 PLC_{FT} occurs for mean diameters over $40 \mu\text{m}$.

Conifers with broader tracheids exhibited higher K_s values (Figure 5A) than those with narrow tracheids. This agrees with the predictions of the Hagen–Poiseuille relationship, which estimates a conduit's flow rate based on its radius raised to the fourth power. Thus, species with high K_s typically have xylem with large diameter conduits and are thus more vulnerable to freezing-induced embolism (Figure 5B).

Pinus contorta roots and stems showed no interaction between PLC_{FT} and minimum freezing temperature (Figure 6, solid symbols). This is expected if bubble formation during freezing is the only cause of PLC_{FT} . In contrast, *G. biloba* stems showed a significant effect of minimum temperature on freezing embolism, with -15°C temperatures causing a two-fold increase in PLC_{FT} over the -5°C treatment (Figure 6, grey symbols). The freezing exotherm in *G. biloba* was between -2 and -3°C (data not shown), indicating that additional PLC_{FT} at the -10 and -15°C temperatures was unrelated to bubble formation in the freezing sap (the sap being already frozen). Thus, the -5°C PLC_{FT} was used in Figure 3.

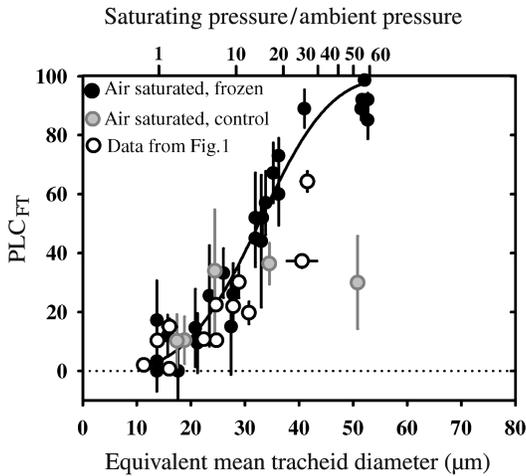


Figure 4. Percent loss of conductivity due to freezing and thawing (PLC_{FT}) in *Abies lasiocarpa* stems saturated with air up to 60 times that of ambient pressure (solid symbols), compared with data from Figure 3 (open symbols). Mean stem tracheid diameter of *A. lasiocarpa* is 13.7 μm , but the equivalent mean tracheid diameter required to hold the same volume of air as saturated stems is on the lower x-axis. Grey symbols represent unfrozen branches ($n \geq 6$; error bars may be smaller than symbols).

Discussion

We found that conifers are as vulnerable to freezing-induced cavitation as angiosperms, and that the PLC_{FT} response is largely a function of conduit diameter rather than volume or pit structure. The centrifuge and air-saturation treatments were used as two independent means of inducing freeze-thaw embolism and showed that the PLC_{FT} rises abruptly when the mean conduit diameter (or equivalent of) exceeds 30 μm . Our analysis suggests that tracheids with a d_c larger than 43 μm will embolize at P_x of -0.5 MPa. Davis et al. (1999) reported similar trends with primarily angiosperm species where conduits with a d_c greater than 44 μm were predicted to cavitate in response to freezing and thawing at this xylem pressure.

Frozen conduit sap has been observed to contain a central, longitudinal series of small air bubbles rather than one large coalesced bubble (Sucoff 1969, Ewers 1985, Robson et al. 1988). This is consistent with the PLC_{FT} being related to conduit diameter, because if the freezing of sap is centripetal, then the bubbles not only should form in the center of the conduit, but their size should be a function of radial air content. If the bubbles coalesce, we would expect the PLC_{FT} of angiosperms to be greater than that of the conifers on account of the volume differences between vessels and tracheids, respectively (Sperry and Sullivan 1992). However, our results show this to be an unlikely pattern of bubble formation.

The r_b previously observed in frozen sap of *Juniperus* and *Pinus* spp. tracheids was about 2 μm (Sucoff 1969, Robson et al. 1988), which, according to Equation 1, means that bubbles of this size would require a P_x^* of only -0.15 MPa. However, the stem tracheid diameters of these species are typically less than 20 μm , so given our current data (Figure 3), these tra-

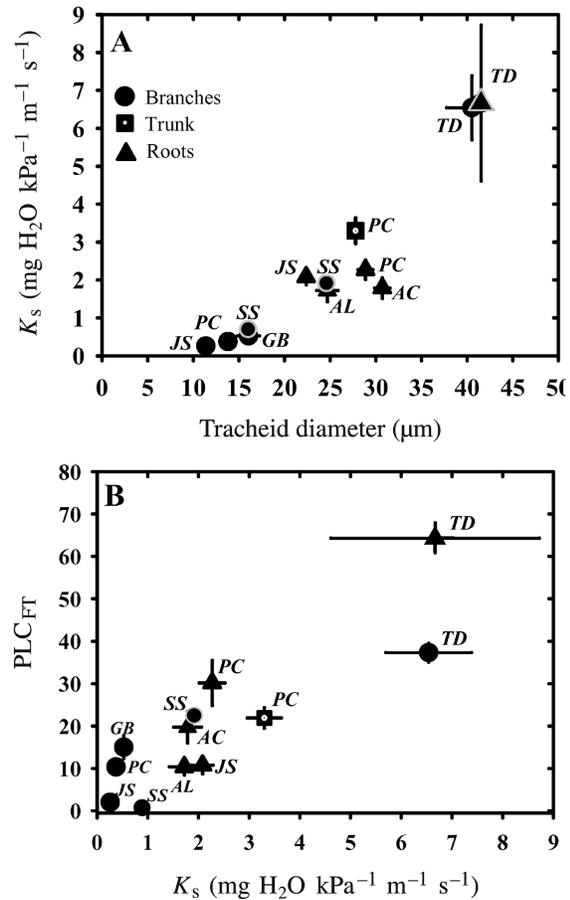


Figure 5. (A) Sapwood specific conductivity of conifer root, trunk and branch wood as a function of mean tracheid diameter. (B) Percent loss of conductivity due to freezing and thawing (PLC_{FT}) versus sapwood specific conductivity ($n \geq 6$; error bars may be smaller than symbols). Abbreviations: AC = *Abies concolor*; AL = *Abies lasiocarpa*; GB = *Ginkgo biloba*; JS = *Juniperus scopulorum*; PC = *Pinus contorta*; SS = *Sequoia sempervirens*; and TD = *Taxodium distichum*.

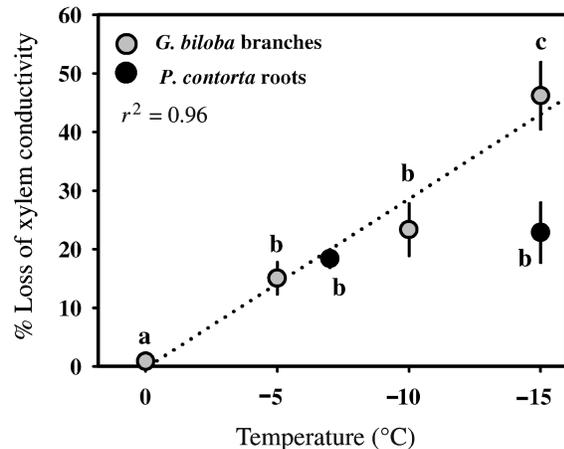


Figure 6. Percent loss of conductivity following freezing and thawing as a function of minimum freezing temperature in branches of *Ginkgo biloba* and *Pinus contorta* roots. Different letters denote significant differences between treatments at $P = 0.05$, Student's t -test ($n = 6$, error bars may be smaller than symbols).

cheids would not be expected to embolize even at -0.5 MPa (Sperry and Sullivan 1992, Davis et al. 1999). Apparently, the air bubbles shrink to less than the critical size (r_b^*) before the ice fully melts and negative pressures are reestablished. Rapid bubble shrinkage would be promoted by the steep diffusion gradient between the bubble and the degassed sap (Sucoff 1969).

Winter embolism values ranging from 35 to 100% have been reported in many temperate conifers (Sperry 1993, Sperry et al. 1994, Sparks and Black 2000, Mayer et al. 2002). Interestingly, mean tracheid diameters of the *Pinus*, *Picea*, *Larix* and *Abies* spp. examined in these studies ranged from only 13 to 18 μm (Panshin and de Zeeuw 1980, Sperry 1993, Sperry et al. 1994). Because these diameters are too narrow to be so highly embolized by freezing and thawing (Figure 3), the observed embolism was probably caused by other factors. Some studies have shown that winter embolism in conifers is related to the total time the xylem is above 0°C (Sparks and Black 2000, Mayer et al. 2002). During warm periods, transpiration and excessive wind and sun exposure can lead to water loss from the shoots (Hadley and Smith 1986, Lemoine et al. 1999, Améglio et al. 2002). This condition, when coupled with the inaccessibility of frozen water in the trunk and the soil, can conspire to significantly reduce stem water content (Sparks and Black 2000) or may cause P_x values to fall below -4 MPa (Lemoine et al. 1999, Mayer et al. 2002). This leads to drought-induced embolism, and even species with narrow tracheids may not be immune to this type of stress.

Conduit diameter is the key variable affecting the PLC_{FT} response, but other factors such as minimum freezing temperature, the P_x during the thaw and the actual rate of thawing may affect the degree to which wood will embolize. The PLC_{FT} is generally unrelated to minimum freezing temperature because bubble formation (and thus Equation 1) is independent of ice temperature (Langan et al. 1997). Hence, the lack of a PLC_{FT} response to minimum temperature in *P. contorta* roots is consistent with this explanation (Figure 6). However, we found that, in stems of *G. biloba*, PLC_{FT} increased as freezing temperatures became progressively lower (Figure 6). Pockman and Sperry (1997) reported similar findings for stems of *Larrea tridentata* (DC.) Cov., and tentatively attributed their results to damage to the living parenchyma tissue (see Ashworth et al. 1997, Ristic and Ashworth 1997). The PLC_{FT} may also be influenced by the magnitude of xylem pressure during the thaw. The d_c of conduits frozen and thawed at P_x below -0.5 MPa has not been empirically investigated, but assuming proportionality between d_c and r_b , Equation 1 suggests that the lower the xylem pressure during the thaw, the smaller the d_c , and the greater the PLC_{FT} . Lastly, Langan et al. (1997) showed that higher rates of sap thawing were consistent with increased PLC_{FT} in *Ceanothus megacarpus* Nutt. Feild and Brodribb (2001) observed similar trends whereby accelerated thaw rates presumably caused a PLC_{FT} response that was greater than the PLC_{FT} in Figure 3. A reasonable explanation for these data may be that the bubbles have less time to dissolve and shrink prior to the complete melting of ice and establishment of negative xylem pressure.

If temperate conifers had tracheids with broader diameters, then they would suffer the stress of freezing-induced cavitation in addition to the winter drought already thought to be responsible for their losses in conductivity. Certainly, large branches of *S. sempervirens*, the trunk of *P. contorta*, and roots in general, have wide-diameter tracheids and thus exhibit a high PLC_{FT} under laboratory conditions. Whether or not these organs embolize over the course of the winter season has not been rigorously documented, but some data show that *P. contorta* roots insulated by a layer of snow suffer little to no embolism when compared with exposed roots (J. Pittermann, unpublished results).

Conduit diameter not only affects PLC_{FT} , but is also coupled to species' K_s , as predicted from the Hagen-Poiseuille equation (Figure 5A; Tyree et al. 1994). Typical results show conifers with narrow tracheids to be resistant to winter embolism, but at the cost of having lower K_s compared with the vessel-bearing angiosperms (Davis et al. 1999, Feild and Brodribb 2001, Feild et al. 2002). Hence, the increased PLC_{FT} of these angiosperm species characterizes the expected trade-off associated with hydraulically efficient conduits. We extend this by demonstrating that these costs also apply to gymnosperms (Figure 5B). Specifically, organs such as roots and trunks, and those species that rarely freeze such as *T. distichum*, exhibit the highest K_s and thus the greatest PLC_{FT} .

Our results imply that, given an equivalent conduit width, conifers are no more resistant to freezing-induced embolism than angiosperms, and the traditional arguments for tracheid-based success of conifers over angiosperms in temperate zones (Wang et al. 1992, Lambers et al. 1998) need to be revised. Specifically, there is no apparent reason why narrow-vessel angiosperms should be any more vulnerable to freezing and thawing than gymnosperms with equivalent tracheid diameters. Feild et al. (2002) hypothesized that, in the Winteraceae clade, the reversal from vessels to tracheids was a derived adaptation to avoid freezing cavitation as species' distributions shifted from tropical, north Gondwanan habitats to south Gondwanan and alpine environments. If Winteraceae xylem was once vessel-based, the puzzle remains as to why these species did not avoid freezing-induced embolism by simply reducing their vessel diameters as some of the Northern Hemisphere temperate angiosperms have done, rather than revert to tracheids. This suggests that there may be additional factors driving the apparent vessel to tracheid reversal in the Winteraceae. Likewise, if narrow vessels are just as resistant to freezing-induced cavitation as tracheids, a factor other than the possession of tracheids may be responsible for the success of conifers in the Northern Hemisphere.

Although latitudinal trends in vessel diameters have been documented in deciduous angiosperms such as *Symplocos*, *Cornus* and *Quercus* (Baas 1990, Schuichi and Bass 2000, Cavender-Bares and Holbrook 2001), similar studies with variously distributed conifer species and their ecotypes have not been reported. It is conceivable then, that the distribution of conifers is also governed by their vulnerability to freeze-thaw embolism, such that species have optimized their xylem anatomy to balance the safety-efficiency trade-offs described

above. Certainly, the Podocarpaceae and Araucariaceae species inhabiting the humid, tropical forests of the Southern Hemisphere would be a robust test of this hypothesis, as they are thought to have evolved in response to light availability rather than sub-zero temperatures (Brodribb and Hill 1997, 1999). In the absence of sub-zero temperatures, we may expect selection to favor large-diameter tracheids that maximize hydraulic conductivity, rather than safety against freezing-induced cavitation.

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