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Freezing-induced xylem cavitation and the northern limit of Larrea tridentata

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Abstract We investigated the occurrence of freezing-induced cavitation in the evergreen desert shrub *Larrea tridentata* and compared it to co-occurring, winter-deciduous *Prosopis velutina*. Field measurements indicated that xylem sap in *L. tridentata* froze at temperatures below *c.* –5°C, and that this caused no measurable cavitation for minimum temperatures above –7°C. During the same period *P. velutina* cavitated almost completely. In the laboratory, we cooled stems of *L. tridentata* to temperatures ranging from –5 to –20°C, held them at temperature for 1 or 12 h, thawed the stems at a constant rate and measured cavitation by the decrease in hydraulic conductivity of stem segments. As observed in the field, freezing exotherms occurred at temperatures between –6.5 and –9°C and as long as temperatures were held above –11°C there was no change in hydraulic conductivity after thawing. However, when stems were cooled to between –11°C and –20°C, stem hydraulic conductivity decreased linearly with minimum temperature. Minimum temperatures between –16 and –20°C were sufficient to completely eliminate hydraulic conductance. Record (>20 year) minimum isotherms in this same range of temperatures corresponded closely with the northern limit of *L. tridentata* in the Mojave and Sonoran deserts.

Key words *Larrea tridentata · Prosopis velutina* · Cavitation · Freezing tolerance · Deserts

Introduction

Freezing tolerance is an important determinant of plant distribution in many habitats (Shreve 1914; George et al. 1974; Sakai and Larcher 1987). Equilibrium freezing (Beck et al. 1984) and supercooling (Gusta et al. 1983; Rada et al. 1985; Ashworth et al. 1988) have been identified as mechanisms that prevent the freezing of living cells at sub-zero temperatures. However, air bubbles in the xylem formed upon freezing can also cause cavitation and consequent disruption of water transport (Hammel 1967; Sperry et al. 1988b; Tyree and Sperry 1989). In this paper we consider the importance of freezing-induced xylem cavitation for the freezing tolerance and distribution of *Larrea tridentata*, an evergreen, drought-tolerant shrub that occurs throughout the warm deserts of North America.

Xylem cavitation refers to the vaporization of water under negative pressure. Cavitation can be nucleated by the aspiration of air bubbles into xylem conduits by critically low xylem pressures (Ψ_{px}) (Zimmermann 1983; Sperry and Tyree 1988; Pockman et al. 1995; Sperry et al. 1996), or by air bubbles formed in situ during freezing (Hammel 1967; Sperry and Sullivan 1992). The resulting vapor- and air-filled ("embolized") conduit no longer contributes to water transport. In the case of freezing, whether or not a bubble nucleates cavitation depends on the balance between the surface tension forces acting to compress it and the negative Ψ_{px} acting to expand it (Tyree et al. 1994). Surface tension forces are given by Laplace's law and equal 2 (t/r) where t is the surface tension of water and r is the bubble radius (Oertli 1971). Thus, if $\Psi_{px} \leq -2$ (t/r), the bubble will nucleate cavitation. Similarly, once cavitation occurs, the conduit cannot refill unless $\Psi_{px} > -2$ (t/r) where r is now the radius of the much larger gas void of the cavitated conduit (Yang and Tyree 1992). Such high Ψ_{px} can arise by root pressure, equilibration of the plant with wet soil (Sperry et al. 1987; Tyree and Yang 1992), and possibly by an auxin-dependent mechanism (Salleo et al. 1996). Such refilling is a seasonal phenomenon in many temperate woody plants, but it may also occur more frequently, especially in herbs (Waring et al. 1979; Tyree et al. 1986; Salleo and LoGullo 1989; Ewers et al. 1991; Lewis et al. 1994; Salleo et al. 1996).

Freezing-induced cavitation could be particularly important for evergreen species, such as *L. tridentata*, that are native to warm, arid habitats in the temperate zone.

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The combination of evergreen phenology and warm winters means that any loss of water transport during intermittent freezes would reduce the ability of the plant to take advantage of the normally warm conditions and could result in desiccation and death of the foliage. Many desert sites receive a significant proportion of their annual precipitation during winter months (Oechel et al. 1972; Sellers and Hill 1974) and *L. tridentata* has been shown to continue gas exchange during the same period (Oechel et al. 1972; W.T. Pockman and J.S. Sperry, unpublished work). The lower Ψ_{px} caused by the arid habitat would make cavitation more likely during a freeze and recovery by root pressure less likely. These factors suggest that (1) *L. tridentata* must avoid significant cavitation during winter in its warm temperate habitat, and (2) that any limits to this avoidance may determine the distribution of this species with respect to temperature.

The most conspicuous evergreen plants of the temperate zone, conifers, avoid freezing-induced cavitation by having small-volume conduits (Sperry and Sullivan 1992). The smaller the conduit, the smaller the radius of air bubbles formed and the lower the Ψ_{px} required to nucleate cavitation. By contrast, many deciduous, vessel-bearing species cavitate extensively due to freezing and either refill their xylem prior to leaf flush or simply replace large cavitated vessels with new ones (e.g., ring-porous species; Sperry et al. 1994). These observations suggest that the predicted avoidance of freezing-induced cavitation in an evergreen such as *L. tridentata* would be associated with small vessel sizes.

The immediate objective of the current study was to determine the resistance to freezing-induced cavitation in *L. tridentata*. For comparative purposes, we also made limited observations on the co-occurring winter-deciduous tree, *Prosopis velutina*. Field studies of the freezing response to a limited range of temperatures were followed up with laboratory studies to determine the response to a wider range of minimum temperatures typical of *L. tridentata*'s entire range. Finally, we evaluated the role of the freezing responses we observed in determining the northern limit to the distribution of *L. tridentata*.

Materials and methods

Research site

All measurements were conducted at the Cienega Creek Natural Preserve, Pima County, Arizona, United States (32°01'N, 110°37'W, elevation: 1036 m) during the period April 1993–March 1996. Plants of a similar size were selected from a single population of *L. tridentata* for use in all experiments. Comparative measurements were also made on plants from a single population of *P. velutina* located within 1 km of the *L. tridentata* site. *P. velutina* at this site was deciduous from mid-November until April.

Xylem embolism

Xylem embolism was defined as the proportion by which the xylem's maximum hydraulic conductivity was reduced by gas blockage (Sperry et al. 1988a). Hydraulic conductivity (k_h) was defined as the mass flow rate through the stem divided by the pressure gradient across the stem. Flow rate was determined by repeated measurements of the flow onto an electronic balance. The initial conductivity (k_i) of each stem was measured and stems were then subjected to repeated 15–30 min flushes at 100 kPa until k_h measured between flushes reached a maximum value (k_m). The percentage embolism was then calculated as:

$$\% \text{ embolism} = 100 \times \left(1 - \frac{k_i}{k_m}\right) \quad (1)$$

Seasonal measurements of xylem embolism of *L. tridentata* and *P. velutina* were conducted from April 1993 to April 1995. At each measurement date, one branch, at least 0.8 m in length, was cut near the ground from each of 15 plants. Branches were triple-bagged with a moist paper towel, transported to the University of Arizona (32 km) and measured within 4 h of collection. One stem segment was cut from each branch underwater to avoid introducing any additional air emboli. Segments were approximately 5 mm in diameter and 100 mm long and were cut at least 0.5 m from the base of the branch. Branches were of sufficient length that few, if any, of the conduits embolized during collection extended to the measured segments. Segments were re-cut underwater with a new razor blade and attached to a tubing manifold to permit the application of a gravity-induced pressure difference across the stem as described above. Embolism measurements of *L. tridentata* branches following laboratory experiments were made using the same procedure.

Xylem pressure

Xylem pressure was measured using a Scholander pressure chamber (Turner 1987). Seasonal pre-dawn (0400–0530 hours) xylem pressure of *L. tridentata* and *P. velutina* was measured from April 1993 to January 1995. Xylem pressure of *L. tridentata* branches was also measured before the freezing treatment in all laboratory experiments.

Field wood temperatures

A datalogger (Campbell Scientific CR-10, Logan, Utah) was used to determine wood temperatures in intact stems in the field from November 1994 to April 1995 (see Sperry et al. 1994). Copper-constantan thermocouples (AWG No. 36) were installed in one stem of each of six *L. tridentata* individuals and two adjacent individuals of *P. velutina*. Air temperature was measured with a thermistor sheltered from direct solar radiation while soil temperature was recorded under the monitored plants using thermistors buried at 150 and 300 mm. Air, soil and wood temperatures were recorded every 2 h until air temperature fell below 2°C. At air temperatures below 2°C wood and air temperature were stored every 15 min. Native embolism was measured in one stem from each plant in November, January, March and April.

Laboratory freezing experiments

To better characterize the freezing response of *L. tridentata* to subfreezing temperatures, stems were frozen in the laboratory while wood temperatures were measured at a higher frequency than was possible in the field. In each experiment, stems were collected using the procedure described above (in the section on xylem embolism). All experiments were performed on stems the same day they were collected. Stems were cut to a length of approximately 0.80 m, a stem segment of *c*. 5 mm diameter was marked *c*. 0.5 m from the stem base for subsequent measurement of xylem embolism and a copper-constantan thermocouple (AWG No. 36) was installed below the marked segment on each stem. The thermocouple was inserted into a small puncture into the outer layer of xylem made using a dissecting needle 200–250 mm below the

marked segment to ensure that it was beyond most, if not all, of the vessels in the marked segment. The installed thermocouple was held in place with adhesive tape at two points to insulate it from the air and prevent shifting during treatment. The temperatures of all stems, a dried wooden dowel, and the air were recorded every 10 s from the start of the experiment until wood temperature was again above 10°C.

Initial experiments were conducted in April 1995 by placing two batches of seven stems, each stem collected from a different plant, in the freezer compartment of a standard kitchen refrigerator. Stems were frozen to –10 or –20°C, held at minimum temperature for 1 h and removed from the freezer. Stems were immediately placed horizontally in a plastic bag on the floor of the laboratory and left to thaw for 1 h without temperature control until the wood reached room temperature. The absence of temperature control resulted in warming rates of 0.93 and 1.35°C min^{-1} (from minimum temperature to 0°C). After thawing, the amount of xylem embolism in the marked segments was determined immediately. Percentage embolism was also determined in unfrozen controls collected at the same time and subjected to the same thermocouple installation process.

The same freezing protocol was repeated in December 1995 using batches of ten stems, each from a different plant, and a controlled temperature chamber similar to that used by Langan et al. (1996). This apparatus permitted greater control of the rate of cooling and warming. The chamber consisted of an inverted, insulated aluminum garbage can equipped with an internal circulating fan, lined with coiled copper tubing and its interior painted black to facilitate heat exchange. Temperature control was achieved using a computer controlled temperature bath (VWR Scientific, model 1157) circulating a 1:1 (v/v) mixture of ethylene glycol and water through the chamber's copper tubing. The stems were placed in the chamber and cooled from a starting temperature of 15°C to the desired temperature. Each one of six batches of stems were exposed in this fashion to one of the following minimum temperatures: –5.6, –8.4, –11.8, –13.9, –16.2 or –18.7°C.

After 1 h at minimum temperature, the chamber temperature was increased. For stems at the lowest minimum temperatures, warming rates approached 1°C min^{-1} until the exotherm temperature was approached (–10°C). Warming rate for all stems was lower from exotherm temperature until wood temperatures were above 10°C. The warming rates from minimum temperature to 0°C ranged from 0.13 to 0.35°C min^{-1} (mean = 0.21°C min^{-1}, SD = 0.08). This compares to warming rates of 0.1–0.2°C min^{-1} measured in the field on the coldest night of the winter.

The results of each freezing treatment were compared with the percentage embolism measured in positive and negative control groups of ten stems each. The positive control consisted of stems cut to length and equipped with a thermocouple as in the experimental groups to test the effect of the thermocouple installation procedure. Positive controls were not subjected to an extended period in the controlled temperature chamber. The negative control was taken as the percentage embolism in stem segments of untreated, field-collected stems.

Additional experiments were performed on batches of seven stems in March 1996 with plant material collected at the field site, triple-bagged with a moist paper towel to prevent dehydration, and shipped overnight to Utah. All measurements were performed within 72 h of the time of collection and stems were kept bagged until measurement. During these experiments, the negative control and the –8°C freezing treatment (1 h) were each repeated as in December. Then, using the same procedure as in the December 1995 experiments, freezing treatments with minimum temperatures of –8.2, –11.7 and –15.7°C for 12 h were imposed on batches of seven stems (except $n = 5$ for –15.7°C) to determine whether the relationship between percentage embolism and minimum temperature was dependent upon the time at minimum temperature. Stems were warmed as above at rates that ranged from 0.12 to 0.23°C min^{-1} (mean = 0.17°C min^{-1}, SD = 0.06).

Xylem anatomy

Xylem vessel lengths were measured using the methods of Zimmermann and Jeje (1981). Intact branches were collected, side branches were excised and the distal tip of a straight section of the branch excised underwater. Filtered water was then forced into the stem for 30 min to refill embolized xylem. After flushing, a dilute (1:200 w/w) paint pigment mixture filtered to 1 μm was applied under 40 kPa air pressure until solution was no longer consumed (usually several days). The method is based on the assumption that the paint mixture flows freely through xylem conduits until pigment particles are filtered out by inter-conduit pit pores causing the conduit to fill with pigment. After injection, branches were cut into 20 mm sections, the ends smoothed with a sliding microtome and the number of paint filled conduits measured. Using these data, vessel length distributions were calculated for branches of eight individuals according to Zimmermann and Jeje (1981).

Vessel diameters were measured using the same stem segments used to measure the loss of k_h following the freezing treatments described below. Thin sections (~15 μm) were cut from stem segments using a sliding microtome. Sections were viewed under a compound microscope (x400) where all conduits within a designated sector of the stem were traced using a *camera lucida* and a digitizing tablet (Donsanto Corp., Micro-Plan II). Maximum diameter and cross-sectional area of 100–200 vessels were measured for sections from each of 13 individuals from the site ($n = 1757$ vessels). Diameter distributions were then calculated using 5- and 10-μm classes based on both the actual percent of vessels in each class (frequency distribution) and on the estimated percent of total conductance contributed by each class (hydraulic distribution). The latter was calculated as the percent contribution of each diameter class to the sum of the fourth power of the radius of all conduits (Sperry et al. 1994). We also calculated the means of both distributions. The mean of the hydraulic distribution equals the sum of the fifth power of the vessel radii divided by the sum of the fourth power of the radii (Sperry et al. 1994). We refer to this as the "hydraulic mean".

Conduit volumes of *L. tridentata* and *P. velutina* were estimated using the vessel length and 5-μm-diameter distributions. Because the effects of the freezing treatment were measured not as a function of number of embolized conduits but as the loss of hydraulic conductivity, the vessel volume distribution was calculated from the hydraulic distribution of diameters. Vessel volume distributions were calculated using two models of the relationship between conduit length and diameter. First, it was assumed that the longest conduits were also the widest (model 1) as shown by Zimmermann and Potter (1982). Based on this assumption, we calculated the volume associated with a given pair of diameter and length classes. We started with the largest diameter and longest length class, and using the middle of each size class for the calculation, worked step-wise through progressively smaller size classes. The percentage of vessels in each volume class was taken to be the smaller of the percentages in each pair of length and diameter classes considered. For example, given a length class with 10% of the total conduits and a diameter class with 15%, 10% of the total vessels fell in the corresponding volume class. The remaining 5% of the total vessels in the diameter class were applied to a volume class calculated using the middle of the initial diameter class and the next shortest length class. This procedure was repeated until all classes of both distributions had been included in the calculation. Since there is only limited empirical basis for the assumption behind model 1 (Zimmermann and Potter 1982), we also calculated the vessel volume distribution based on the more conservative assumption that vessel diameter is random with respect to vessel length (model 2). In this case the diameter distribution was applied to each length class to calculate the range of volumes associated with that class.

Comparison of freezing response with *Larrea* distribution

To test the hypothesis that the northern limit of the distribution of *Larrea tridentata* is associated with freezing-induced cavitation, we compiled temperature data from 1201 sites in the seven states

(Arizona, California, Colorado, Nevada, New Mexico, West Texas and Utah) of the American southwest. Data were obtained from a CD-ROM database (ClimateData, EarthInfo Inc., Boulder, Colo.) and long-term weather records (Ruffner 1985). The area included was substantially larger than the range of *L. tridentata* to avoid edge effects in the area of interest. Only sites with 20 or more years of temperature data were included. A triangulation method was used to generate record minimum temperature isotherms across the region using the latitude and longitude of each station and its record minimum temperature (JMP v. 3.1, SAS Institute). Data were not corrected for elevation or geographic features so isotherms may cross mountainous regions where temperature data were not available.

Results

Freezing and embolism in the field

Significant changes in native embolism in *Larrea tridentata* were not associated with periods of freezing temperatures from 1993 to 1995 (Fig. 1a) even though pre-dawn Ψ_{px} in *L. tridentata* was between –3 and –4 MPa during the winter (Fig. 1b). In contrast, percentage embolism in *Prosopis velutina* increased to nearly 100% during both winters of the study while pre-dawn Ψ_{px} in *P. velutina* was higher than in *L. tridentata* (Fig. 1).

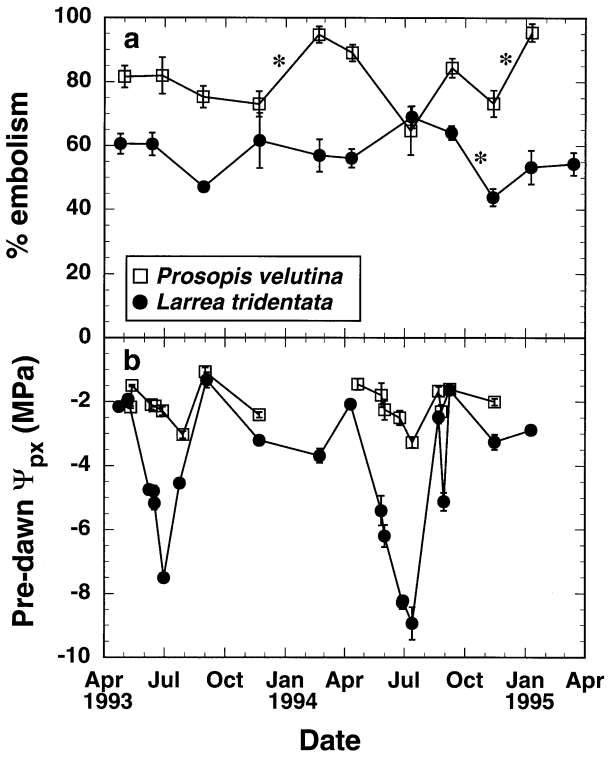

Fig. 1 **a** Seasonal pattern of embolism in *Larrea tridentata* (ANOVA, F = 3.0806, P = 0.0022; Tukey-Kramer HSD, P<0.05, marked with asterisk) and *Prosopis velutina* and **b** seasonal predawn xylem pressure (Ψ_{px}) of *L. tridentata* from April 1993 to December 1995 (±SEM). A Student's *t*-test showed the increase in embolism in *L. tridentata* in August–November 1993 was significant (P<0.05). The increase, if real, was slight and probably did not result from freezing conditions since the increase in *P. velutina* embolism did not appear until after November

When site temperatures were continuously monitored during the winter of 1994–1995, the air temperature fell below 0°C on 27 occasions (Fig. 2a). In every case, the minimum air temperature was between 0 and –5°C except on 20 November 1994 when it fell to approximately –7°C. Though wood temperature closely followed air temperature, air temperature at night was approximately 1°C higher. This was probably because of the shelter above the air thermistor. The only direct evidence of stems freezing was apparent exotherms recorded in *L. tridentata* (Fig. 3a) and *P. velutina* (data not shown) during the night of 20 November 1994. The exotherm can be seen as a brief increase in stem temperature where the air temperature decreases steadily (Fig. 3a, inside rectangle) and as a sharp decrease in the difference between air and wood temperature (Sakai and Larcher 1987). Minimum soil temperatures never fell below 4.5°C at 150 mm and 7.3°C at 300 mm (data not shown).

During the same winter, the only significant differences in percentage embolism in the temperature-monitored *L. tridentata* plants were between November and April (Fig. 2b). The percentage embolism was also significantly correlated with the number of freeze/thaw events preceding the measurement date (r = 0.436, n = 41, P<0.05) when all stems measured during the period were considered (thermocouple-equipped plants and neighbors). Though this increase was slight and exotherms were observed only on one night, we cannot eliminate the possibility that these data are evidence for a small cumulative effect of repeated freezing and thaw-

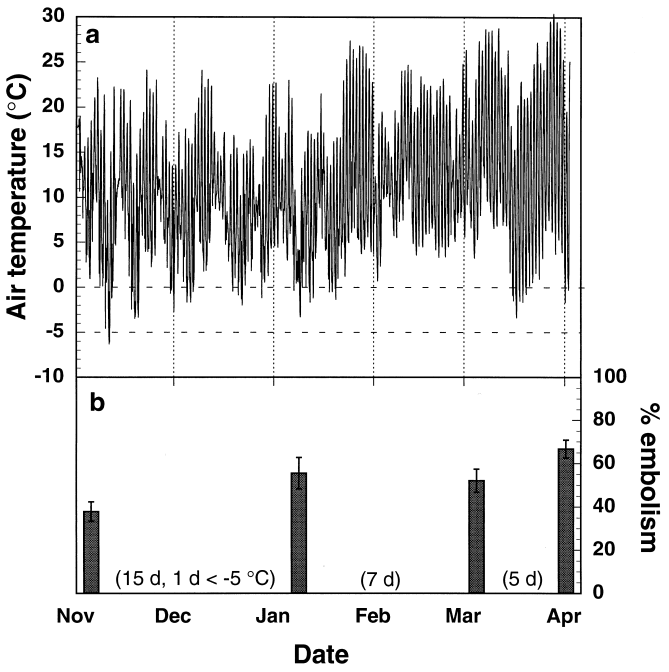

Fig. 2 **a** Canopy air temperature and **b** percentage embolism in xylem of *L. tridentata* from November 1994 to April 1995. The number of days with air temperatures recorded below 0°C is noted between embolism measurements. The only groups that differed were measurements made in November 1994 and April 1995 (ANOVA, F = 4.1634, Tukey-Kramer HSD, P<0.05)

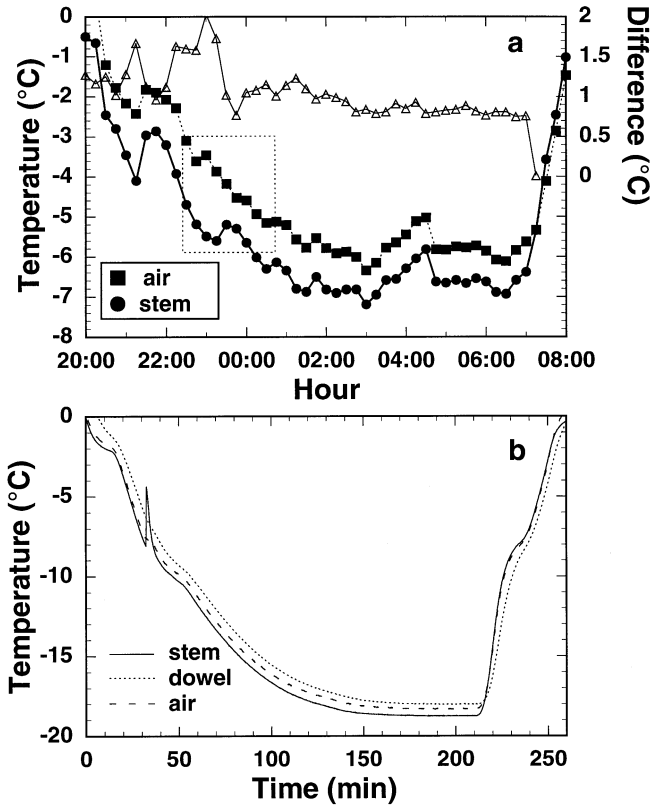

Fig. 3 **a** Field measurements of wood and air temperature (*left y-axis, circles and squares*) and the difference between air and wood temperature (*right y-axis, triangles*) on the night of 20 November 1994. A freezing exotherm (inside *rectangle*) was revealed by an increase in wood temperature without a corresponding change in air temperature and, at the same time, a sharp decrease in the difference between air and wood temperature. Pre-dawn Ψ_{px} measured several days earlier was –3.1 MPa (SD = 0.6). **b** Laboratory measurements of stem, dried dowel and air temperature during a typical freezing experiment. An exotherm occurred at –8°C. The apparent endotherm during thawing is an artifact of a pause in the program controlling the temperature bath

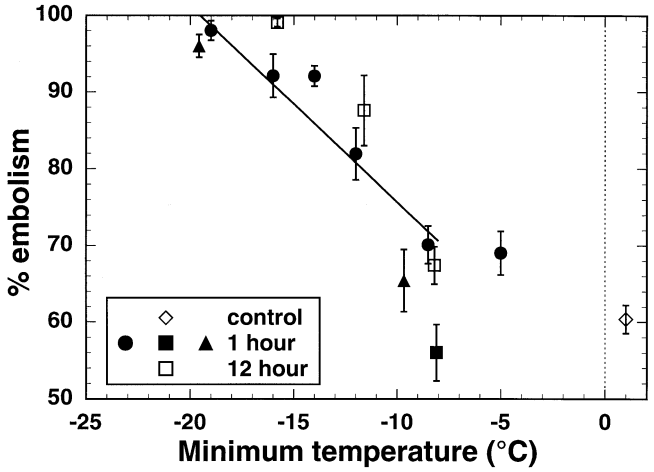

Fig. 4 Percentage embolism in *L. tridentata* stems after freezing as a function of minimum wood temperature during controlled freezing experiments. Control measurements from all experiments were not significantly different and were pooled (*open diamond*). Stems were frozen for 1 h (*triangles* April 1995, *circles* December 1995, *solid squares* March 1996) and 12 h (*open squares* March 1996). Stems cooled to temperatures of –11°C or below were significantly different from controls while those cooled to –9°C or above were not different (ANOVA, $F = 26.2052$, $P<0.0001$, Dunnett's method, $P<0.05$). The slopes of linear regressions of 1 and 12 h treatments in which exotherms were detected were not significantly different. A linear regression through these pooled data is shown above ($r = –0.658$, $P<0.01$, $n = 85$)

ing over the course of the winter. Nevertheless, the increase in percentage embolism in *L. tridentata* was small compared to that observed in *P. velutina* during the same period (Fig. 1a). From these data we conclude that for *L. tridentata*, little embolism resulted from freezing under field conditions during the period measured.

Laboratory freezing experiments

Our initial experiments confirmed field results in that percentage embolism in stems cooled to –10°C (Fig. 4, triangles) was not significantly different from unfrozen control stems (Fig. 4, diamond). However, stems frozen to –19.5°C (triangles) had significantly higher embolism than the control and not different from 100%. Wood temperature data (not shown) suggested the occurrence of freezing exotherms but these results were difficult to interpret because of the fluctuations in air temperature as the freezer cycled on and off.

Results from freezing trials in December 1995 and March 1996 confirmed and extended these initial observations (Fig. 4). Exotherms were readily detected in all stems cooled to –8°C or below (Fig. 3b) while none were seen in stems cooled to –5°C. This agreed with field data which showed freezing only when temperatures dropped below –5°C (Fig. 3a). No embolism above control values was detected in stems cooled to between –5 and –9°C despite the occurrence of freezing in stems cooled below –8°C. Stems cooled to –11°C or below exhibited a linear increase in embolism with decreasing minimum temperature and reached 100% between –17 and –19°C. There were no significant differences between the slope of the relationship in the 1 and 12 h treatments (Fig. 4).

The results above suggested that minimum temperature was the primary factor determining the extent of embolism. Although mean Ψ_{px} before freezing differed between April (–4.3 MPa, SD = 0.28) and December, 1995 (–5.4 MPa, SD = 0.37) and March, 1996 (–3.6 MPa, SD = 0.92), there was no relationship between Ψ_{px} and the percentage embolism following freezing. The rates of cooling and warming were also not important. Cooling rates were between 0.14 and 0.24°C min^{-1} (mean = 0.17°C min^{-1}, SD = 0.04) and were not correlated with the percentage embolism (cooling: $r = 0.51$, $n = 9$, $P>0.05$). Warming rates from minimum temperature to 0°C ranged from 0.12 to 1.35°C min^{-1} across all experiments and were not correlated with percentage embolism ($r = 0.19$, $n = 12$, $P>0.05$). There was also no correlation when warming rates were calculated from exotherm tem-

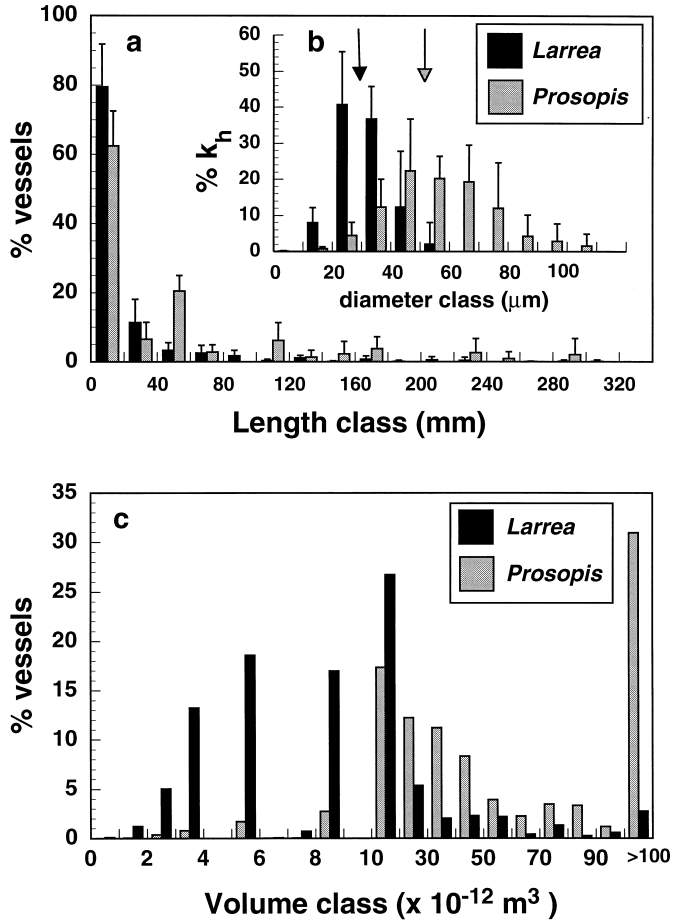

Fig. 5a–c Xylem conduit dimensions of *L. tridentata* (*black bars*) and *P. velutina* (*gray bars*). **a** Xylem conduit length and **b** diameter distributions. **c** Calculated vessel volume distribution. Diameter distributions show 10-μm classes reflecting the contribution of each class to total hydraulic conductivity (k_h) based on the Hagen-Poiseuille relationship (Zimmermann 1983). *Arrows* indicate the hydraulic mean diameter of *L. tridentata* (*black arrow*) and *P. velutina* (*gray arrow*). Volume distributions are based on the assumption that conduit diameter was randomly distributed with respect to length (model 2, see text)

perature to 0°C (r = 0.02, n = 12, P>0.05). Finally, the total time that stems were frozen (vs. time at minimum temperature) was not correlated with percentage embolism after treatment (r = 0.22, n = 71, P>0.05).

Xylem anatomy

In *L. tridentata* and *P. velutina*, 60–80% of the conduits were shorter than 40 mm (Fig. 5a). However in all stems of both species a small population of vessels, larger in *P. velutina* than in *L. tridentata*, extended up to lengths of 320 mm. It was likely that a small number of conduits of both species were not completely filled with paint because injection of freshly collected stems with low pressure compressed air (40 kPa) resulted in the appearance of air bubbles through segments longer than 320 mm (W.T. Pockman, unpublished work). The mean vessel diameter across the 13 *L. tridentata* individuals sampled was 19.7 μm (SD = 1.8) while the hydraulic mean was 28.4 μm (SD = 2.4, Fig. 5b, black arrow). The mean vessel diameter in *P. velutina* was 30.2 μm (SD = 2.7) and the hydraulic mean was 51.0 μm (SD = 8.2, Fig. 5b, gray arrow).

The two methods for estimating conduit volume produced similar results. Only the more conservative model 2 results are shown in Fig. 5c. Over 80% of the conduits in *L. tridentata* had a volume less than 2×10^{-11} m^3 while slightly more than 20% of those in *P. velutina* fell in the same range. Over 30% of the conduits in *P. velutina* had volumes greater than 1×10^{-10} m^3 compared to less than 3% in *L. tridentata*.

Larrea distribution with respect to temperature

Laboratory experiments indicated that temperatures between –16 and –20°C were sufficient to completely embolize *L. tridentata* xylem. There was a close correspondence between minimum isotherms (20 year record minimum) in this temperature range and the northern limit of *L. tridentata* (Fig. 6, solid line) in the Mojave and Sonoran deserts. The correspondence was particularly close across Arizona where the density of weather stations was higher than in southern Nevada and eastern California. In southeastern Arizona and in New Mexico and Texas the same correspondence was not evident.

Discussion

The patterns of seasonal embolism (Fig. 1a) confirmed our hypothesis that *L. tridentata* can avoid freezing-induced cavitation. This was particularly remarkable because winter pre-dawn Ψ_{px} between –3 and –4 MPa (Fig. 1b) would favor cavitation. Notably, the deciduous *Prosopis velutina* growing within 1 km became almost completely embolized during both winters of the study (Fig. 1a). The resistance of *L. tridentata* to freezing-induced cavitation was confirmed in the laboratory studies for minimum temperatures above –11°C.

Qualitatively, the data support the hypothesis that *L. tridentata*'s greater resistance to freezing-induced cavitation relative to *P. velutina* was because of its much smaller vessel volumes (Fig. 5c). Quantitatively, however, the *L. tridentata* vessel volumes were still too large to explain its avoidance of cavitation. Theoretical predictions of embolism based on Ψ_{px} and conduit volume overestimate the embolism observed experimentally (Hammel 1967; Sperry and Sullivan 1992). However, an empirically-derived relationship between embolism and conduit volume based on coniferous, diffuse- and ring-porous trees (Sperry et al. 1994) predicts that conduits larger than 10^{-12} m^3 would be at least 50% embolized at –2 MPa. This includes 99% of the vessels in *L. tridentata* (Fig. 5c). Apparently, the air bubbles in thawing *L. tri-*

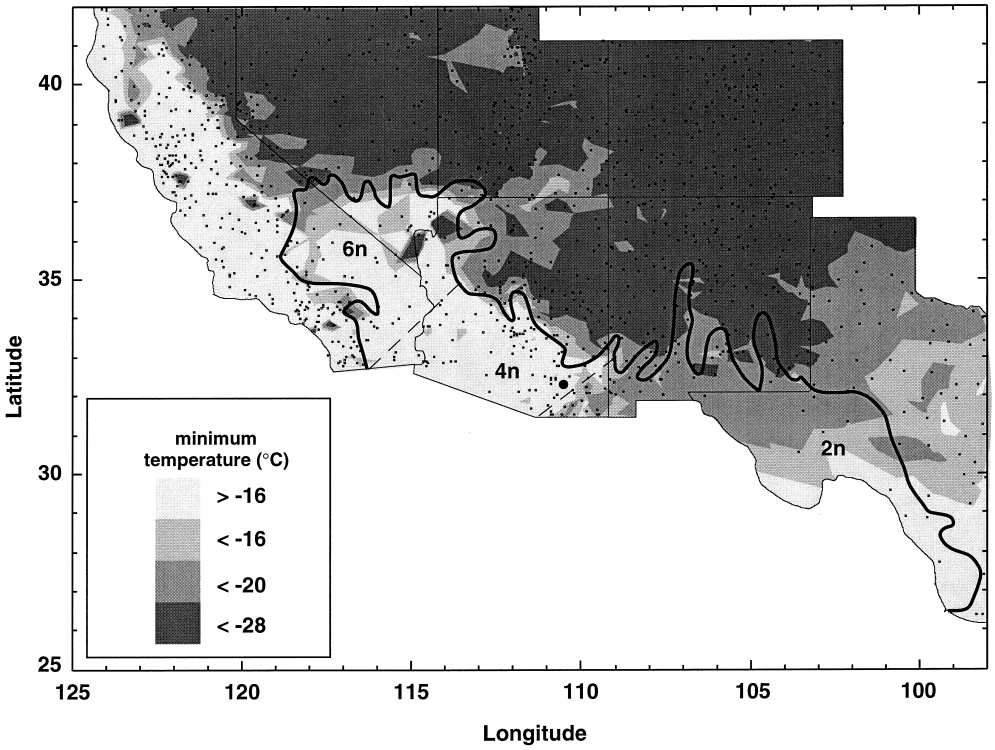

Fig. 6 The distribution of *L. tridentata* compared with record minimum temperature isotherms across the American southwest. Isotherms were calculated with temperature data from 1201 stations (*squares*). The mean period analyzed per station was 40.3 years (SD = 16) and the most common shared period of record was 1948–1989. The *solid line* is the approximate limit to the distribution of *L. tridentata* and the *broken lines* across the distribution indicate the approximate divisions between the diploid, tetraploid and hexaploid races (Hunziker et al. 1977). The location of the study site is labelled with a *solid circle*

dentata vessels were smaller than those in comparable-sized vessels of these tree species.

A number of factors, acting in concert, may be responsible for the smaller than expected bubble sizes in *L. tridentata*. During thawing, Ψ_{px} can be close to or above atmospheric pressure owing to the expansion of water during freezing (Robson and Petty 1987) with the result that bubbles may shrink. Bubble dissolution will also be favored by the de-gassed state of the xylem sap during the thaw (Sperry and Sullivan 1992). In addition, if a few conduits did cavitate, the water released to the rest of the xylem could help mitigate pressures and allow a longer period for the bubbles to dissolve (Sucoff 1969). Why these processes, which in principle could be common to all plants, should result in more complete dissolution of bubbles in *L. tridentata* xylem than in other species of comparable vessel volume is unknown.

The increase in embolism as a function of declining minimum treatment temperature (Fig. 4) is also difficult to explain. If the bubbles formed by freezing are the only cause of embolism, the minimum temperature below the freezing point should be unimportant. This conclusion is supported by laboratory experiments with *Ceanothus megacarpus* (Langan et al. 1996) and field studies of winter embolism in diffuse-porous trees (Sperry et al. 1994). However, other studies agree with our results for *L. tridentata* in showing an effect of minimum temperature on embolism. *Quercus ilex* of the southern mediterranean basin developed increasing embolism with decreasing minimum temperature (Lo Gullo and Salleo 1993), as did *Rhus laurina* and *R. ovata* of the California chaparral (S.D. Davis and F.W. Ewers, unpublished work).

There are a number of possible explanations for an interaction between minimum temperature and embolism. In *Q. ilex*, LoGullo and Salleo (1993) suggested that interfacial effects decreased the freezing point of xylem sap with decreasing vessel diameter, causing incremental freezing of xylem as temperatures dropped. However, this is unlikely because water-filled pores must be narrower than 0.1 μm before they depress the freezing point relative to bulk water (Ashworth and Abeles 1984), and vessel diameters are orders of magnitude larger than this limit (Fig. 5b). A more rapid thaw rate associated with lower minimum temperatures might inhibit bubble dissolution and trigger more embolism (Langan et al. 1996). However, the cooling and thawing rates were not related to the patterns we observed.

The temperature dependence might arise from the fact that the water potential of ice decreases steeply with declining temperature (Rajashekar and Burke 1982; Beck et al. 1984). As the water potential of apoplastic ice decreases, water is drawn from surrounding xylem parenchyma into the xylem conduits and intercellular spaces where it freezes. As a result, the colder the ice, the more dehydrated the surrounding cells become. Upon thawing, the water potential gradient into the living cells would be determined by the minimum temperature during the freezing episode. A colder freeze would result in a steeper gradient, and a more rapid uptake of apoplastic water during the thaw. A more rapid uptake could result in a more rapid establishment of low Ψ_{px} during thawing, and thus more cavitation.

Freezing injury to xylem parenchyma could also contribute to the observed temperature dependence of embolism. Cell injury could indirectly cause embolism if it

nucleated cavitation in the xylem after thawing or interfered with bubble dissolution during thawing. Although we did not assess freezing-induced cell injury in our experiments, cell death and embolism were correlated for *Rhus laurina* and *R. ovata* (S.D. Davis and F.W. Ewers, unpublished work). If embolism is linked to cell injury or death, frost acclimation of cells (Sakai and Larcher 1987) could cause seasonal changes in the effect of minimum temperature on embolism formation. In our case, however, the embolism response in April, December, and March was similar (Fig. 4: compare triangles, circles and open squares).

Although the mechanism underlying the observed embolism response in *L. tridentata* is unclear, the response itself (Figs. 2, 4) suggests that freezing-induced cavitation may play an important role in determining the northern limit to the distribution of this evergreen species. This conclusion is further supported, at least for its western range, by the close relationship between the northern border of the actual distribution of polyploid *L. tridentata* and the region representing a record minimum temperature of between –16 and –20 °C (Fig. 6). *L. tridentata* in this region (Fig. 6) are reported to be either tetraploid or hexaploid (Yang 1970; Hunziker et al. 1977). The lack of such a relationship at the eastern and western edges of the *L. tridentata* distribution would suggest that other factors such as precipitation (Beatley 1974a) are more important in those regions.

The correspondence between distribution and minimum temperature observed across Arizona, California, Nevada, and Utah is not evident in New Mexico where *L. tridentata* would appear to be tolerant of colder temperatures. Interestingly, this part of *L. tridentata*'s range is composed of diploid populations (Fig. 6). We would predict that experiments similar to ours performed on a diploid *L. tridentata* population in New Mexico would show a greater tolerance to freezing without cavitation. Previous work has shown that the size of some cells increases with ploidy (Stebbins 1971). If this pattern applied to xylem conduit volumes, it would be consistent with the expectation of increased resistance to freezing-induced cavitation of the diploid race. Though it is only a qualitative observation here, the role of ploidy in freezing tolerance bears further study in light of previous reports of correlations between ploidy and stress tolerance (Robichaux and Canfield 1985).

Our findings are consistent with previous studies and anecdotal reports of the freezing tolerance of *L. tridentata*. Using 10 years of climatic data collected over a wide area near the northern limit of *L. tridentata* in Nevada, Beatley (1974b) found that *L. tridentata* did not occur at sites where the mean minimum temperature during the study period was below –17.8°C. This value agrees with the limit that we would predict assuming that induction of 100% cavitation is the defining criterion. In particular, *L. tridentata* was excluded from sites subject to low elevation temperature inversions that maintained air temperatures lower than adjacent higher elevation sites (Wells and Shields 1964; Beatley 1974b). Temperatures below –17°C have been shown to kill *L. tridentata* shoots, but the plants can grow back from the root crown (Cottam 1937; Fosberg 1938; Valentine and Gerard 1968). Although re-sprouting would mitigate the consequences of infrequent dieback from cavitation, any regular occurrence of dieback would exhaust slowly accumulating reserves.

Though our experiments indicate that freezing-induced cavitation is an important determinant of the northern limit of *L. tridentata*, damage to the leaf tissue may also contribute. If leaves were killed by freezing but meristematic and xylem tissue survived then regrowth from apical buds would seem likely. In contrast, if leaf cells survived and the xylem were completely cavitated then we would predict that the leaves would die rapidly as a result of dehydration (Sperry and Sullivan 1992; Sperry et al. 1993; Sperry and Pockman 1993). Following the loss of all leaf area, dieback to the root crown where freezing temperatures were more moderate (see Results) would not be unexpected. The latter pattern was observed following severe freezes in Utah and New Mexico (Fosberg 1938; Valentine and Gerard 1968).

Our work leaves unanswered the question of what is responsible for the high native percentage embolism (~60%, Fig. 1a) in the population of *L. tridentata* that we studied. The freezing patterns we observed (Figs. 2–4) and the record minimum temperature data for an extended period (Fig. 6) suggest that the study population is unlikely to experience temperatures sufficient to induce extensive cavitation. Nevertheless, it is possible that some cavitation occurs with repeated freeze/thaw events (Fig. 2a) and that the native embolism we observed is the result of the accumulation of such minimal cavitation over many years (the lifetime of the stems measured). Though the native embolism could also be attributed to water stress-induced cavitation, the absence of significant changes in percentage embolism (Fig. 1) during the summer suggests that this is not the case. Whatever the source of the embolism, *L. tridentata* presents an excellent experimental system for future work on the role of freezing-induced xylem cavitation in limiting the distribution of evergreen desert plants.

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