

Xylem cavitation caused by drought and freezing stress in four co-occurring *Juniperus* species

Cynthia J. Willson^{a,*} and Robert B. Jackson^{a,b}

^aDepartment of Biology, Duke University, Durham, NC 27708, USA

^bNicholas School of the Environment and Earth Sciences, Duke University, Durham, NC 27708, USA

Correspondence

*Corresponding author,
e-mail: cjw@duke.edu

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Previous studies indicate that conifers are vulnerable to cavitation induced by drought but in many cases, not by freezing. Rarely have vulnerability to drought and freezing stress been studied together, even though both influence plant physiology and the abundance and distribution of plants in many regions of the world. We studied vulnerability to drought- and freezing-induced cavitation, along with wood density, conduit reinforcement, tracheid diameter, and hydraulic conductivity, in four *Juniperus* species that typically occupy different habitats, but uniquely co-occur at the same site in Arizona, AZ. We combined drought with a freeze-thaw cycle to create freezing-induced vulnerability curves. All four species demonstrated greater vulnerability to drought + freezing- than to drought-induced cavitation alone ($P < 0.0001$). Mean tracheid diameter was correlated with vulnerability to drought + freezing-induced cavitation ($r = 0.512$, $P = 0.01$). The vulnerability to cavitation of each species followed expected rankings based on relative moisture within each species' natural distribution. Species with naturally drier distributions showed greater resistance to both drought- and drought + freezing-induced cavitation. Even conifer species with relatively small tracheid diameters can experience xylem embolism after a single freeze-thaw cycle when under drought stress.

Introduction

Drought and freezing are important determinants of plant distribution. Both can cause xylem cavitation, followed by the entry of air from surrounding tissues, leading to an embolized, or air-filled, xylem conduit that becomes non-functional (Sperry and Sullivan 1992). For this reason, plant water transport, growth, and survival are limited by xylem cavitation (Jackson et al. 2000, Sperry and Tyree 1990, Sperry et al. 1994, Tyree and Dixon 1986). Drought-induced cavitation has been proposed as a limiting factor in the distributions of both angiosperms and conifers (Brodribb and Hill 1999,

Pockman and Sperry 2000). Freezing-induced cavitation has also been suggested to influence treeline and latitudinal limits of species distributions (Mayr et al. 2003b, Pockman and Sperry 1997, Sparks and Black 2000). Nevertheless, drought- and freezing-induced cavitation are rarely studied together, despite their co-occurrence in many habitats in nature. The goal of our research was to use four co-occurring, related species to explore links between species distributions, xylem anatomy, and vulnerability to both drought- and freezing-induced cavitation.

Although both drought- and freezing-induced cavitation are related to water potential (ψ) in the xylem, they

Abbreviations – P50, absolute value of the xylem water potential at 50% loss of conductivity; PLC, percent loss of conductivity.

occur via different mechanisms. Drought stress causes xylem cavitation when ψ decreases below xylem-specific thresholds such that air enters from adjacent air-filled and cavitated conduits ('air seeding', Zimmermann 1983). In conifers, drought-induced cavitation occurs when the thickened torus is displaced from its sealing position. Theoretically, the structure of the torus-margo complex in conifers determines vulnerability thresholds for drought-induced cavitation (Hacke et al. 2004). Alternatively, freezing-induced cavitation occurs when xylem sap freezes and bubbles form centripetally in conduit lumens, because air is soluble in liquid but not in ice (Robson et al. 1988, Sucoff 1969, Zimmermann 1983). Upon thawing, the air bubbles may either re-dissolve or expand to fill the conduit and nucleate cavitation. This outcome depends on surface tension forces, xylem ψ , and the bubble radius (Davis et al. 1999, Sperry and Sullivan 1992, Sperry et al. 1994). A bubble is more likely to expand following a freeze-thaw if the xylem ψ present before freezing is low (Yang and Tyree 1992). Larger xylem conduits contain more air in solution and have larger diameter bubbles that more easily expand during a thaw (Davis et al. 1999, Pittermann and Sperry 2003). The bubbles formed in smaller conduits more readily re-dissolve upon thawing (Davis et al. 1999, Hammel 1967, Robson et al. 1988, Sucoff 1969). Freezing-induced cavitation in conifers is generally avoided, because tracheids are small in diameter (<30 μm in most species) compared with vessels of angiosperms.

Conduit diameter affects not only freezing-induced cavitation but also hydraulic conductivity as described by the Hagen-Poiseuille equation (McElrone et al. 2004, Tyree et al. 1994). Plants with narrow conduits are likely to be resistant to freezing-induced cavitation. This will, however, probably result in lower hydraulic conductivities of conifer xylem relative to vessel-bearing angiosperm xylem (Davis et al. 1999, Feild and Brodribb 2001, Feild et al. 2002, Maherali et al. in press). Another potential tradeoff involves the mechanical vs hydraulic function of xylem. For conifers, greater resistance to drought-induced cavitation is associated with thicker cell walls in relation to lumen diameter to withstand compressive forces and protect against implosion (Hacke et al. 2001). Greater wall thickness per unit diameter in conifers translates into greater wood density and, presumably, higher construction cost. Conduit construction vs safety from drought-induced cavitation and conduit diameter vs safety from freezing-induced cavitation tradeoffs are well supported (Davis et al. 1999, Hacke et al. 2001, Pittermann and Sperry 2003). How these tradeoffs influence hydraulic transport across taxa is unclear (Maherali et al. 2004).

The only experimental evidence for a conifer with narrow (<30 μm) conduit diameters vulnerable to a single freeze-thaw cycle is for *Juniperus scopulorum*, which showed small but significant effects at low ψ (Sperry and Sullivan 1992). Sperry and Sullivan (1992) proposed that other drought-resistant conifers might also exhibit vulnerability to freezing. Few studies have evaluated physiological differences between *Juniperus* spp., a generally drought-resistant group of conifers. Although they typically occupy semi-arid habitats, they are also found in mesic habitats, from sea level to near timberline and, in the New World, from British Columbia to Mexico. We studied cavitation as a result of both drought and freezing stress in a natural population of four co-occurring *Juniperus* species in northern Arizona, AZ. Because *Juniperus* spp. in the western United States differ in distribution based on elevation and latitude, we hypothesized that species' vulnerability to either drought- or freezing-induced cavitation would differ according to the degree of drought or freeze-thaw events they normally experience and that this would parallel the qualitative ranking shown in Fig. 1.

We measured vulnerability to xylem cavitation and associated anatomical characteristics in plants of each species to test four main hypotheses. First, species that typically occupy the more arid habitats of the four species will be less vulnerable to drought-induced cavitation. Second, wood density and conduit wall reinforcement will increase as vulnerability to drought-induced cavitation increases. Third, species that typically occupy the colder habitats of the four species will be less vulnerable to freezing-induced cavitation. We combined drought with freezing to create freezing-induced vulnerability curves. Fourth, tracheid diameter will increase as vulnerability to freezing-induced cavitation increases. Many *Juniperus* spp. are expanding into or invading more arid environments throughout the western and central United

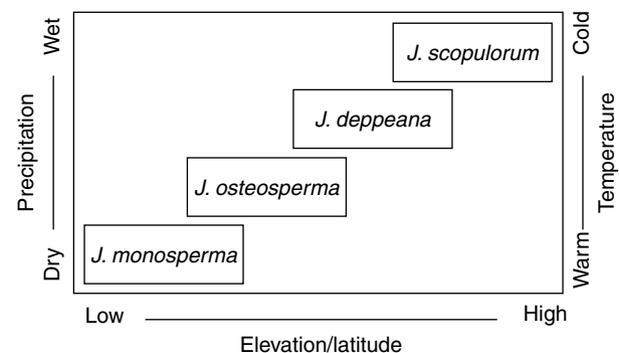


Fig. 1. Segregation of *Juniperus* species along elevation/latitude, moisture, and temperature gradients, based on data from Thompson et al. (1999).

States (Jackson et al. 2002). One of the major goals of our study is to provide clues toward understanding each species' distribution and, potentially, their ability to survive in newly invaded habitats.

Materials and methods

Plant material

We studied four co-occurring juniper species, *Juniperus deppeana* Steud., *Juniperus monosperma* (Engelm.) Sarg., *Juniperus osteosperma* (Torr.) Little and *J. scopulorum* Sarg., at Walnut Canyon National Monument in northern Arizona, AZ (35°10'N, 111°31'W; 2042 m). Mean annual precipitation for the site is 470 mm. Rainfall distribution is bimodal with peaks in March and August. Mean minimum and maximum temperatures are -9°C (January) and 28°C (July). An assemblage of Great Basin, montane, and desert southwest plants occurs at Walnut Canyon National Monument, which offers a unique opportunity to study four *Juniperus* species from diverse habitat types that are growing side by side. The woodland also contains *Pinus edulis* and *Pinus ponderosa*.

In August 2003, we collected 6–7 branches from each of six adult trees per species, with a minimum of 36 branches per species. All plant material was collected before 11:00 h local time to minimize drought stress. Canopy heights varied from 2 to 5 m, and branches were collected from the bottom third of the canopy. Branches were selected to have diameters of 0.7–1.1 cm and a minimum length of 30 cm without side branches. Branches were immediately enclosed with damp paper towels, triple bagged in plastic bags, and placed in a cooler to inhibit desiccation during transit to the University of New Mexico. Vulnerability curves were started in the laboratory within 3 days of collection.

Drought-induced vulnerability curves

The relationship between xylem tension and embolism can be described by a vulnerability curve. To develop drought- and freezing-induced vulnerability curves, we used the dehydration method, where branches with leaves intact are left to dehydrate in the laboratory until a desired water potential is reached (e.g. Sperry and Sullivan 1992, Sperry et al. 1988). Embolism was quantified as a decrease in xylem hydraulic conductivity (K_H) after either a drought stress or a drought stress plus freeze-thaw treatment. To measure K_H , we used a modification of the 'adjacent segment' method (Sperry and Tyree 1990). Along each branch, three 5-cm-long segments separated by 2–3 cm were marked with '1' for

basal, '2' for middle, and '3' for most proximal. Segment 1 of the branch was excised immediately upon arrival at the laboratory, and hydraulic conductivity (K_{H1}) was measured to determine a maximum K_H value. Segment 2 was cut after the drought treatment, and segment '3' was cut after the drought plus a freeze-thaw (drought + freeze) treatment. After the segment was cut underwater and 1 cm of bark removed from each end, the ends were shaved with a fresh razor blade and mounted in a tubing manifold to measure hydraulic conductivity as described in Sperry et al. (1988). Hydraulic conductivity (K_H , $\text{kg m MPa}^{-1} \text{ s}^{-1}$) is defined as the mass flow rate of water through a segment divided by the pressure gradient along the segment. It was measured with deionized, degassed, and filtered (0.22 μm) water under a gravity-induced pressure head of 5–6 kPa with an electronic balance connected to a computer. Because flushing conifer stems has been reported to be problematic or ineffective (Sperry and Tyree 1990), we conducted a pilot study, in which we flushed a subset of stems (collected in the morning during the August monsoon to minimize native emboli) and found negligible native embolism. Therefore, we did not flush stems in this study. Average tracheid lengths for *Juniperus* species are 2 mm or less (Panshin and de Zeeuw 1980); hence, no embolism induced by cutting should reach each segment.

After measuring segment 1 to obtain K_{H1} , stems were allowed to air dry to create a drought-induced vulnerability curve. After air drying to desired levels, stems were sealed in dark plastic bags for at least 90 min for equilibration. After equilibration, leaf water potentials (ψ) were measured on 1 or 2 branchlets (approximately 2 cm long) per stem with a Scholander pressure chamber (PMS Instruments, Corvallis, OR). Then segment 2 was cut underwater, and K_{H2} was measured. The equation percent loss of conductivity (PLC) = $(1 - K_{H2}/K_{H1})100$ yielded the PLC resulting from drought stress (PLC_{drought}). After removing segment 2, the branch was subjected to a freeze-thaw cycle.

Freezing-induced vulnerability curves

In addition to drought-induced vulnerability curves, where we measured only the effects of negative ψ , we also created freezing-induced vulnerability curves, where we determined the combined effects of negative ψ followed by a freeze-thaw cycle. Freezing and thawing took place in the freezing chamber described by Martinez-Vilalta and Pockman (2002), similar to that illustrated by Langan et al. (1997). Prior to beginning our study, we tested whether there was any chamber effect on water potentials or hydraulic conductivity. We

placed 10 stems inside the chamber and maintained temperature at approximately 10°C overnight. There was no significant difference in water potentials or hydraulic conductivity before and after the approximately 12-h cycle in the chamber with no freezing treatment. For the freeze-thaw treatment, the water bath controlling the chamber temperature was programmed for cooling and heating rates of 1°C min⁻¹ above 0°C, equilibration at 0°C for 30 min, and freezing and thawing rates of 0.2°C min⁻¹ below 0°C. The freezing and thawing rates were similar to those of Pittermann and Sperry (2003). Time at minimum freezing temperature of -15°C was 90 min. After the freeze-thaw treatment, the third segment, 3, was cut underwater from each sample and its conductivity (K_{H3}) measured. Water potentials after treatment were measured for a subset of branches to verify that ψ did not change from the pre-freeze-thaw value. The PLC of segment 3 was obtained after the branch had undergone two treatments, dehydration followed by a freeze-thaw event (PLC_{freeze}), and was calculated as PLC_{freeze} = (1 - K_{H3}/K_{H1})100. Thus, the term freezing-induced cavitation used throughout this article refers to a vulnerability curve produced by a drought treatment followed by a freeze-thaw (drought + freezing) treatment.

Specific conductivities and Huber value

Specific hydraulic conductivity (K_S , kg m⁻¹ MPa⁻¹ s⁻¹; a measure of conducting efficiency and sapwood permeability) was calculated as maximum K_H divided by the cross-sectional xylem area of the segment. Leaf-specific hydraulic conductivity (K_L , kg m⁻¹ MPa⁻¹ s⁻¹) was calculated as maximum K_H divided by the distal leaf area supported by the segment. Leaf area was determined from a projected leaf area : leaf dry mass relationship based on a subsample of leaves per stem. The Huber value (HV), the ratio of stem area to distal leaf area supported (Tyree and Ewers 1991), was calculated as the cross-section of conducting tissue (m²) divided by leaf area distally supported (m²).

Xylem conduit dimensions and wood density

To examine the relationship between cavitation resistance and conduit diameters, we measured tracheid diameters on segments used for vulnerability curves. Transverse sections (20–30 µm thick) were cut from 12 branches per species using a sliding microtome (American Optical Co., Buffalo, NY). The sections were stained with Toluidine Blue, then rinsed in deionized water and mounted in glycerol on a microscope slide. Images (at four randomly selected locations along

each of 3–4 radial axes 90° apart) were captured for one section per stem with a Nikon CoolPix 990 digital camera (Nikon Inc., Melville, NY) mounted on a light microscope (Nikon E400). A minimum of 500 tracheids was analysed per section. After these images were downloaded to a computer, we measured lumen area of all intact tracheids with image analysis software (SCION IMAGE v. β4.02 for Windows, Scion, Frederick, MD). Because the tracheids were roughly square rather than circular in shape, tracheid diameter (d , in µm) was determined to be equal to the side of a square with an area equal to the lumen cross-section. To determine the functional significance of conduit size distribution, we calculated hydraulic mean diameters (d_h , in µm) as described in Kolb and Sperry (1999).

To examine the relationship between cavitation resistance and construction costs, we also measured the reinforcement of conduit walls against implosion, and a related trait, wood density. Conduit wall reinforcement [$(t/b)^2$] was measured using the same images used for determining d and d_h (Hacke et al. 2001). We measured maximum span across a tracheid (b) and thickness of the double wall between the pair (t) on a minimum of 50 pairs of tracheids with diameters within 2 µm of d_h using SCION IMAGE. Wood density (D_t ; dry weight per fresh volume, g cm⁻³) was measured on the same segments used for vulnerability curves. Segments 2.5-cm-long were cut from stems without heartwood. The bark was peeled away, and fresh volume was determined as the volume of a cylinder. The segments were then oven dried at 75°C for 72 h to obtain dry weight.

Statistical analyses

Statistical analyses were carried out using SAS (v.9.1, SAS Institute Incorporated, Cary, NC) and JMP IN software (v.5.1, SAS Institute Incorporated). P_{50} values represent the absolute value of the ψ inducing a PLC of 50% on a vulnerability curve. P_{50} values were estimated by fitting the following sigmoidal function using the non-linear mixed model procedure in SAS: PLC = 100 / {1 + exp[a(P-b)]}, where P is the tension in the xylem, a is proportional to the slope of the vulnerability curve and b is P_{50} (Pammenter and Vander Willigen 1998). Unless otherwise stated, significant differences were determined with one-way ANOVA for species (*J. scopulorum*, *J. deppeana*, *J. osteosperma*, *J. monosperma*), followed by multiple comparisons with Tukey's HSD using JMP ($\alpha = 0.05$). Relationships between $P_{50\text{drought}}$ or $P_{50\text{freeze}}$ and median climate values for each species' distribution (Thompson et al. 1999) or between $P_{50\text{drought}}$ or $P_{50\text{freeze}}$ and xylem anatomical traits were analysed with Pearson correlation tests using JMP.

Results

All four juniper species were more vulnerable to drought + freezing-induced cavitation than to drought-induced cavitation alone (Fig. 2; two-sided

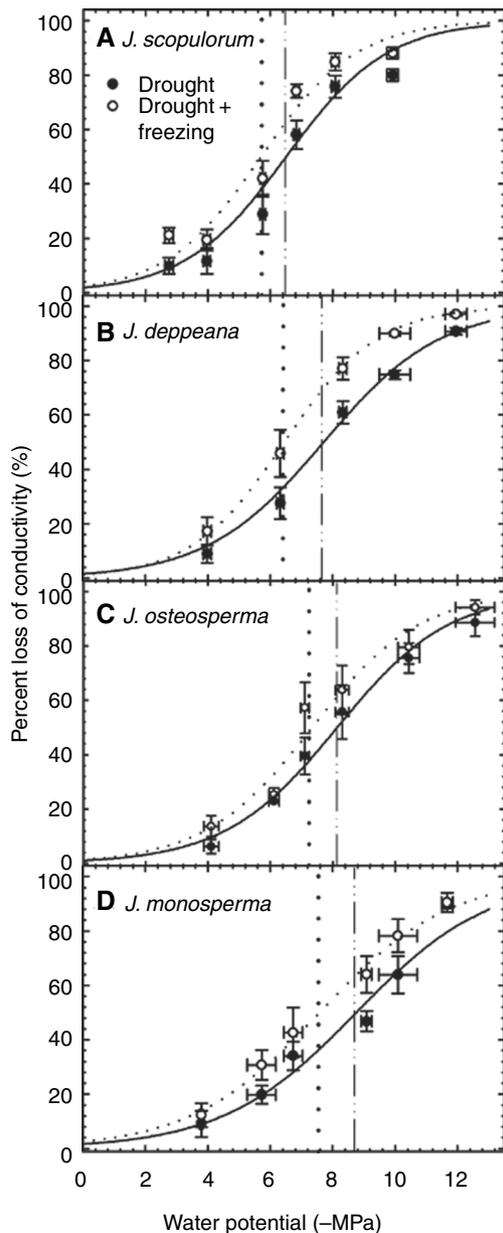


Fig. 2. Drought- and freezing-induced vulnerability curves for four co-occurring *Juniperus* species, showing the relationship between xylem tension (–MPa) and corresponding loss of hydraulic conductivity. Data points were determined with the dehydration method with a minimum of 36 branches per site and per species. The dotted vulnerability curves are freezing-induced, and the solid curves are drought-induced. Vertical lines cross vulnerability curves at P_{50} , the xylem tension inducing 50% loss of conductivity. For each species, the vertical line on the left indicates $P_{50\text{freeze}}$, and the vertical line on the right indicates $P_{50\text{drought}}$.

paired *t*-test $P < 0.0001$). When $P_{50\text{drought}}$ and $P_{50\text{freeze}}$ are compared, species were more vulnerable to freezing (drought + freeze) as follows: *J. scopulorum* was 0.82 MPa, *J. deppeana* was 1.34 MPa, *J. osteosperma* was 0.57 MPa, and *J. monosperma* was 1.22 MPa more vulnerable to freezing than to drought (Fig. 2).

Vulnerability to drought-induced cavitation ($P_{50\text{drought}}$) followed the order expected by elevation/latitude for the typical distribution for each species, as predicted in Fig. 1. Of all the species, *J. scopulorum* was the most vulnerable to drought-induced cavitation and was significantly more vulnerable than *J. osteosperma* and *J. monosperma* (Fig. 3A; $P = 0.0019$). The four species decreased in vulnerability to both drought- and freezing-induced cavitation as follows: *J. scopulorum* > *J. deppeana* > *J. osteosperma* > *J. monosperma* (Fig. 3A). As with drought-induced cavitation, *J. scopulorum* was most vulnerable to freezing-induced cavitation and significantly more vulnerable than *J. osteosperma* and *J. monosperma* (Fig. 3A; $P = 0.0048$).

The same trend in species order observed for vulnerability to drought- and freezing-induced cavitation was seen for conduit wall reinforcement [$(t/b)^2$; cf. Fig. 3A, B]. The most vulnerable species, *J. scopulorum*, had the lowest conduit wall reinforcement, whereas the most resistant species, *J. monosperma*, had significantly greater wall reinforcement (Fig. 3B; $P = 0.036$). Three of the four species, *J. deppeana*, *J. osteosperma*, and *J. monosperma*, increased in wood density in a manner similar to that observed for $(t/b)^2$, whereas *J. scopulorum* had higher wood density than expected (Fig. 3C; $P = 0.0028$). Neither $P_{50\text{drought}}$ nor $P_{50\text{freeze}}$ were significantly correlated with $(t/b)^2$, however (data not shown; $P > 0.14$ for each), nor with wood density ($P > 0.30$ for each).

As with wood density, tracheid diameters (d and d_h) of *J. scopulorum* strayed slightly from the trend seen among all species for P_{50} (cf. Fig. 3A, D). In conifers, wood density and tracheid diameter are commonly related, with smaller diameters leading to greater density. The species with the highest wood density (Fig. 3C), *J. monosperma*, had the smallest d and d_h (Fig. 3D). Similarly, the species with the lowest wood density, *J. deppeana*, had the largest d and d_h . Overall, wood density and d_h were negatively correlated (data not shown; $r = -0.476$, $P = 0.016$). Also, wood density and K_s were negatively correlated, reflecting the role of d_h in hydraulic conductivity (data not shown; $r = -0.459$, $P = 0.021$).

Three of the four species showed a trend of decreasing tracheid diameters with decreasing vulnerability to

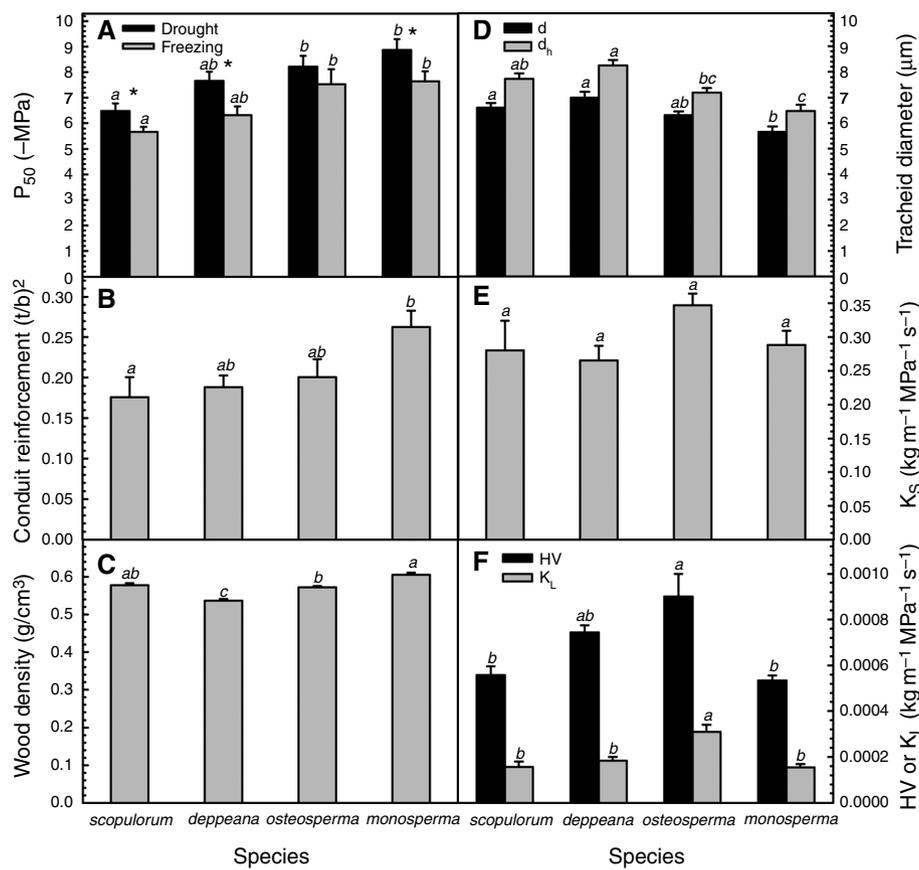


Fig. 3. (A) P_{50} values (xylem tension in $-MPa$ corresponding to a 50% loss of conductivity) from drought- and freezing-induced vulnerability curves, (B) conduit wall reinforcement, (C) wood density, (D) mean tracheid diameter (d) and hydraulic mean diameter (d_h), (E) specific conductivity (K_S), and (F) leaf-specific hydraulic conductivity (K_L) and Huber value for stems of four co-occurring *Juniperus* species (± 1 SE). Different letters above bars shaded similarly, or asterisks between bars shaded differently (panel A), indicate statistically significant differences.

freezing-induced cavitation (cf. Fig. 3A, D). Because patterns among species were similar for both drought and drought + freezing stresses, tracheid diameter size also decreased with decreasing vulnerability to drought-induced cavitation. Hydraulic diameters were approximately 15% larger than mean tracheid diameters for the four species (Fig. 3D). Overall, P_{50} values for vulnerability to both freezing- and drought-induced cavitation were significantly correlated with hydraulic diameter such that greater resistance was associated with smaller d_h values (Fig. 4A). Only vulnerability to freezing, however, was significantly correlated with mean tracheid diameter (Fig. 4B).

Specific conductivity (K_S) was not significantly different among species, as it varied widely by branch (Fig. 3E). HV and leaf-specific conductivity (K_L) increased with resistance to cavitation for three of the four species (Fig. 3F; $P < 0.0001$). There was no evidence for a tradeoff between conductivity (K_S or K_L) and either $P_{50drought}$ or $P_{50freeze}$ (data not shown; $r < 0.255$ and $P > 0.23$ for all).

Discussion

Many conifers with narrow conduit diameters are vulnerable to drought-induced cavitation, but do not show

hydraulic conductivity losses following freezing (Feild and Brodrigg 2001, Hammel 1967, Sperry and Sullivan 1992, Sperry et al. 1994, Sucoff 1969). In *Juniperus*, on the other hand, we found that greater cavitation results when drought is followed by a freeze-thaw cycle than when drought occurs alone (Fig. 2). We demonstrated this phenomenon by examining vulnerability curves created by drought and a combination of drought + freezing stress in four co-occurring *Juniperus* spp., which are among the most resistant conifers to drought-induced cavitation (Maherali et al. 2004). In accordance with a previous study of *J. scopulorum* (Sperry and Sullivan 1992), our results suggest that *Juniperus* spp. experience greater embolism because of combined drought and freezing stress than to drought stress alone. As drought stress before a freeze-thaw cycle increased, a freeze-thaw cycle in *Juniperus* spp. appears to induce cavitation, whereas in most other conifers, drought-induced cavitation would have already occurred.

Our results suggest that differences in water stress adaptation between four *Juniperus* spp. may contribute to their different habitats and distributions. Although we could not eliminate possible differences in rooting depth

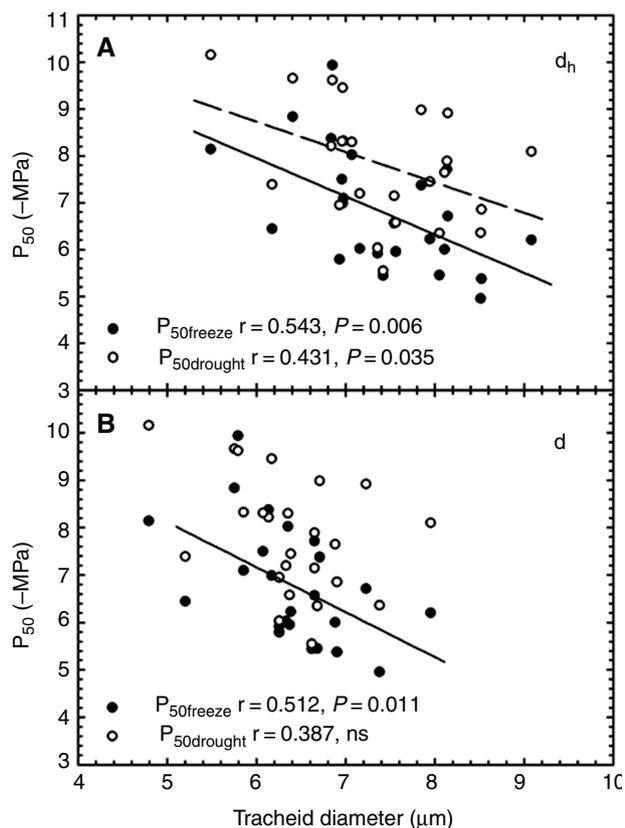


Fig. 4. Correlations between $P_{50drought}$ or $P_{50freeze}$ and (A) hydraulic mean diameter (d_h) and (B) mean tracheid diameter (d) of stems for four species.

or microclimate among the four species in our study, we could eliminate macro-scale differences in substrate and climate by selecting a study site where the four species naturally co-occur. We predicted that *J. scopulorum*, which usually occurs either at low elevations at higher latitudes or at higher and more mesic elevations at low latitudes, would be the least drought resistant and most freezing resistant (Fig. 1). In contrast, we predicted that *J. monosperma*, typically found at low elevations at low latitudes, would be the most drought resistant and least freezing resistant, while *J. deppeana* (high elevations at low latitudes) and *J. osteosperma* (dry summers and cold, wet winters) would exhibit intermediate resistance. As predicted, species that typically occupy drier environments were more resistant to drought-induced cavitation (Figs 1 and 2). Results for freezing vulnerability, however, did not follow expected species rankings (Figs 1 and 2). Rather, all the four species were more vulnerable to drought + freezing than to drought to a similar degree, 0.7–1.3 MPa (Fig. 3A). In studies utilizing natural populations and common gardens, similar differences in vulnerability to drought-induced cavitation between species, subspecies and even varieties

have been shown to result from genetic differentiation rather than phenotypic plasticity (Kavanagh et al. 1999, Kolb and Sperry 1999).

Neither $P_{50drought}$ nor $P_{50freeze}$ were correlated with median annual temperature, moisture index, median July temperature or median January temperature within the distribution of each species (data not shown). The lack of correlation between P_{50} and these climate data is probably because median values may not capture critical differences in species distributions. Distribution limits may be better correlated with climatic extremes (e.g. Brodribb and Hill 1999). For example, Pockman and Sperry (1997) found that the northern limit of distribution in *Larrea tridentata* corresponded with record minimum temperatures. In the mountainous terrain where many juniper species grow in the western United States, nearby weather stations may not always be close enough to give accurate estimates of climate. Moreover, junipers often occur on rocky substrates. Accordingly, in *Juniperus* spp., median values for climate data may not accurately reflect water limitation nor correspond to cavitation resistance.

Conduit diameter is a critical factor in determining freezing-induced cavitation. In other studies of plants subjected to a single freeze-thaw event, conifers and angiosperms with mean conduit diameters $<30 \mu\text{m}$ do not cavitate, $>30 \mu\text{m}$ cavitate extensively, and greater than approximately $43\text{--}44 \mu\text{m}$ cavitate completely (Davis et al. 1999, Pittermann and Sperry 2003). Those freeze-thaw studies were conducted under conditions of essentially no water stress ($\psi = -0.5 \text{ MPa}$). In contrast, we examined the response of conductivity to a single freeze-thaw event over a range from mild to high water stress. With mean conduit diameters of approximately $6 \mu\text{m}$, the species in our study were well under the approximately $30 \mu\text{m}$ threshold observed to induce losses in conifers, and in fact, our results under little to mild water stress (approximately 0 to -4 MPa) are similar to those of Pittermann and Sperry (2003). Thus, the vulnerability to freezing-induced cavitation of the *Juniperus* spp. in our study is not due to the species having larger tracheids than other conifers. Another trait related to conduit diameter, hydraulic conductivity (K_S or K_L) was highly variable among branches and species (Fig. 3F). Although we found no support for a tradeoff between resistance to cavitation and hydraulic conductivity, we demonstrated an association of greater resistance to freezing-induced cavitation with smaller tracheid diameters (Fig. 4).

Our study of four co-occurring *Juniperus* species subjected to drought stress suggests that a single freeze-thaw is sufficient to induce freezing cavitation if the water potential of the stem is very low, as is often the case in a

drought-tolerant species. Other laboratory studies on drought-stressed conifers found no loss of conductivity induced by one or a few freeze-thaw cycles, perhaps because for almost all conifers with relatively narrow conduit diameters studied, the xylem pressure required for bubble expansion is more negative than that causing drought-induced cavitation first (Sperry and Sullivan 1992, Sperry et al. 1994). More recent studies indicate that freezing-induced cavitation can occur in conifers with relatively narrow tracheids but only after a combination of drought stress and numerous cumulative freeze-thaw cycles (e.g. Sparks et al. 2001). For example, *Picea abies* and *Pinus cembra* had P_{50} values 0.6 and 0.4 MPa more vulnerable compared with drought stress alone after 50 freeze-thaw cycles and 1.8 and 0.8 MPa more vulnerable after 100 freeze-thaw cycles (Mayr et al. 2003a). These shifts are similar in magnitude to those after only a single freeze-thaw cycle in angiosperms (Davis et al. 1999) and in our study (Fig. 3A). In studies where xylem embolism is correlated with the number of freeze-thaw cycles, there may be an additive effect of small cavitation events from each cycle.

The freezing vulnerability observed in *Juniperus* spp. may be related to an interactive effect of drought and freezing. At low to moderate drought stress (e.g. approximately 0 to -4 MPa), there was little difference between vulnerability curves created by drought alone and by a combination of drought + freezing (Fig. 2). As drought stress before the freeze-thaw increased ($\psi < \text{approximately } -4$ MPa), *Juniperus* spp. experienced greater losses of hydraulic conductivity when subjected to a freeze-thaw cycle than when subjected to drought alone (Fig. 2). Freezing-induced cavitation is favored by greater bubble size, which is a function of conduit diameter and also by lower ψ (Yang and Tyree 1992). We might have expected that the freezing- and drought-induced vulnerability curves would have continued to diverge as ψ decreased (Fig. 2). We instead found, for $\psi < \text{approximately } -4$ MPa, that the drought + freeze curves showed a relatively constant approximately 10–20% greater loss of conductivity compared with drought alone. Ice has a much lower ψ than water. As a result, extracellular ice crystals in wood extract water and lower the ψ of liquid water in neighboring conduits, leading to further dehydration. Some species may have cell walls that resist the subsequent reduction in cellular volume, thereby limiting the extent of dehydration (Pearce 2001). The dehydrating effect of ice and the distribution of conduit diameters in the stem may be contributing factors, but exactly how drought and freezing stress interact to affect xylem cavitation warrants further investigation.

The suite of anatomical characteristics predicted among species generally held for three of the four

species, excluding *J. scopulorum*. Conduit reinforcement, wood density, and P_{50} increased while d and d_h all generally decreased in the following order: *J. scopulorum* > *J. deppeana* > *J. osteosperma* > *J. monosperma* (Fig. 3). *J. scopulorum* presented an exception for wood density, d and d_h . *J. scopulorum* may differ from the other three species because of the evolutionary relationships of the four species. *J. scopulorum* belongs to a different phylogenetic group than the other three species based on the presence or absence of fine leaf margin serration. The evolutionary relationships of traits involved in xylem structure and function are a promising avenue for further work to understand variation in hydraulic traits among taxa (e.g. Maherali et al. 2004, Reich et al. 2003).

In summary, we found that freezing-induced cavitation can occur after a combination of drought and a single freeze-thaw event in conifers that are highly resistant to drought-induced cavitation. We also found that vulnerability to drought-induced cavitation was more related than freezing-induced cavitation to *Juniperus* species distribution. Although conifers may be relatively resistant to freezing stress because of their dependence on small-diameter tracheids for both support and water conduction, even species with small tracheid diameters can be susceptible to freezing-induced cavitation.

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