American Journal of Botany 89(12): 1916–1924. 2002.

THE VULNERABILITY TO FREEZING-INDUCED XYLEM CAVITATION OF *LARREA TRIDENTATA* (ZYGOPHYLLACEAE) IN THE CHIHUAHUAN DESERT[1]

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The temperature dependence of freezing-induced xylem cavitation was studied in a Chihuahuan desert population of *Larrea tridentata* (Zygophyllaceae). Field measurements of wood temperature and xylem embolism were combined with anatomical studies and laboratory measurements of embolism in stem and root samples frozen under controlled conditions. Our laboratory experiments corroborated the previously observed relationship between minimum freezing temperature and embolism. The area of the low-temperature exotherms produced during the freezing treatments was correlated with the resulting embolism, suggesting that the freezing of water inside parenchyma cells is associated with the occurrence of xylem embolism. In the laboratory experiments, embolism in stems increased only at temperatures below −14°C. Although this meant that the studied population was more resistant to freezing-induced xylem embolism than a previously studied population from the Sonoran desert, the impact of freezing was nevertheless greater because of much lower environmental temperatures. This result suggests that dieback associated with periodic extreme freezes may contribute to limiting the present distribution of *L. tridentata* in central New Mexico. Although laboratory experiments showed that root xylem embolism increased after freezing to less negative minimum temperatures than stems (significant effects at $T = -7$°C), root embolism in the field was lower than shoot embolism in accordance with measured soil temperatures throughout the study.

Key words: central New Mexico; freezing; *Larrea tridentata*; low-temperature exotherm; water transport; xylem embolism; Zygophyllaceae.

Low temperatures and, in particular, freezing injury, have been reported as the main factors limiting plant distribution in many habitats (Burke et al., 1976; Sakai and Larcher, 1987; Woodward, 1987). While supercooling (Rada et al., 1985) and other mechanisms (e.g., Beck et al., 1984) delay the freezing of water in living plant cells, freezing in xylem conduits (the apoplast) generally occurs within a few degrees below 0°C. Among the consequences of apoplastic freezing are cell dehydration and collapse (Rajashekar and Lafta, 1996) and the disruption of long-distance water transport as a result of xylem embolism (Zimmermann, 1983; Tyree and Sperry, 1989). The vulnerability to freezing-induced xylem embolism and its ecological significance have been studied in a number of species, including conifers (Sperry and Sullivan, 1992; Sperry et al., 1994), diffuse-porous (Sperry and Sullivan, 1992; Hacke and Sauter, 1996; Langan, Ewers, and Davis, 1997), and ring-porous species (Cochard and Tyree, 1990; Sperry and Sullivan, 1992; Hacke and Sauter, 1996).

Freezing-induced xylem embolism occurs because the solubility of air is lower in ice than in liquid water. As a result, freezing forces air out of the xylem sap solution forming bubbles (Lybeck, 1959; Sucoff, 1969; Zimmermann, 1983). On thawing, these bubbles can either re-dissolve or nucleate the cavitation of water inside the conduits depending on the balance between the surface tension forces and xylem pressure (Sperry and Sullivan, 1992; Davis, Sperry, and Hacke, 1999). The cavitation of water inside a conduit is followed by the entry of air from surrounding tissues until the conduit becomes air-filled or embolized (Tyree and Sperry, 1989). An embolized conduit no longer contributes to water transport unless it is refilled by liquid water. In principle, refilling can only occur if xylem pressure is close to the atmospheric pressure. However, active refilling has been recently observed even at high xylem tensions (Salleo et al., 1996; Zwieniecki and Holbrook, 1998; Tyree et al., 1999), and possible mechanisms have been proposed (Salleo et al., 1996; Holbrook and Zwieniecki, 1999; Zwieniecki and Holbrook, 2000; but see Steudle, 2001).

We might expect that roots should be more vulnerable to freezing-induced cavitation than branches. Roots tend to have much wider conduits than branches (Zimmermann, 1983; Ewers et al., 1997), and the relationship between conduit size and vulnerability to embolism caused by freezing is well established (Tyree, Davis, and Cochard, 1994; Davis, Sperry, and Hacke, 1999). Although the soil is a good insulator, minimum soil temperatures can easily fall below zero near the surface, even in temperate areas. In a recent study, Jaquish and Ewers (2001) showed that seasonal levels of embolism remained much lower in surface roots than in stems for two ring-porous species. However, our knowledge about the hydraulic limitations posed by belowground tissues, particularly in relation to freezing, is still very limited.

Pockman and Sperry (1997) studied the vulnerability to freezing-induced xylem embolism in stems of creosote bush (*Larrea tridentata* Cov.), and related it to the northern limit of the species distribution. *Larrea tridentata* is an evergreen, drought-tolerant shrub that lives throughout the warm deserts of North America (MacMahon, 2000). Pockman and Sperry (1997) found that the temperatures that caused a complete xylem embolism in the laboratory (−16° to −20°C) correspond-

[1] Manuscript received 21 February 2002; revision accepted 27 June 2002.

The authors acknowledge field assistance by M. Mangirón and the support of the staff of Sevilleta LTER. M. Mangirón, Dr. J. Piñol, and two anonymous reviewers provided valuable comments on an earlier version of the manuscript. This study was supported by NSF through the Sevilleta LTER (DEB 9411976 and DEB 0080529) and by USDA 98-35100-7025 to WTP. JM benefited from an FI grant and specific financial support for the stay in New Mexico from the Catalan Government.

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ed closely to the minimum recorded temperatures in the northern limit of the range of *L. tridentata*. However, the relationship did not hold for the Chihuahuan desert, the coldest of the three warm deserts of southwestern USA, where *L. tridentata* occurs in spite of lower mean annual and extreme minimum temperatures. The authors suggested that this different behavior in the Chihuahuan desert might indicate lower vulnerability to freezing-induced cavitation in this region, possibly related to the existing differences in ploidy between the populations of the three deserts (Hunter et al., 2001). Additionally, the loss of hydraulic conductivity was a function of minimum freezing temperature; below about −11°C xylem embolism increased linearly with minimum temperature. This result is difficult to explain because, if bubbles remaining in xylem conduit lumens after a freeze-thaw cycle are the cause of embolism, then once ice formation occurs in the xylem the minimum temperature below the freezing point should be unimportant.

In this study we explore the vulnerability to freezing-induced xylem embolism in a *L. tridentata* population from the Chihuahuan desert. Our objectives were (1) to test whether this population was more resistant to freezing-induced embolism than the one studied by Pockman and Sperry (1997); (2) to explain the relationship between embolism and minimum temperature found by Pockman and Sperry (1997); and (3) to assess the possible role of the vulnerability of the root system in the response to freezing temperatures. We combined field measurements of xylem embolism in a natural population in central New Mexico with laboratory measurements of xylem embolism of roots and branches under carefully controlled freezing conditions.

MATERIALS AND METHODS

Study site—All field monitoring and most measurements were conducted at the Five Points area of the Sevilleta National Wildlife Refuge, an NSF Long Term Ecological Research site in central New Mexico, USA (34°22′ N, 106°4′ W, elevation = 1610 m above sea level). The long-term (1961–1990) mean temperature is 13.3°C, and annual rainfall averages 242 mm. The number of days with subzero temperatures per year ranged from 103 to 160 between 1989 and 2000 (Moore, 1989–2000). The region is a transition zone for a number of biomes, including the Great Plains grassland, the Chihuahuan desert, and the Colorado Basin shrub-steppe. *Larrea tridentata* expanded its distribution in this area in the early 20th century, probably as a result of a northward invasion (Buffington and Herbel, 1965; but see Betancourt et al., 2001). With the exception of several isolated populations, the area represents the northern limit of *L. tridentata* and contains some of the populations of *L. tridentata* living under the coldest conditions (Pockman and Sperry, 1997). Plants of similar size (ranging between 0.9 and 1.5 m in height) from a single population were selected for use in all experiments. The study lasted from October 2000 to May 2001.

Field monitoring—A datalogger (Model CR-10X, Campbell Scientific, Logan, Utah, USA) and copper-constantan thermocouples were used to record stem and root wood temperature and air and soil temperature at the Five Points population. Wood thermocouples, secured with epoxy glue, were installed in small holes into the outer layer of xylem as described in Pockman and Sperry (1997). From late October (day of year [DOY] = 296) to early December 2000 (DOY = 337) we measured the wood temperature of stems about 8 mm in diameter from nine different individuals using copper-constantan thermocouples (AWG No. 36). Additional thermocouples were placed in a piece of dry *L. tridentata* wood as a reference for temperatures observed in living stems and in the soil (about 10 cm deep) under one of the monitored plants. Air temperature was measured with a thermistor sheltered from direct solar radiation. In early December 2000 (DOY = 337), additional thermocouples were placed in plants in the same area and thereafter the monitoring included ten different individuals. In four plants two thermocouples were placed in the same stem, one proximal (about 8 mm in diameter) and the other distal (about 5 mm in diameter). In the other plants we maintained the proximal thermocouples installed in October. Soil temperature was also measured under two studied plants, with thermocouples (AWG 24) at 10-, 20-, and 30-cm depth. In each profile, thermocouples were also installed in two roots (7–10.5 mm in diameter) at depths between 10 and 20 cm. The air thermistor was substituted by four air thermocouples (AWG 24) shielded from direct solar radiation (about 1.25 m in height). Another thermocouple was also added in a second piece of dry wood.

From late October to early December, temperatures were recorded every 2 h while air temperature was above 0°C. When air temperature fell below 0°C all the temperatures were measured every 10 s and the 5-min mean was stored. Beginning in early December we stored 2-min averages to capture exotherms and endotherms associated with freezing and thawing.

Freezing exotherms were revealed by sharp increases in wood temperature without a parallel change in air or dry wood temperatures. The starting points were identified during later data analysis by means of a semi-automatic procedure, but it was impossible to analyze them in the same detail as in the laboratory experiments (see below) because of the lower frequency of storage and the much higher temporal variability of air temperature in the field.

Water potentials—We used a Scholander pressure chamber (PMS Instruments, Corvallis, Oregon, USA) to measure leaf water potentials (Turner, 1987) in the field and the laboratory (see ***Laboratory freezing experiments***). In the field, predawn (0430–0530 solar time) and midday water potentials (1130–1230 solar time) were measured through the study period in two terminal stems of each of the ten monitored plants. Water potentials were also measured in the laboratory before each freezing treatment in all of the experiments. A single profile of soil psychrometers was installed in the study area in early February 2001 (DOY = 42) to monitor soil water potential at different depths. The sensors were placed at 15, 30, 50, and 80 cm.

Sampling for native xylem embolism and freezing experiments—We defined xylem embolism as the percent loss in hydraulic conductivity (in relation to the maximum) due to gas blockage of xylem vessels. Native xylem embolism refers specifically to the percent loss of conductivity under field conditions (Sperry, Donnelly, and Tyree, 1988).

Native xylem embolism of the stems was measured through the study period in the Five Points population. At each measurement date, ten different individuals within a radius of 100 m from the monitored plants were sampled. We cut one stem per plant for determination of native embolism, and two additional stems were cut from each plant if freezing experiments were to be carried out (Langan, Ewers, and Davis, 1997; see below). This procedure was used to increase our control over laboratory experiments, since initial measurements showed that the variability in native embolism within plants was approximately half of that among plants ($N = 4$ plants and $N = 5$ stems per plant). Stems were of similar size and at least 0.8 m in length. This length was great enough that few, if any, of the conduits embolized during collection extended to the measured segments (the mean of maximum vessel length was 0.35 m, $N = 3$; air-injection method, Ewers and Fisher, 1989). Measured plants were never sampled again in the following measurement dates. Branches and a damp towel were sealed in three plastic bags (''triple-bagged''), carried to the laboratory, and measured within 3–4 h of collection.

Root native embolism was measured only once, in connection with a freezing experiment. Five plants were excavated, and three roots per plant were cut at the distal end at least at 0.5 m from the crown. To minimize introduction of air into the root segment to be measured, the entire root crown of each plant with the roots attached was triple bagged with a damp towel and carried to the University of New Mexico. Once in the laboratory the proximal ends of the roots (as close as possible to the crown) were cut underwater. Considering that the mean of maximum vessel length for roots was 0.46 m ($N = 3$, air-injection method, Ewers and Fisher, 1989), it is likely that some of the conduits embolized during collection reached the measurement segment in the shortest roots. However, it is unlikely that any of them completely crossed the segment and the ones that reached it were probably very few. Indeed, no

effect of the total length of the sampled roots was found on percent embolism, although root length varied from 0.5 to 1 m. Measurements were carried out within 24 h of collection.

Measurement of xylem embolism—From each stem or root, one segment (about 7 mm in diameter) was cut underwater, recut with a new razor blade, and attached to the tubing manifold for measurement. Segment length was approximately 150 mm for stems and 200 mm for roots. Hydraulic conductivity (K_h) was calculated as the water flow rate through a wood segment divided by the pressure gradient across it (Sperry, Donnelly, and Tyree, 1988). The flow rate under known pressure differences (about 5.5 kPa for stems and 5 kPa for roots) was determined by repeated measurements of the flow of filtered (0.2 μm), deionized water onto an electronic balance attached to a computer. Flow rates were measured before and after flushing the segments at about 100 kPa for 30 min to remove all embolisms, and the percent loss of conductivity due to xylem embolism (PLC) was then calculated as $100(1 - K_{h,before}/K_{h,after})$. Specific conductivity (K_S) was calculated as the hydraulic conductivity per unit cross sectional area of the measured segment (without bark). This protocol was used to measure native embolism and embolism that occurred in laboratory freezing experiments.

Laboratory freezing experiments—Freezing experiments were carried out under controlled conditions using a procedure similar to Pockman and Sperry (1997). Samples were collected four times from mid-November to mid-December. The number of individuals sampled was ten (stems) or five (roots; only one sampling). From the three stems or roots collected per individual, one was used as a control, and the other two were subjected sequentially to different freezing treatments. All the experiments were performed the same day of collection (stems) or the day after (roots). Rather than rehydrate samples in water prior to imposing a freezing treatment, we elected to perform laboratory experiments at field water potential to maintain parity with field data. The water potential was measured in all stems just before treatment and, in a subsample, it was also measured shortly after treatment. There was no significant difference between field water potentials and the ones measured before and after laboratory treatments (one-way ANOVA, $P = 0.111$), suggesting that our laboratory measurements were representative of field conditions.

A segment of about 7 mm in diameter was marked in each stem/root, and a copper-constantan thermocouple (AWG No. 36) was installed into the outer layer of xylem (Pockman and Sperry, 1997) 5–10 cm below the segment. Thermocouples were held in place with adhesive tape. The temperatures of wood, air, and a piece of dry wood were measured every 5 s and averaged every 30 s (Datalogger Model CR-7, Campbell Scientific).

Temperature control was achieved with a temperature chamber (Langan, Ewers, and Davis, 1997) attached to a temperature bath (VWR Extra Low Temperature Circulator, Model 1197, VWR Scientific Products, Niles, Illinois, USA). The temperature chamber consisted of an inverted, insulated, black plastic garbage can (0.17 m^3), lined with about 35 m of coiled copper tubing. The fluid in the circulating bath was continuously passed through the copper tubing to control chamber temperature.

In each experiment, batches of ten stems or five roots were placed inside the temperature chamber and exposed to the desired treatment. Minimum (air) temperatures were 3.5°, −4°, −11°, −14°, −17° or −22°C for stems; and −2° or −7°C for roots. All treatments started and finished at 10°C. Cooling and warming rates were 1°C/min for temperatures above 0°C and 0.1°C/min below 0°C. Target minimum temperatures were maintained for 90 min before chamber temperature began to increase. Previous experiments showed no significant effects of time at minimum temperature (Pockman and Sperry, 1997).

Exotherms were identified in the manner described for field data. Typically two exotherms were apparent, which we termed high temperature and low temperature exotherms (HTEs and LTEs, respectively; Fig. 1). To calculate their exact duration and area, we plotted the temperature difference between each stem and the corresponding dry wood sample (cf. differential thermal analysis; Burke et al., 1976) and identified the starting and end points of the exotherms. The area between a line linking these two points and the temperature curve was considered to be the area of the exotherm (Fig. 1b).

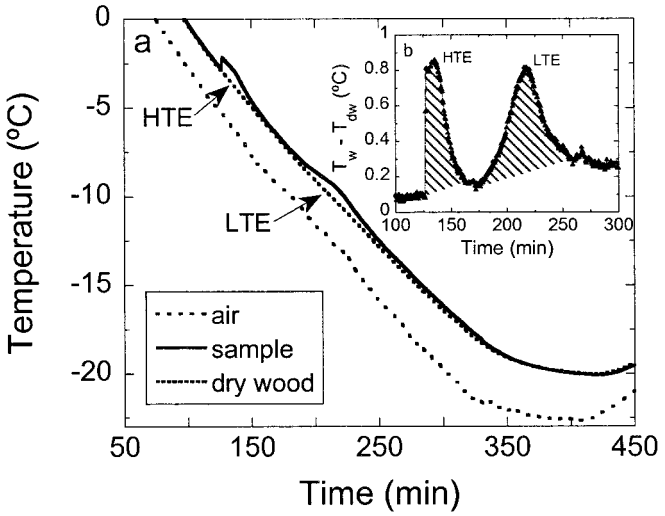

Fig. 1. (a) Example of laboratory measurements of air, stem, and dry wood temperatures during a typical freezing experiment. The high temperature and low temperature exotherms (HTE and LTE, respectively) are marked by arrows. (b) Difference between stem (T_w) and dry wood temperature (T_{dw}) during the same experiment. The filled polygons correspond to the exotherm areas that were used in the analyses.

After treatment, a wood segment was cut underwater from each sample and its percent loss of conductivity due to xylem embolism (PLC) and K_S were measured as explained in ***Measurement of xylem embolism***. In all cases the time span between thawing and measurement was similar, approximately equal to 90 min. The resulting values were normalized relative to the values measured on the control from the same plant in the following way: relative $K_h = (100 - PLC_{treatment})/(100 - PLC_{control})$; relative $K_S = K_{S,treatment}/K_{S,control}$. These relative variables were used in the analyses to compare the different treatments. We chose not to compare the PLC of the same segments before and after freezing because this procedure would have hydrated the measured segments during the initial measurement making the results difficult to compare with field measurements at much lower water potentials.

Xylem anatomy—Vessel diameters were measured on ten stems and eight roots from the Five Points population. Transverse sections were cut from segments collected for native embolism measurements using a rotary microtome (Model 820, American Optical, Buffalo, New York, USA). The sections were stained with toluidine blue (0.05%) to improve contrast, mounted in glycerol, and viewed with a compound microscope (Nikon Eclipse E400, Nikon, Tokyo, Japan) at 100× (stems) or 50× (roots). Two representative regions from the outermost rings, situated 90° apart, were photographed from each section with a digital camera (Nikon Coolpix 990, Nikon). The images (black and white) were transferred to a computer and analyzed with Scion Image (v. β4.02 for Windows, Scion, Frederick, Maryland, USA). Within each image all open vessels with a diameter larger than 7.4 μm (stems) or 8.8 μm (roots) were sampled. These values were selected in each case to maximize the agreement between the visually identified vessels and the ones selected by the computer. For each selected conduit the program determined the total cross sectional area and perimeter. At least 300 conduits were measured from each section.

Vessel-anatomy measurements were also carried out on ten stems from the same population studied by Pockman and Sperry (1997) at the Cienega Creek Nature Reserve, Arizona (32°01′ N, 110°37′ W). The purpose of these measurements was to test whether there were differences in the obtained diameter distributions because of the different methods used in the two studies. Two variables were used to characterize the xylem anatomy of each population: the mean vessel diameter (d [in micrometers]) and the mean hydraulic di-

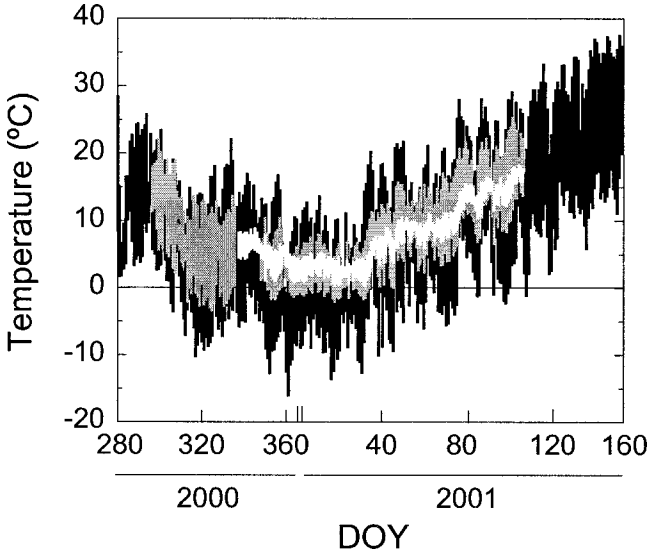

Fig. 2. Daily maximum and minimum air (black) and soil temperatures at a depth of 10 (gray) and 30 cm (white) during the study period at the Five Points site. DOY = day of year.

ameter (d_h [in micrometers]). The hydraulic diameter was calculated considering that hydraulic conductivity is proportional to vessel diameter raised to the fourth power. The following expression was used to calculate the hydraulic mean diameter for each segment: $\Sigma\ d^5_i\ /\ \Sigma\ d^4_i$ (Sperry et al., 1994).

Data analysis—Two-way ANOVAs with repeated measurements were used to compare the freezing temperatures at which exotherms were observed at proximal and distal parts of the stems (only the exotherms that were detected at both parts of the stems were considered). The field measurements of PLC from consecutive sampling dates were compared by means of *t* tests. The laboratory experiments were analyzed in two ways: PLC and K_S data from each treatment were compared to their corresponding controls using repeated measurements *t* tests, and differences between relative K_h and relative K_S among treatments were evaluated using one-way ANOVA. The attributes of the exotherms were compared using one-way ANOVA when comparing tissues or treatments (only for stems) or one-way ANOVA with repeated measurements when comparing HTEs with LTEs. The vessel diameters of different tissues and populations were compared by means of *t* tests. All variables were normalized when not normally distributed (arcsine transformation in the case of PLC and logarithmic for the rest). It was considered that a difference was marginally significant whenever $0.10 > P > 0.05$.

RESULTS

Temperature measurements in the field—During the study period (182 d) minimum air temperature (T_{air}) was below 0°C on 129 d and fell below −10°C on 13 d (Fig. 2). For the days with below-zero temperatures, maximum T_{air} was below zero only three times, and the mean difference between maximum and minimum daily temperature was 18.3°C. The minimum recorded air temperature was −16.0°C, reached on 28 December 2000 (DOY = 363). As expected, thermal oscillations were smaller in the soil than in the air, and these oscillations decreased with depth below the surface (Fig. 2). The number of days with below-zero soil temperatures was 52 d at 10 cm, 12 d at 20 cm, and none at 30 cm. The minimum temperature measured in the soil was −3.3°C (at 10 cm).

Freezing exotherms were only detectable in the field when the storage frequency was increased to 2-min averages (from early December onwards). For that period, exotherms were observed in stems between −1.5° and −10.2°C (mean = −5.4°C, $N = 400$). Water froze at approximately the same time (within our 2-min resolution) on the proximal and distal part of the stem ($P = 0.586$). Since temperatures were slightly lower at the proximal parts, freezing occurred there at an average of 0.7°C lower temperatures ($P = 0.003$). No difference was observed across individuals in either the time or the temperature at which freezing took place ($P > 0.25$ in both cases). On average, sap freezing was detected once every 3 d in the monitored stems and was never observed in roots.

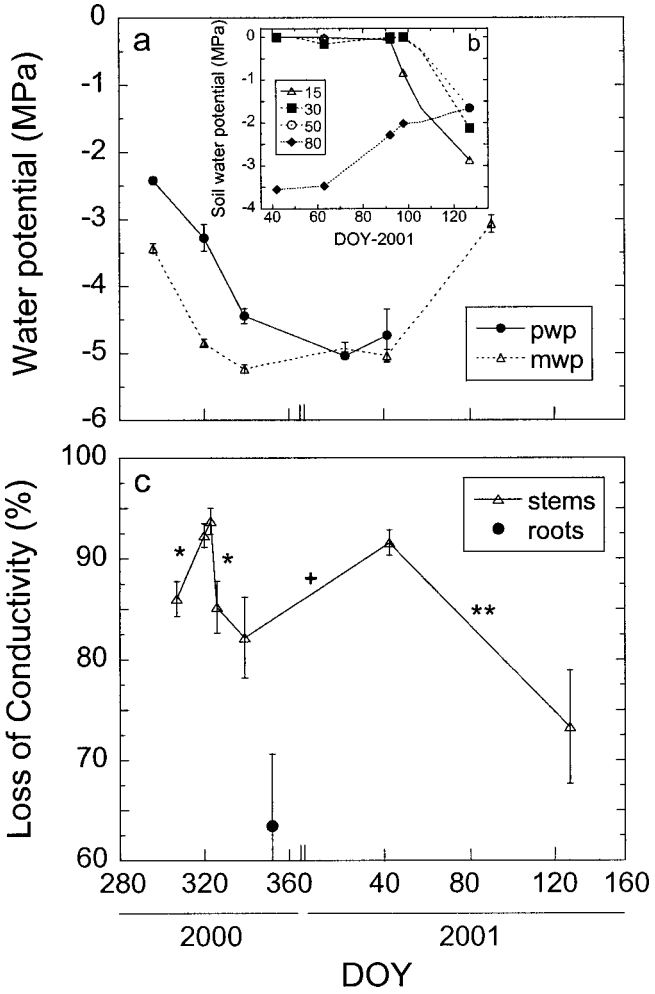

Fig. 3. Seasonal pattern of predawn (pwp) and midday (mwp) leaf water potentials (a), soil water potentials at different depths (in centimeters) (b), and percent loss of conductivity (PLC) due to xylem embolism (c) (means ± SE). Asterisks and the plus sign mark significant differences between two consecutive embolism measurements ($^+P < 0.1$, $^*P < 0.05$, $^{**}P < 0.01$; *t* test, $N = 10$). DOY = day of year.

Water potentials and native embolism—Leaf water potentials were low (less than −2.5 MPa at predawn and less than −3.5 at midday) throughout most of the study period (Fig. 3a). These low values were observed despite wet surface soil during most of the period. Soil psychrometer measurements beginning in early February indicated that soil water potential at depths of 50 cm and above was indeed high (Fig. 3b). However, plant water potential did not increase until March (before DOY = 91), when air and soil temperatures increased (Fig. 2).

Measured levels of native embolism were high (PLC always >70%) and did not show a sharp decrease until the advent of warm temperatures at the end of the study period (Fig. 3c). Only two freezing episodes ($T_{air,min} = -3.4$°C) were recorded before the first native embolism measurements. Since freezing episodes of −3.4°C were not sufficiently cold to cause exotherms in stem xylem, it is unlikely that they caused the observed PLC values of >80% by early November (see below). After this first measurement only one significant increase in PLC was detected (DOY = 320) and was associated with the first temperatures below −10°C. A second increase was observed between December and early February (DOY = 42), which was marginally significant ($P = 0.058$). The decrease in PLC observed in late November (DOY = 326) coincided with consistently low temperatures (daily minimums always less than −7°C and down to −8.6°C) and was not associated with any precipitation event. In the only native embolism measurement in roots (DOY = 351), PLC was 63.4 ± 7.2, much lower than the values measured in stems.

Freezing experiments—Although sampling for laboratory experiments (stems) was concentrated between mid-November and early December to minimize changes in field conditions at sampling time, the water potential of laboratory specimens nevertheless decreased significantly during the sampling period ($P < 0.001$). However, this decrease was probably not critical because: (1) the decrease in water potential was quantitatively small (about 0.7 MPa); (2) water potentials (in the field and in the laboratory) were low enough that we would expect nearly complete cavitation following a freezing-thaw cycle (Davis, Sperry, and Hacke, 1999); (3) in the control stems water potential was uncorrelated with native embolism ($r = 0.25$, $P = 0.218$); (4) the minimum temperature imposed in each freezing experiment followed no pattern with respect to date; and (5) treatment effects were evaluated in relation to the controls sampled at the same time.

In the laboratory, two types of freezing exotherms were observed in most samples (Fig. 1). The first (HTE) started at temperatures between −1.5 and −7.3°C in stems ($N = 48$) and between −3.8° and −5.3°C in roots ($N = 5$). The second (LTE) started at −5.9° to −9.5°C in stems ($N = 38$) and at −4.5° to −5.4°C in roots ($N = 4$). Except in the $T = -22$°C treatment, LTEs lasted approximately until the minimum treatment temperature was reached. High temperature exotherms occurred at lower temperatures in roots ($P = 0.037$), whereas for LTEs the reverse was true ($P < 0.001$). No significant differences between tissues were observed in the area of LTEs or HTEs. Low temperature exotherms were characterized by having longer duration, less amplitude, and higher area than HTEs ($P < 0.001$ in all cases) (Fig. 1b). While there was no difference among subzero treatments in the area of the HTE ($P = 0.428$), the area of the second exotherm increased as a function of the minimum temperature from −4°C, where no LTE was observed, to −22°C ($P = 0.010$; Fig. 4b).

No increase in PLC was detected for temperatures down to −14°C. Stems frozen to −17° or −22°C had significantly higher embolism than the control (Fig. 4a). This result is qualitatively similar to the pattern reported in Pockman and Sperry (1997), although their stems were more vulnerable to freezing-induced embolism. Roots were more vulnerable to cavitation than stems, with a significant increase in PLC at −7°C (Fig. 4a). Although the effect was never significant ($P > 0.05$), all treatments down to −11°C tended to have higher conductivity

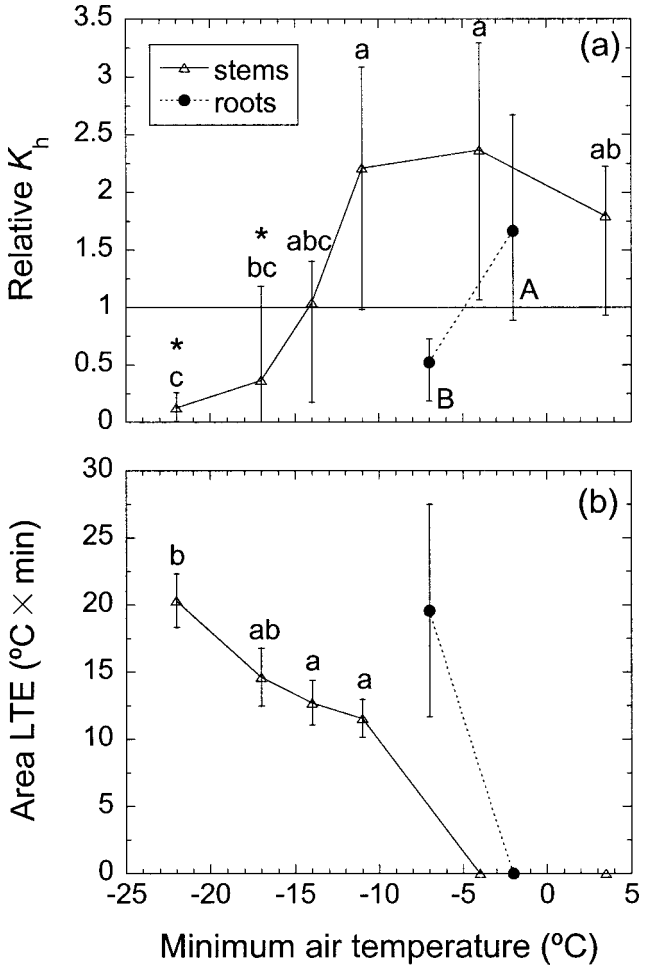

Fig. 4. Results of the freezing experiments. (a) Percent conductivity relative to controls (relative K_h) after each treatment (controls = 1) for stems and roots from the Five Points site. Since the variable was not normally distributed, the points represent the estimated mean, and the error bars the 95% confidence intervals. Asterisks indicate the presence of significant differences in relation to the controls (*$P < 0.05$; t tests). (b) Mean area of the low temperature exotherms (LTEs) observed after each treatment (means ± SE). In both (a) and (b) different letters (lowercase for stems and uppercase for roots) denote significant differences between treatments (one-way ANOVA).

than the control (Fig. 4a). This effect was reduced when we plotted the absolute conductivity after each treatment instead of the value relative to the control (data not shown).

There was a significant correlation between the average area of the LTE and the relative K_h of each treatment ($P = 0.015$) (Fig. 5). These data (Fig. 5) may suggest a threshold effect more than the linear relationship that we have assumed. However, since analysis of the data with nonzero LTE area alone would be dominated by a single point ($T = -11$°C) and since we lack any other evidence to support a threshold effect, we have retained the simpler linear model and added the best fit considering only the points with nonzero LTEs. From either perspective our main point, namely that there is a relationship between the area of LTE and embolism, would remain unaffected. The freezing treatments had no effect on maximum K_S ($P = 0.277$ for stems, and $P = 0.192$ for roots).

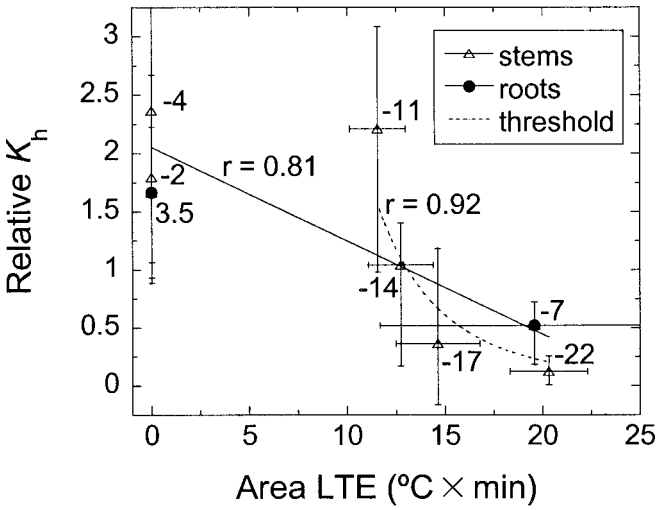

Fig. 5. Relationship between the mean area of the low temperature exotherm (LTE) and the percent conductivity in relation to the controls (relative K_h) observed after each treatment. The numbers indicate the minimum temperatures (in degrees Celsius) corresponding to each treatment. The linear fit combining all stems and roots and the power fit considering only the cases with nonzero LTEs (threshold effect) are shown. Error bars show SE in the case of the area of LTEs and 95% CI for relative K_h.

Xylem anatomy—Although the differences were quantitatively small, actual vessel diameters and hydraulic diameters were higher in roots than in stems (Table 1 and Fig. 6a–b). Between populations, vessel diameters tended to be slightly larger in the stems of Sonoran individuals. The difference was only significant for mean diameters (Table 1). Our anatomy measurements for the Arizona population were similar though significantly different ($P = 0.030$) to the ones given by Pockman and Sperry (1997) (mean diameter = 21.5 ± 0.6 μm vs. 19.7 ± 0.5 μm, respectively). This discrepancy may be due to the fact that we only measured the vessels in outermost rings while Pockman and Sperry (1997) measured entire sectors.

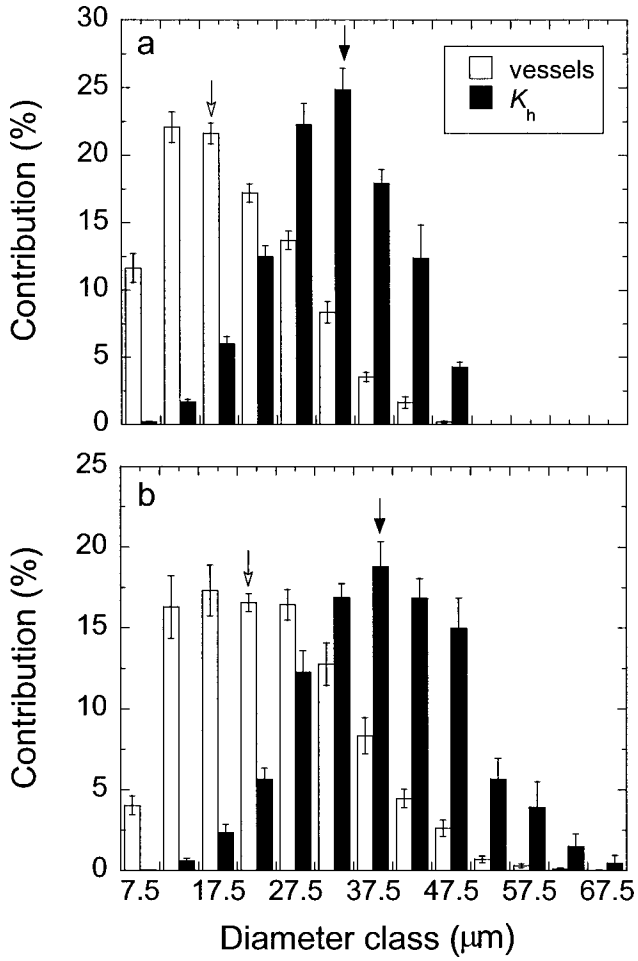

Fig. 6. Vessel diameter distributions (in 5-μm size classes) of stems (a) and roots (b) from the Five Points population (means ± SE). Both the percent contribution to the total number of vessels and to the overall hydraulic conductivity (K_h) are represented. Arrows mark the mean vessel diameter and the mean hydraulic diameter.

DISCUSSION

Comparison of the Chihuahuan and Sonoran populations—The absence of freezing-induced embolism at temperatures down to −14°C and a linear increase in embolism with decreasing minimum temperature below this threshold value (Fig. 4a) are functionally similar to the general cavitation response observed in *L. tridentata* in the Sonoran desert (Pockman and Sperry, 1997). Consistent with predictions based on long-term minimum temperature data (Pockman and Sperry, 1997), the New Mexico population studied here was more resistant to embolism than the southern Arizona population. Whereas in the Sonoran desert population the decrease in PLC was already apparent at temperatures of about −12°C, in our case the decrease started between −14° and −17°C (Fig. 4a). This difference in vulnerability was consistent with the smaller vessels in the New Mexican population (Table 1). Our laboratory results were qualitatively corroborated by the fact that increases in PLC in the field were only observed after temperatures around −11°C or lower. Temperatures below −11°C occur on average 15 times per year at the study site, and yearly minimums range between −14° and −20°C (Moore, 1989–2000). Clearly, the Sevilleta population is more frequently subjected to minimum temperatures that cause freezing-induced cavitation than the Sonoran population, where yearly minimums range between 0° and −12°C (Santa Rita Experimental Range weather station, 1950–2001), and temperatures fell be-

TABLE 1. Anatomy results. d = diameter; d_h = hydraulic diameter. Numbers in parentheses are standard errors. Significant differences between stems and roots or between populations (stems) are denoted by an asterisk (t test). *$P < 0.05$, ***$P < 0.001$.

Site	Tissue	N	Mean d (μm)	Maximum d (μm)	Mean d_h (μm)
Sevilleta, New Mexico	Stem	10	19.9 (0.4)	49.0	31.3 (0.5)
Sevilleta, New Mexico	Root	8	24.7 (0.3)***	67.3	38.4 (0.8)***
Cienega Creek, Arizona	Stem	10	21.5 (0.6)*	55.3	31.6 (0.9)^{ns}

low −5°C only once during the 1994–1995 winter. The difference in vulnerability to freezing-induced xylem embolism between both populations is probably not large enough to compensate for the existing climatic differences.

Because temperatures sufficient to cause freezing-induced cavitation occur more frequently in central New Mexico than in the Sonoran desert, it is not surprising that we found very high levels of native embolism (>80% in stems for most of the winter; Fig. 3c). In principle, both water stress and freezing are possible causes of this embolism. Pockman and Sperry (1997) measured predawn water potentials as low as −9 MPa during the summer without any increase in native embolism. Available data (Sevilleta LTER data 1989–1992; J. Medeiros and W. T. Pockman, unpublished data) indicate that minimum water potentials are similar in the Chihuahuan population. *Larrea tridentata* is known to remain photosynthetically active at very low xylem water potentials (about −8 MPa) (Odening, Strain, and Oechel, 1974). All of these data suggest that this species is very resistant to drought-induced embolism and that water stress alone is not the main cause of the high levels of native embolism in the study population. However, it is well known that water stress or, more precisely, xylem water potential interacts with freezing stress (Langan, Ewers, and Davis, 1997). Winter leaf water potentials were lower in our Chihuahuan population than in the Sonoran population studied by Pockman and Sperry (1997). This may have contributed to the higher levels of native embolism we found. In any case, our measurements both in the field and in the laboratory showed that, at least at the low water potentials that prevailed during the study period, freeze-thaw events can cause substantial increases in xylem embolism. This evidence supports the interpretation that the high levels of native embolism are mainly the result of the cumulative effect of repeated freeze-thaw events over the lifetime of the stems.

The high embolism levels that we observed suggest that the studied population is close to the limit of its ability to cope with freezing stress. In mid-November (DOY = 323), when the highest levels of embolism were measured, 30% of the stems presented a PLC >95%. The amount of dead standing biomass in the population, which reflects tissue mortality in past years, was as much as 50% of the stems in the studied population (M. Mangirón, Center for Ecological Research and Forestry Applications [CREAF], Spain, unpublished data). Shoot mortality as a result of freezing stress has been reported in the chaparral shrub *Rhus laurina* (Boorse, Ewers, and Davis, 1998). Qualitatively fewer dead stems were observed in the Sonoran desert population studied by Pockman and Sperry (W. T. Pockman, personal observation). The decreasing temperatures observed with increasing latitude in central New Mexico, and the associated increased accumulation of xylem embolism predicted by our results, are likely to contribute to limiting the present distribution of *L. tridentata*.

Dynamics of freezing-induced xylem embolism—The existence of two non-overlapping exotherms in a wood sample is usually interpreted in the following way (Burke et al., 1976; Gusta, Tyler, and Chen, 1983): when temperature reaches the freezing point of sap inside xylem conduits the sap freezes and there is a first exotherm (HTE). As temperature decreases below that point cellular water migrates to the vessels and intercellular spaces, where it freezes. The properties of the cell wall and the freezing point depression of cell sap caused by solutes protect cellular water from freezing and, together with the water potential of ice, regulate the rate of cell dehydration (Rajashekar and Lafta, 1996). Eventually, intracellular freezing occurs in xylem parenchyma and a second exotherm (LTE) is observed. If we accept this scenario, the increase in the area of the LTE with decreasing temperature can be interpreted as the result of freezing injury to increasing numbers of parenchyma cells as temperatures decline below −14°C. Consistent with this interpretation are the lack of a correlation between minimum freezing temperature or xylem embolism and the area of HTEs and the differences in shape that we observed between the two exotherms (HTE sharper than LTE), which may result from a faster progression of freezing in the apoplast (HTE) than in the symplast (LTE).

The effect of minimum temperature on embolism formation has long been recognized for conifers (Hammel, 1967) but only recently has been reported in some species of angiosperms (Lo Gullo and Salleo, 1993; Pockman and Sperry, 1997; this study). Our results suggest that freezing of living cells, most likely the xylem parenchyma, causes the progressive increase in embolism with decreasing temperature below a threshold of −14°C (Fig. 5). This implies that living cells contribute to the maintenance of xylem function following the apoplastic freezing identified by an HTE (e.g., Fig. 5; −11°C), and that this contribution is diminished as minimum temperature declines.

However, the possible role of parenchyma cells in the formation/removal of xylem embolism remains unclear. One possibility is that refilling occurs passively as a result of the positive pressures associated with the expansion of water during ice formation (Hammel, 1967). Winter stem xylem pressures can increase dramatically during freeze-thaw events (Améglio et al., 2001). To the extent that this positive pressure persists during thawing before the water column is placed under tension, it could contribute to the dissolution of bubbles before they can seed cavitation. Refilling by this mechanism might be diminished or offset by declining minimum temperature in two ways (Pockman and Sperry, 1997). First, increased dehydration of living cells during freezing and correspondingly more rapid water uptake by these cells during subsequent thawing might diminish the duration and effect of positive pressure transients. Second, and more likely, increased intracellular freezing and cell injury with decreasing minimum temperature could nucleate cavitation in surrounding xylem conduits if the rupture of cell membranes causes the entrance of cytoplasmatic materials into vessel lumens. The latter interpretation is supported by the increase in LTE area with decreasing minimum temperature (Fig. 4) and the decline in hydraulic conductance with increasing LTE area (Fig. 5).

An alternative interpretation of the association between intracellular freezing and xylem embolism is provided by recent work suggesting that parenchyma cells may contribute actively to the integrity of the conducting system following drought-induced cavitation (Salleo et al., 1996; Holbrook and Zwieniecki, 1999; Zwieniecki and Holbrook, 2000). If vessels can be hydraulically isolated, by gas entrapment in pits or another mechanism, as proposed to explain refilling at low water potentials (Holbrook and Zwieniecki, 1999), the local generation of positive or near-positive hydrostatic pressures might be sufficient to force gas bubbles into solution. Living parenchyma cells adjacent to the vessels might cause the required positive pressures, although the mechanism is not well understood (Holbrook and Zwieniecki, 1999). This interpretation is also consistent with our observations that (1) in the field there was

a significant decrease in PLC concurrent with low water potentials (less than −3 MPa) and minimum temperatures down to −8.6°C (Fig. 3c), and (2) laboratory treatments showed a consistent (though never significant) decrease in PLC at temperatures down to −11°C (Fig. 4a). However, our data do not provide enough information to distinguish with certainty between this active role of xylem parenchyma in refilled embolized xylem conduits and the passive mechanisms described above. It should be also noted that even the association between cell freezing, LTEs and xylem embolism remains tentative since no direct measurement of cell mortality was carried out after the treatments.

Vulnerability of roots vs. shoots—Roots were more vulnerable to freezing-induced cavitation than stems (Fig. 4a). According to the mechanisms discussed above, it is likely that this greater vulnerability in roots resulted from the greater susceptibility to freezing of their parenchyma cells and from their wider vessels (Fig. 6) (Davis, Sperry, and Hacke, 1999). It should be noted, however, that the difference in vessel diameter between stems and roots was relatively small compared to other species (e.g., Hacke, Sperry, and Pittermann, 2000; Martínez-Vilalta et al., in press). In spite of the greater vulnerability to freezing of roots and the fact that soil temperatures reached considerably low values (Fig. 2), exotherms were never observed in the field-monitored roots, and their native embolism was lower than in stems. These results are in agreement with other studies (Jaquish and Ewers, 2001) suggesting that roots are less limited by freezing-induced embolism than stems. In fact, it is likely that the 63.4 PLC that we measured in roots was mostly caused by water stress alone because (1) soil temperatures at 10 cm dropped below −5°C (approximately the mean exotherm temperature for roots) only once in the last 10 yr, the absolute minimum being −5.3°C (data from the Deep Well weather station, at similar altitude and 5 km from the study site; Moore, 1989–2000); and (2) predawn water potentials remained very low during the period studied (Fig. 3a). Although we did not study the vulnerability to drought-induced embolism in *L. tridentata* roots, the measured water potentials will be enough to cause severe embolism in the roots of most woody species (e.g., Hacke, Sperry, and Pittermann, 2000; Martínez-Vilalta et al., in press). The low predawn water potentials (Fig. 3a) together with the observed pattern of soil water potentials at different depths (Fig. 3b) suggest that for some reason surface roots were hydraulically disconnected from the soil. Our results show that this was not caused by lack of hydraulic conductivity in the xylem of the woody roots that we measured.

Conclusions—Our results show that *L. tridentata* populations from the Chihuahuan desert are limited by freezing. Although resistance to freezing-induced xylem embolism is higher than in the Sonoran population studied by Pockman and Sperry (1997), the impact of freezing is also greater because of the much lower temperatures. Roots were more vulnerable to freezing-induced cavitation than stems; however, stems were more affected by freezing than roots because of the thermal buffering effect of the soil. Our laboratory experiments suggest that the freezing of water inside parenchyma cells is associated with the occurrence of xylem embolism. These results are consistent with other studies that point to an active role of living cells in maintaining long-distance water transport (Holbrook and Zwieniecki, 1999) and provide a reasonable explanation for the temporal dynamics of embolism and refilling observed in the field. This finding opens new perspectives in the study of the impact of frost acclimation on the vulnerability to xylem embolism. However, more detailed studies are needed to clarify the possible role of living cells in freezing-induced cavitation and water transport in general.

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