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**Root and stem xylem embolism, stomatal conductance, and leaf turgor in *Acer grandidentatum* populations along a soil moisture gradient**

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**Abstract** The objective of this study was to determine how adjustment in stomatal conductance ( $g_s$ ) and turgor loss point ( $\Psi_{tlp}$ ) between riparian (wet) and neighboring slope (dry) populations of *Acer grandidentatum* Nutt. was associated with the susceptibility of root versus stem xylem to embolism. Over two summers of study (1993–1994), the slope site had substantially lower xylem pressures ( $\Psi_{px}$ ) and  $g_s$  than the riparian site, particularly during the drought year of 1994. The  $\Psi_{tlp}$  was also lower at the slope ( $-2.9 \pm 0.1$  MPa; all errors 95% confidence limits) than at riparian sites ( $-1.9 \pm 0.2$  MPa); but it did not drop in response to the 1994 drought. Stem xylem did not differ in vulnerability to embolism between sites. Although slope-site stems lost a greater percentage of hydraulic conductance to embolism than riparian stems during the 1994 drought ( $46 \pm 11\%$  versus  $27 \pm 3\%$ ), they still maintained a safety margin of at least 1.7 MPa between midday  $\Psi_{px}$  and the critical pressure triggering catastrophic xylem embolism ( $\Psi_{pxCT}$ ). Root xylem was more susceptible to embolism than stem xylem, and there were significant differences between sites: riparian-roots were completely cavitated at  $-1.75$  MPa, compared with  $-2.75$  MPa for slope roots. Vulnerability to embolism was related to pore sizes in intervessel pit membranes and bore no simple relationship to vessel diameter. Safety margins from  $\Psi_{pxCT}$  averaged less than 0.6 MPa in roots at both the riparian and slope sites. Minimal safety margins at the slope site during the drought of 1994 may have led to the almost complete closure of stomata ( $g_s = 9 \pm 2$  versus  $79 \pm 15$  mmol m<sup>-2</sup> s<sup>-1</sup> at riparian site) and made any further osmotic adjustment of  $\Psi_{tlp}$  non-adaptive. Embolism in roots was at least partially reversed after fall rains. Although catastrophic embolism in roots may limit the minimum  $\Psi$  for gas exchange, partial (and reversible) root embolism may be adaptive in limiting water use as soil water is exhausted.

**Key words** Xylem embolism · Xylem cavitation · Turgor maintenance · Stomatal conductance · Drought tolerance

**Introduction**

A number of studies have suggested that stomata regulate transpiration to maximize stomatal conductance on the one hand while preventing critically negative xylem pressures causing excessive xylem cavitation on the other (Tyree and Sperry 1988; Jones and Sutherland 1991; Meinzer and Grantz 1990; Cochard et al. 1992; Meinzer 1993; Sperry et al. 1993; Saliendra et al. 1995). If this is generally true, the resistance of a species to xylem cavitation determines the potential range of water stress over which that species can maintain gas exchange. This could clarify our understanding of how plants are adapted to water stress, and how they may respond to changing climatic regimes.

Xylem cavitation is the abrupt transition of xylem water from a metastable liquid state to vapor, and it leads inevitably to an air-filled, or embolized, xylem conduit and reduced xylem hydraulic conductance (Tyree et al. 1994). When cavitation occurs in a transpiring plant, positive feedback ensues between decreasing hydraulic conductance and decreasing xylem pressure ( $\Psi_{px}$ ). For a given initial hydraulic conductance of xylem ( $k$ ) and soil water potential ( $\Psi_s$ ), there is a critical maximum transpiration rate and minimum (most negative) xylem pressure beyond which this positive feedback becomes unstable and catastrophic, or "runaway," cavitation and embolism occurs that eliminates xylem transport (Tyree and Sperry 1988; Jones and Sutherland 1991). This minimum xylem pressure ( $\Psi_{pxCT}$ ) is the value of  $\Psi_{px}$  that maximizes the transpiration rate ( $E$ ) for steady-state conditions in the following equation:

$$E = (\Psi_s - \Psi_{px}) k(\Psi_{px}) \quad (1)$$

where  $k(\Psi_{px})$  is the function describing the relationship between  $k$  and  $\Psi_{px}$  (a "vulnerability curve," Jones and

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Sutherland 1991). As emphasized by Jones and Sutherland (1991), considerable cavitation can develop in some plants without  $\Psi_{px}$  reaching  $\Psi_{pxCT}$ .

The studies cited above all imply more or less directly that without stomatal regulation,  $\Psi_{px}$  would exceed  $\Psi_{pxCT}$ . Accordingly, xylem pressures are predicted to be near or even within the cavitation range under transpirational conditions. However, many species can grow over a wide range of soil water availability and acclimate to drier conditions by altering tissue water relations and stomatal behaviour (Turner and Jones 1980; Morgan 1984). Does susceptibility to cavitation also change across these gradients so that the safety margin from critical values of cavitation remains nearly equal for all populations? Or does cavitation only limit gas exchange for the driest populations? There is no information on this issue.

This paper seeks to answer this question for adjacent populations of *Acer grandidentatum* growing in riparian and slope habitats in northern Utah. This tree is relatively shallow-rooted and shows large differences in water status between these habitats (Dina 1970). We compared stomatal conductance and transpiration, tissue water relations, and xylem embolism (loss of hydraulic conductance) resulting from cavitation in stems and roots at both sites to determine how these traits adjusted in response to differing soil moisture. The importance of stomatal regulation of transpiration for avoiding critical values of embolism was determined by estimating the minimum safety margin from catastrophic xylem embolism. In terms of xylem pressure, this safety margin was the difference between the minimum  $\Psi_{px}$  and  $\Psi_{pxCT}$ . We made our comparison across two summers, one of which was exceptionally cool (1993, 4.1°C below average, precipitation 7% below average, June–September), and the other exceptionally hot and dry (1994, 4.6°C above average, precipitation 74% below average) for the study site area.

## Methods

### Study sites

The study was done in the Red Butte Canyon Research Natural Area, east of Salt Lake City, Utah (c. 111°48' W 47°N; elevation 1640 m). The riparian site was immediately upstream from Red Butte Reservoir along Red Butte Creek, a perennial stream. The slope site was approximately 0.25 km north of the riparian site.

### Xylem pressure, transpiration, and stomatal conductance

Leaf xylem pressure ( $\Psi_{px}$ ) was measured using a pressure bomb (P.M.S. Instruments, Corvallis, Oregon, USA) on three leaves per tree at both sites several times throughout the study. The same ten trees, 10–15 m in height, were repeatedly measured at the riparian site in shady and exposed situations. Five exposed trees, 2–3 m in height, were measured at the slope site. Pre-dawn measurements were taken between 0500 hours and 0630 hours, and mid-day readings were taken between 1300 hours and 1400 hours on clear days.

Stomatal conductance ( $g_s$ ) was measured using a null-balance porometer (Licor 1600, Licor Inc., Lincoln, Neb.). Measurements were taken of five leaves from each of three exposed trees per site. Transpiration ( $E$ ) at ambient humidity was also measured with the porometer. Although porometer measurements of  $E$  do not necessarily reflect in situ values because of alterations of boundary layer and evaporative gradient (McDermitt 1990), our interest was in comparative rather than absolute values. We only report values from exposed trees where boundary layer and evaporative gradients would have been approximately the same (and altered to equal extents by the porometer).

### Vulnerability to xylem embolism

Embolism refers to the blockage of xylem from cavitation, and for most purposes the terms "cavitation" and "embolism" are functionally synonymous. "Vulnerability curves" show the percentage decrease in hydraulic conductance of xylem from embolism as a function of minimum (negative) xylem pressure. This is the basis for defining the function  $k(\Psi_{px})$  in Eq. 1 where  $k$  is hydraulic conductance. For the same material, the same vulnerability curve is obtained whether embolism is induced by negative xylem pressure in dehydrated stems, or by positive air pressure injected into the vascular system of hydrated stems where xylem pressure was atmospheric. This is because cavitation occurs by air entering intact xylem conduits through inter-conduit pits, and whether the air is pulled through pits by negative xylem pressure or pushed through by air pressure, the pressure difference required is equal (Sperry and Tyree 1990; Sperry et al. 1991; Cochard et al. 1992b; Sperry and Saliendra 1994; Crombie et al. 1985; Jarbeau et al. 1994).

We used the positive air pressure method to measure most vulnerability curves in hydrated stems and roots, but also compared results using negative xylem pressures in dehydrated stems.

### Air pressure method

The air pressure technique has been described in detail by Sperry and Saliendra (1994) (see also Cochard et al. 1992b). Briefly, a stem or root segment was placed within an air-tight steel chamber with each end protruding, and the upstream end attached to a supply of filtered (0.2  $\mu$ m) solution of HCl in distilled water (pH = 2). The low pH inhibited microbial growth on the inner walls of the tubing which otherwise causes rapid clogging of the xylem (Sperry et al. 1988). Tests showed no effect of HCl relative to distilled water alone on the results (Sperry and Saliendra 1994). The hydraulic conductance of the xylem was determined from the mass flow rate of solution divided by the pressure difference across the segment when the solution source was raised above it. Mass flow rate was measured by collecting flow-through from the segment in pre-weighed vials filled with absorbent paper or cotton. The portion of segment in the chamber was then exposed to increasingly high air pressures. Air entered the vascular system through cut side-branches (or cut lateral roots) and/or petiole stubs. After each interval of pressure injection, the air blockage (= embolism) was quantified from the percent the hydraulic conductance decreased relative to the initial value. Control stems that were not pressurized showed less than 10% deviation in hydraulic conductance relative to their initial value over a typical measurement period with no systematic increase or decrease with time.

The hydraulic pressure difference used during conductance measurements was c. 8 kPa for stems, but was reduced to 2 kPa for roots when we found the higher pressure was sufficient to displace air from vessels that were embolized by the air pressure and continuous through the segment. This was not a problem for stems because stem vessels were shorter than in roots so fewer of them extended through the stem segment. Stem vessels were also narrower (19.6  $\pm$  1.3  $\mu$ m;  $n$  = 15 stems) than root vessels (55.0  $\pm$  7.1  $\mu$ m;  $n$  = 12 roots) and required greater water pressure to displace air from any vessels open at both ends of the segment. From the capillary equation, this displacement pressure was

14.7 kPa for the average stem vessel versus 5.2 kPa for root vessels.

Branches for the air pressure experiments were harvested in the field in lengths of c. 1 m and brought to the laboratory in plastic bags. Segments 150 mm long and between 5.0 and 8.8 mm in diameter were cut from the center of these branches under water to avoid blocking additional vessels with air and to avoid including vessels that were air-blocked during harvesting. Three stems from different trees per site were sampled in July, August, and September during the summer of 1993 to detect any seasonal changes in cavitation resistance.

Roots for air pressure experiments were cut from the tree in lengths of at least 0.5 m. The cuts were made underwater by bending the roots into a shallow water-filled tray. This was done to minimize air blockage from the cut ends of the segments which was more of a problem in roots than stems because of their longer vessel lengths (Zimmermann and Potter 1982) and the difficulty of harvesting long root pieces. Root segments were transported to the lab in distilled water, and recut to lengths ranging from 209 mm to 395 mm and basal (distal) diameters ranging from 3.8 mm to 13.8 mm. Roots of this size probably functioned mainly in axial transport and had a limited role in water uptake owing to their low surface area/volume ratios and well-developed periderm. Six roots from different trees per site were sampled in August 1994.

Root segments were flushed with HCl solution at c. 100 kPa for at least 30 min before vulnerability curves were measured. This insured that the initial hydraulic conductance prior to air injection represented the no embolism situation. Stems were not flushed prior to measurement, but apparently refilled substantially prior to vulnerability curve measurement because loss of conductance was commonly observed at pressure differences they had already experienced in the field. As noted in Results, this agreed with independent measurements of the loss of conductance in the field ("native" embolism, see below).

"Average embolism pressure" was calculated from vulnerability curves of root and stem segments using the method of Sperry and Saliendra (1994). Vulnerability curves show the cumulative distribution of loss of conductance versus pressure. They can be re-plotted as a regular (non-cumulative) distribution of loss of conductance versus pressure increment. The mean of this distribution gives the average embolism pressure for the material. Means were calculated using the mid-point of each pressure increment.

#### Negative pressure method

For stems, we compared vulnerability curves measured with positive air pressure to curves measured using negative xylem pressures in dehydrated stems. For this latter method, we collected intact branch tips (from riparian site trees) and dehydrated them in the pressure bomb to targeted xylem pressures (Cochard et al. 1992b). Branches were kept in plastic bags for 1–2 h to allow cavitated and vapor-filled vessels to become air-filled. Segments 100–150 mm long were cut from these branches underwater and the amount of embolism determined from the percentage their hydraulic conductance was below the maximum value after air in vessels was dissolved by high pressure (c. 100 kPa) flushes of solution through the segment (Sperry et al. 1988).

Dehydrating branches with the pressure bomb also may induce embolism by injection of air into the vascular system. Thus, correspondence of air pressure and bomb-dehydration results would show that dehydration caused no *additional* embolism above what was caused by air pressure alone.

#### Determination of critical xylem pressure

The lowest (most negative) allowable xylem pressure ( $\Psi_{pxCT}$ ) was obtained by finding the maximum  $E$  in Eq. 1 and then solving for  $\Psi_{px}$ . The vulnerability curve provided the relation between hydraulic conductance and xylem pressure [ $k(\Psi_{px})$ , Eq. 1]. We converted the percentage loss of hydraulic conductance to actual con-

ductance values using an arbitrary maximum hydraulic conductance. While this returns an arbitrary value of  $E$  in Eq. 1, it does not alter the value of  $\Psi_{pxCT}$  which is the only parameter we report. We assumed that  $\Psi_{px}$  equalled predawn  $\Psi_{px}$ .

Equation 1 can be solved for maximum  $E$  using analytical methods when  $k(\Psi_{px})$  is a relatively simple function (Jones and Sutherland 1991). We used third-order polynomial fits to root and stem vulnerability curves and resorted to the iterative procedure detailed in Tyree and Sperry (1988). A computer program was written to take values of  $E$ ,  $\Psi_s$ , and maximum  $k$  and do the following: (1) solve for  $\Psi_{px}$  given maximum  $k$  and  $\Psi_s$ , (2) determine the loss in  $k$  from  $k(\Psi_{px})$ , (3) solve for the new  $\Psi_{px}$  at the new  $k$ , (4) repeat steps 2 and 3 until  $\Psi_{px}$  remained stable, (5) increment  $E$  and repeat steps 1–5 until the program failed to find a stable  $\Psi_{px}$  (after 1000 iterations). The last stable  $\Psi_{px}$  was taken as  $\Psi_{pxCT}$ . The same value was obtained from the program and from analytical methods when simplified (linear) functions for  $k(\Psi_{px})$  were used.

Our analysis of Eq. 1 and its predicted limits for  $E$  and  $\Psi_{px}$  apply only for steady-state flow through the plant under conditions of constant source water potential (i.e., constant  $\Psi_s$ ) as  $E$  is incrementally increased. In the plant, deviations from steady-state flow could allow both  $E$  and  $\Psi_{px}$  to temporarily exceed steady-state limits without inducing catastrophic cavitation. Drift in source water potential as  $E$  increased during the day would cause variation in  $\Psi_{pxCT}$ . Nevertheless, we feel this approach provides a useful quantitative benchmark for evaluating the limits on steady-state water flux resulting from cavitation. In this context it is preferable to choosing an arbitrary cavitation threshold based on subjective analysis of the vulnerability curve.

#### Vessel anatomy

Each segment used for vulnerability curves was stored in a freezer for later measurement of inner vessel diameter distribution. Vessels were measured using a light microscope interfaced with a Micro-plan II image analysis system (DonSanto Corp., Natick, Mass.) and a computer. For each segment, a minimum of 300 vessels from current years growth were measured. The percentage of the total sum of vessel radii to the fourth power ( $\Sigma r^4$ ) was determined for vessels in 10  $\mu$ m diameter classes. This gives a hydraulically weighted diameter distribution assuming hydraulic conductance of a vessel is proportional to its radius to the fourth power as predicted by Poiseuille's law (Zimmermann 1983). The mean of this distribution is  $\Sigma r^5$  divided by  $\Sigma r^4$ . We report hydraulically weighted diameters because they provide the most direct comparison with embolism vulnerability which was determined from hydraulic conductance measurements.

To compare the conducting efficiency of roots from riparian and slope sites, we divided the hydraulic conductance of ten root segments from each site by the transverse area of the segment. The hydraulic conductance used in these calculations was the maximum value obtained after embolism removal (see "native embolism," below). Transverse root area was calculated from root diameter assuming a circular cross section.

Inter-vessel pit membranes of roots and stems were observed with a scanning electron microscope (SEM; Hitachi S-450). Longitudinal sections were cut with a sliding microtome, dehydrated in progressively stronger ethanol concentrations, and critical-point dried. Samples were coated with gold-palladium prior to observation.

#### Native embolism measurements

Branches and roots were collected in the field in the same manner as for the air pressure experiments described above. Segments of the same length and diameter were cut from these branches and roots, and the amount of embolism was determined as the percent their initial hydraulic conductance was below the maximum value obtained after a series of 100 kPa flushes of HCl solution through

the segments (Sperry et al. 1988). This represented an estimate of how much embolism was present in situ ("native" embolism).

Embolism was measured in this manner on five branch segments (one per tree) from each site three times per summer. Root embolism was measured on five roots per site (one per tree) at two times during late summer of 1994, and once again at the slope site after fall rains in 1994.

Root embolism measurements made before the fall rains were minimum estimates because the pressure head used during the hydraulic conductance measurements (c. 8 kPa) was sufficient to displace air from vessels open at both ends. The initial conductance so obtained was too high depending on the number of embolized vessels longer than the segment. Reducing the pressure head to 2 kPa in subsequent measurements avoided air displacement. As mentioned above, this was not a problem in stems because vessels were shorter and narrower than in roots. In a separate series of experiments we embolized roots with air pressure and determined that the embolism measured at the higher hydraulic pressure head under-estimated actual values anywhere from 8.7 to 22.0 percentage points. In the absence of embolism, hydraulic conductance was independent of the pressure head.

#### Pressure-volume analysis

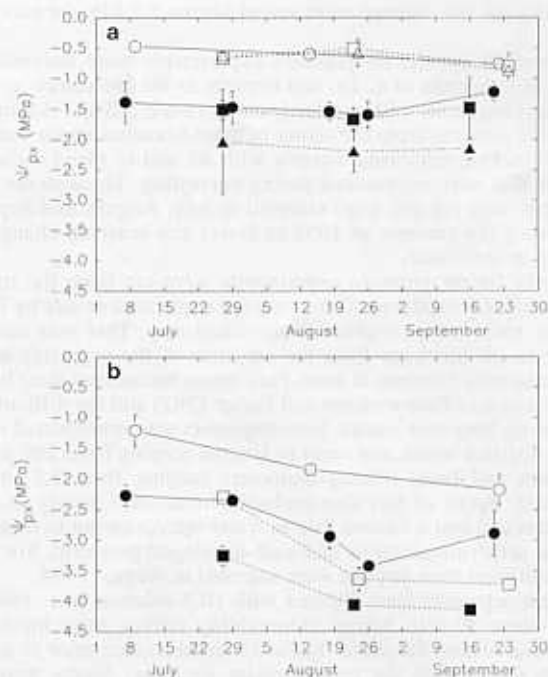
Shoots approximately 1 m in length from a total of six trees per each site were harvested, bagged, and brought into the laboratory for pressure-volume analysis during mid-summer (1993, slope and riparian sites) and late summer (1994, slope site only). Shoot tips were recut underwater and allowed to briefly rehydrate (less than 30 min) in distilled water before constructing pressure-volume curves on individual leaves using the bench-top dehydration method (Ritchie and Hinckley 1975). Bulk tissue estimates of osmotic potential, cell wall elastic modulus, and turgor loss point were made using the methods of Schulte and Hinckley (1985) and software provided by Dr. P.J. Schulte.

## Results

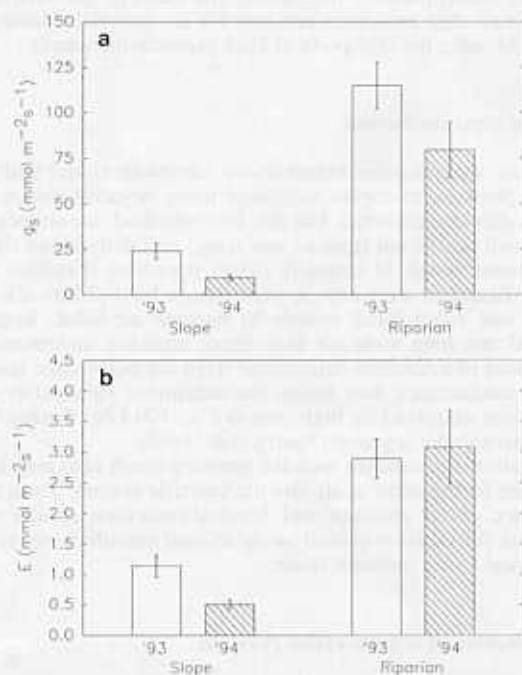
Riparian and slope sites differed substantially in predawn and midday  $\Psi_{px}$  (Fig. 1a, b) during both summers. Values at the riparian site were constant during the summer and between years. At the slope site, predawn and midday  $\Psi_{px}$  tended to decrease throughout the growing season; values were considerably lower during the hot and dry summer of 1994 than during the cool summer of 1993 (Fig. 1b, compare circles and squares).

The drier conditions at the slope site in late summer limited stomatal conductance and transpiration relative to the riparian site even during the cool conditions in 1993 (Fig. 2; late August–early September data). The limitation was especially severe during the 1994 drought when stomata were nearly closed in slope trees (Fig. 2a, hatched bars).

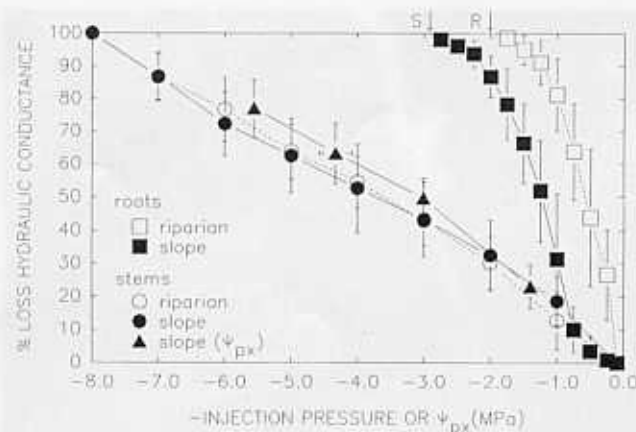
Turgor loss point ( $\Psi_{tlp}$ ) averaged  $-1.9 \pm 0.2$  MPa ( $n = 6$ ; all means given are  $\pm 95\%$  confidence limits) at the riparian site versus  $-2.9 \pm 0.1$  MPa ( $n = 10$ ) at the slope site. This was sufficiently low for leaves to be near or above the  $\Psi_{tlp}$  at both sites for most of 1993. The one exception was 26 August 1993 when slope site leaves were c. 0.4 MPa below  $\Psi_{tlp}$ . The difference in  $\Psi_{tlp}$  between sites resulted from lower bulk tissue osmotic potential rather than changes in cell wall elasticity (data not shown). There was no difference in slope-site  $\Psi_{tlp}$  be-



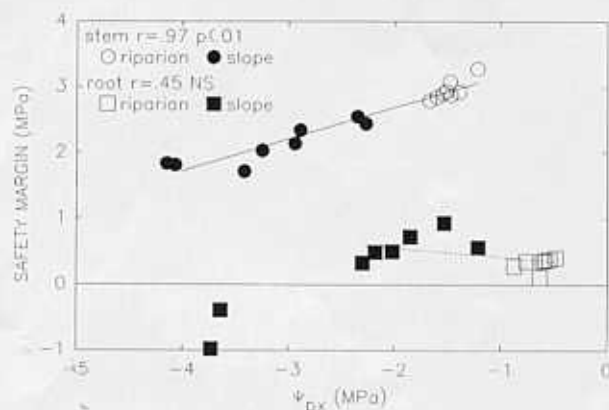
**Fig. 1a, b** Seasonal leaf xylem pressure ( $\Psi_{px}$ ) in *Acer grandidentatum* from July to September in 1993 (circles, solid lines) and 1994 (squares, triangles, dashed lines). Open symbols predawn values, midday values closed symbols. Error bars are 95% confidence limits ( $n = 5$ ). **a** Riparian site. Circles shaded trees, triangles exposed trees (1994 data only). **b** Slope site. All trees exposed



**Fig. 2 a** Stomatal conductance ( $g_s$ ), **b** transpiration ( $E$ ) at midday under clear conditions for 1 September 1993 (open bars  $\pm 95\%$  confidence limits,  $n = 15$ ) and 23 August 1994 (shaded bars,  $n = 15$ )



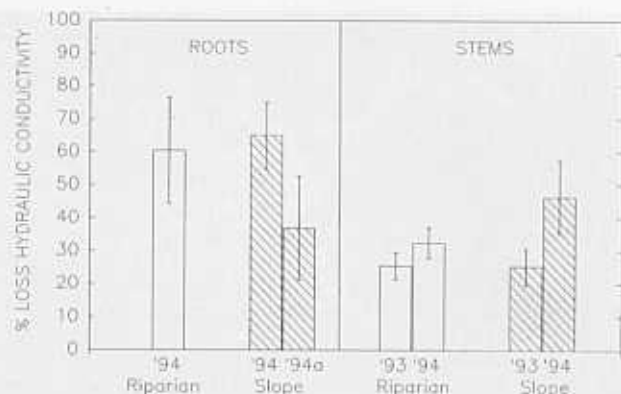
**Fig. 3** Vulnerability curves showing the percentage loss in hydraulic conductance versus xylem pressure in dehydrated stems or the negative of the air pressure injected into hydrated stems. Curves are for roots (squares,  $n = 6$ ) and stems (circles,  $n = 9$ ; triangles,  $n = 3-6$ ) of slope (solid symbols, solid lines) and riparian (open symbols, dashed lines) trees. All curves were obtained from the air pressure method except for one curve from slope tree stems (triangles) which was obtained using negative pressures in dehydrated stems ( $\Psi_{px}$ ). Arrows on upper x axis indicate average turgor loss points for riparian (R, right arrow) and slope (S, left arrow) leaves. Error bars are 95% confidence limits



**Fig. 4** Safety margin (midday  $\Psi_{px} - \Psi_{px(CT)}$ ) of stems (circles) and roots (squares) at riparian (open symbols) and slope (closed symbols) sites versus predawn  $\Psi_{px}$ . Safety margins were calculated from the midday and predawn  $\Psi_{px}$  data in Fig. 1 using vulnerability curves in Fig. 3. Stems had larger safety margins than roots, although the margin decreased with increasing drought ( $r = 0.97$ ;  $P < 0.01$ ) because vulnerability to cavitation was the same at both sites. Safety margins in roots were small (positive values averaged  $0.59 \pm 0.20$  MPa) and constant at both sites because of adjustment in vulnerability to cavitation. Negative safety margins were predicted in roots at the slope site at the end of the 1994 drought

tween years despite the 1994 drought (mean given is pooled across years). As a result, both midday and predawn  $\Psi_{px}$  was substantially below the bulk-tissue turgor threshold during late summer 1994 (Fig. 1b).

Stem xylem at both sites had the same vulnerability to xylem embolism and there was no change in this property during the season (Fig. 3; site curves represent pooled seasonal data). The average embolism pressure in stems



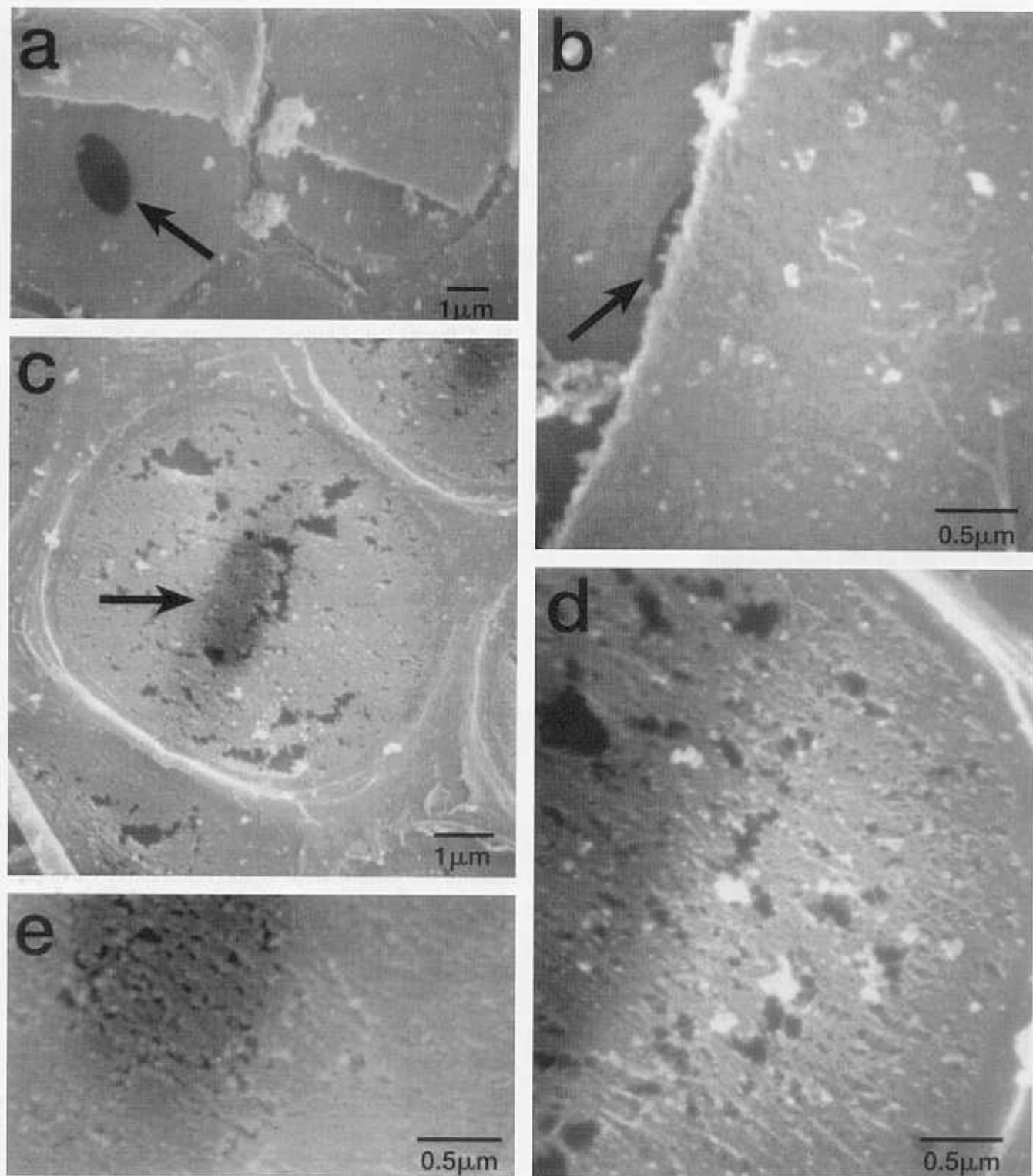
**Fig. 5** Percent loss of hydraulic conductance ( $\pm 95\%$  confidence limits) for root (left panel,  $n = 5-12$ ) and stem (right panel,  $n = 27-31$ ) segments measured immediately after collection in the field ("native embolism") from mid-August to September in years indicated (open bars riparian site trees, hatched bars slope site trees). A single October measurement on roots from slope trees collected after a prolonged fall rain in 1994 is also shown (1994a, root)

was  $-3.7 \pm 0.4$  MPa ( $n = 18$ ). The same vulnerability curve was obtained whether positive air pressure or negative xylem pressure was used (Fig. 3, compare circles and triangles) indicating that dehydration induced no more embolism than predicted from air pressure results.

Stem xylem had large margins of safety between midday  $\Psi_{px}$  and  $\Psi_{px(CT)}$  at both sites throughout the study (Fig. 4, circles). Although the safety margin decreased with increasing drought from c. 3 MPa at the riparian site (Fig. 4, open circles) to 1.7 MPa at the slope site at the end of the dry 1994 summer (Fig. 4, solid circles), the stomatal closure observed at that time (Fig. 2a) was not necessary to avoid catastrophic embolism in stems.

Despite the large safety margins from catastrophic embolism, the shallow slope of the vulnerability curve predicted significant xylem embolism at field  $\Psi_{px}$ . Based on an estimate of minimum stem  $\Psi_{px}$  of  $-3.9$  MPa (average of predawn and midday  $\Psi_{px}$ ) for slope trees during August–September 1994, we predicted  $52 \pm 17\%$  loss of conductance in stem xylem from the stem vulnerability curve (Fig. 3). This agreed with the average native embolism over this time:  $46 \pm 11\%$  ( $n = 30$ ; Fig. 5). Minimum stem xylem  $\Psi_{px}$  in riparian trees was estimated at  $-1.1$  MPa for both summers which corresponded to a predicted  $14 \pm 9\%$  loss of conductance (Fig. 3). This was somewhat below ( $P < 0.01$ ) the average native embolism over both summers:  $27 \pm 3.3\%$  ( $n = 62$ ; Fig. 5). This could have resulted from incomplete refilling of stem xylem prior to measuring the air pressure curve. Stems at the slope site were more cavitated than at the riparian site ( $P < 0.001$ ) only during the drought year of 1994 (Fig. 5).

Root xylem was more vulnerable than stem xylem at both sites (Fig. 3). Unlike stems, root vulnerability curves differed between sites: complete loss of conductance was predicted at  $\Psi_{px}$  of  $-1.75$  at the riparian site versus  $-2.75$  MPa at the slope site (Fig. 3). This corre-



**Fig. 6a-e** Inter-vessel pit membranes viewed with the scanning electron microscope in stem of a slope tree (**a, b** same view in low versus high magnification), root from riparian tree (**c, d** same view in low versus high magnification), and root from slope tree (**e**, high magnification). Low magnification views in **a, c** show pit membranes exposed in face view by removal of the secondary wall layer above the membrane. The secondary wall and pit border

below the membrane is intact and can be seen where the membrane was torn by sectioning (**a, b**). *Arrows* in **a, b** indicate the pit aperture leading to the vessel lumen. The pit aperture can also be seen in **c, d, e** through the relatively thin and porous membrane in roots (*arrow* in **c**; *shadows* in **d, e**). Comparison of membranes at same magnification in stem (**b**) and roots (**e, d**) shows their relative porosity

sponded closely with turgor loss points at both sites (Fig. 3, arrows on upper  $x$  axis). Average embolism pressure was  $-0.6 \pm 0.1$  MPa ( $n = 6$ ) in riparian roots versus  $-1.3 \pm 0.1$  MPa ( $n = 6$ ) in slope roots.

The margin of safety from catastrophic embolism in roots was much less than in stems (Fig. 4, compare circles and squares). Excepting the two instances where a negative safety margin was predicted (Fig. 4, solid squares below the zero line), the safety margin in roots averaged  $0.59 \pm 0.20$  MPa and was constant across riparian and slope sites (Fig. 4, squares) owing to the adjustment in cavitation vulnerability. This is a maximum estimate of the safety margin because we assumed midday root  $\Psi_{px}$  was equal to predawn  $\Psi_{px}$ . The same assumption predicts that roots at the riparian site should have had  $55 \pm 16\%$  ( $n = 6$ ) loss of conductance from embolism for average predawn  $\Psi_{px}$  of  $-0.68 \pm 0.06$  MPa ( $n = 30$ ) during the summer of 1994 (Figs. 1, 3). This did not differ from the minimum (see Methods) average native embolism during the same summer:  $60 \pm 16\%$  ( $n = 12$ ; Fig. 5). These small safety margins and high native embolism levels indicated that stomatal regulation of transpiration was necessary to avoid catastrophic embolism in root xylem. They also indicated that the difference in the vulnerability curve between sites was adaptive: if slope roots had the same vulnerability curve as riparian roots,  $\Psi_{pxCT}$  would have been regularly exceeded at the slope site.

Even with their greater resistance to cavitation, slope roots should have developed  $\Psi_{px}$  in excess of  $\Psi_{pxCT}$  at the end of the 1994 drought (Fig. 4, solid squares with negative safety margin). Predawn  $\Psi_{px}$  at this time (average =  $-3.7 \pm 0.1$  MPa) dropped below the  $\Psi_{px}$  predicted to cause complete embolism ( $-2.75$  MPa, Fig. 3). Although this was consistent with the nearly complete midday stomatal closure in slope trees (Fig. 2), it was not consistent with the native xylem embolism which was only  $65 \pm 10\%$  ( $n = 10$ ; Fig. 5). This discrepancy between native and predicted embolism necessarily resulted from imprecision in measurement of (1) root vulnerability curves, (2) native embolism, or (3) minimum root  $\Psi_{px}$ . We suspected the last possibility. Although we did not compare air-pressure with negative-xylem-pressure methods for root vulnerability curves, the two methods agreed for stems (Fig. 3) and there was no obvious reason why the same would not be true for roots. We do know our estimate of native embolism in roots could have been between 8 and 22 percentage points too low (see Methods); but this would have influenced all such measurements, not just those for slope roots. Minimum root  $\Psi_{px}$  was estimated from predawn leaf  $\Psi_{px}$ . This could have given overly negative estimates because of the lack of equilibration between soil and leaf  $\Psi$  overnight. This would have been exacerbated under drought conditions because of low hydraulic conductances. Unfortunately, we did not make independent measurements of soil  $\Psi$  for comparison with our predawn  $\Psi_{px}$  measurements.

The extensive embolism observed in roots (Fig. 5) was at least partially reversed following extensive rains amounting to 52.1 mm over 6 days. One week after this

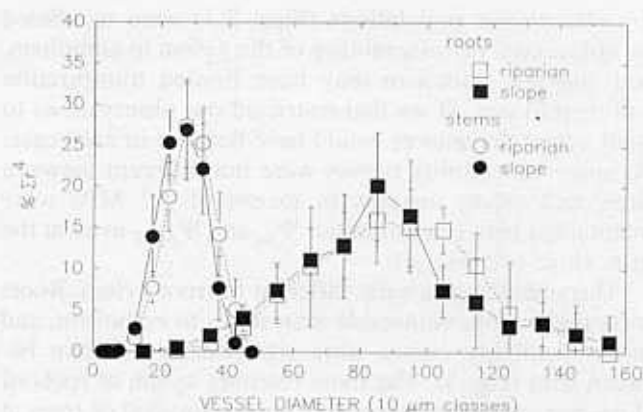


Fig. 7 Distribution of estimated percentage of hydraulic conductance ( $\% \Sigma r^4$ ) contributed by vessels of  $10 \mu\text{m}$  diameter classes for roots (squares,  $n = 6$ ) and stems (circles,  $n = 8$ ) from slope (solid symbols) and riparian (open symbols) trees. Error bars are 95% confidence limits

rain, native embolism in roots at the dry site was  $37 \pm 16\%$  ( $n = 5$ ) versus  $65 \pm 10\%$  ( $n = 10$ ) before the rain (Fig. 5, "1994a").

Inter-vessel pit membrane structure in roots and stems was consistent with roots being the most vulnerable to embolism. Pit membranes in roots of both sites were conspicuously more porous (Fig. 6c,d,e) than stems (Fig. 6a,b) as predicted if cavitation was caused by air-seeding through pit membrane pores. The average maximum pore diameter per vessel can be calculated from the average embolism pressure (obtained from vulnerability curves) using the capillary equation (Sperry and Tyree 1990). Predicted pore diameters were  $0.32 \mu\text{m}$  for riparian roots,  $0.19 \mu\text{m}$  for slope roots, and  $0.072 \mu\text{m}$  for stems. The largest pores in regions of the pit membranes that were intact and not obviously damaged by preparation were between c.  $0.15$  and  $0.5 \mu\text{m}$  in diameter for roots (Fig. 6c,d). Pores were not visible in most stem pit membranes (e.g., Fig. 6a, b), although the expected pore diameter of  $0.072 \mu\text{m}$  was above the estimated  $0.03 \mu\text{m}$  limit of resolution. More quantitative comparisons of pore sizes between sites was not warranted from our limited sampling.

Other than roots having larger vessel diameters than shoots (Fig. 7), there was no relationship between vessel diameter and vulnerability to embolism. Hydraulically-weighted vessel diameters were the same in slope- and riparian site roots (hydraulic means =  $81.2 \pm 7.7 \mu\text{m}$  ( $n = 6$ ) versus  $84.5 \pm 10.4 \mu\text{m}$  ( $n = 6$ ), respectively) despite their differences in vulnerability (Fig. 3). Furthermore, roots from slope and riparian sites had equal hydraulic conductances per transverse root area ( $10.9 \pm 3.9$  versus  $12.0 \pm 5.4 \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ m}^{-2}$ , respectively;  $n = 10$ ).

## Discussion

Our main questions were whether the significant differences in  $\Psi_{dp}$  and  $g_s$  between riparian and slope A.

*grandidentatum* populations (Figs. 2,3) were paralleled by differences in vulnerability of the xylem to embolism, and whether embolism may have limited transpiration and therefore  $g_s$ . If we had restricted our observations to stem xylem the answer would have been no in each case, because vulnerability curves were not different between sites, and safety margins in excess of 1.7 MPa were maintained between minimum  $\Psi_{px}$  and  $\Psi_{pxCT}$  even at the drier slope site (Fig. 4).

The situation was quite different for root xylem. Roots were much more vulnerable than stems to embolism, and the vulnerability curves were significantly different between sites (Fig. 3). The more resistant xylem of roots of slope trees appeared necessary for the survival of trees at this drier site. Safety margins from catastrophic embolism in roots averaged less than 0.6 MPa at both sites during 1993 and the first part of 1994. Near the conclusion of the 1994 drought, roots may even have reached  $\Psi_{pxCT}$  at the slope site, although native embolism was only 65% (loss of conductance) rather than the expected 100%. Certainly the stomata were almost completely closed at this time (Fig. 2) and even if  $\Psi_{pxCT}$  had not been exceeded the margin of safety would have been minimal.

These results suggest that gas exchange was limited by root rather than stem embolism. It also may explain why there was no adjustment of  $\Psi_{ip}$  to lower values during the drought of 1994 relative to the previous summer. If stomatal closure was necessary to prevent catastrophic xylem embolism or further loss of water following extensive root cavitation, and if stomata respond to changes in leaf turgor, raising leaf turgor (by lowering  $\Psi_{ip}$ ) and possibly causing stomatal opening would have been maladaptive.

Although complete embolism of xylem may only occur during exceptional stress, the results indicated that significant *partial* embolism was typical for *A. grandidentatum*. Although root and stem xylem tended to be more embolized at slope than at riparian sites, embolism occurred extensively even at the riparian site (Fig. 5). This resulted from the shape of the vulnerability curves: rather than embolism occurring in a threshold manner in a narrow pressure range, it occurred gradually over a broad pressure range. As a result,  $\Psi_{px}$  could drop well within the cavitation range without reaching  $\Psi_{pxCT}$  and causing runaway cavitation (see also Tyree and Sperry 1988; Jones and Sutherland 1991). In contrast, stem xylem of a strict riparian species of the same area, *Betula occidentalis*, has a threshold-type vulnerability curve and embolism in the stem xylem is avoided except during extreme soil drought (Sperry and Saliendra 1994; Saliendra et al. 1995). However, the root xylem of this species actually has a vulnerability curve identical to that for *A. grandidentatum* roots at the riparian site (Fig. 3), and embolism was significant in roots in the field (Sperry and Saliendra 1994).

Why should embolism occur so extensively and be so limiting? There is no straightforward answer. However, several possible explanations have been offered. Jones and Sutherland (1991) emphasized that some embolism was required to support maximum stomatal conductance

(i.e., to approach maximum  $E$  and  $\Psi_{pxCT}$ ) when vulnerability curves have a shallow slope. However, this begs the question of why some species like *A. grandidentatum* have shallow-sloped curves. One possibility is that there is a disadvantage to having "safe" xylem because it is less efficient in water conduction. Having some efficient, yet vulnerable, xylem would maximize conducting efficiency when conditions were favorable but result in inevitable loss of conductance as soil  $\Psi$  dropped. However, this safety versus efficiency trade-off (Zimmermann 1983) has not been confirmed either across species (Tyree and Dixon 1986; Sperry et al. 1994; Tyree et al. 1994), or even within species (Sperry and Saliendra 1994), although it can be seen within an individual (Sperry and Saliendra 1994). The present study supports the lack of a trade-off, because although roots of slope trees were more resistant to embolism than in riparian trees (Fig. 3), they had equal vessel diameters (Fig. 7) and equal hydraulic conductances per transverse root area.

Perhaps there is an advantage to the reduced hydraulic conductance that results from partial embolism. Lower hydraulic conductance means lower volume flow rates of water for the same driving force. Stomatal regulation of leaf water status controls and may reduce the driving force as soil dries (Jones 1990). Superimposing partial embolism on stomatal regulation provides an additional means for reducing water use during incipient and prolonged drought. Reduced uptake of water would prolong its availability in the absence of rain, and somewhat paradoxically could increase the long-term soil-plant hydraulic conductance. The reason for this is that if water was extracted too rapidly from a drying soil, positive feedback between reduced soil water content and soil hydraulic conductance would break hydraulic continuity between root and soil water. This is analogous to runaway embolism in xylem. Avoiding this loss of hydraulic contact with soil water may restrict the ability of plants to compete for soil water during a drought simply by increasing their rate of water uptake.

The preferential development of partial embolism in roots (rather than stems) may be adaptive because embolism in roots would be more reversible after the drought when soil is wet and root xylem pressures approach or exceed atmospheric (e.g., Fig. 5). Reversibility of embolism would allow the cycle to repeat during a subsequent drought. Drought and refilling cycles could be seasonal or even diurnal in period. In principle, the same advantages of partial embolism could apply to riparian as well as slope sites. In riparian situations, droughts may be more short-term and involve shifting water tables and/or local draw-down of soil water adjacent to roots. Opportunities for embolism reversal would be more frequent (perhaps every night) for riparian site roots; however we did not investigate this.

During extreme drought, complete embolism in the smaller roots could also represent a survival mechanism. By hydraulically isolating plant from soil, complete embolism in absorbing roots and reduced transpiration through leaf-drop and/or stomatal closure would allow



root-collar and stem  $\Psi$  to remain higher than soil  $\Psi$  and preserve some of the hydraulic conductance in these organs. Wet conditions would then allow regrowth of the root system. This occurs in the context of normal growth in desert succulents with CAM photosynthesis (Nobel and North 1993). Although we sampled only a narrow range of root diameters in this study, other work has shown that smaller roots of *Pseudotsuga menziesii* were more vulnerable than larger ones (T. Ikeda and J.S. Sperry, unpublished work).

The exceptional vulnerability of roots to embolism seen in this and an earlier study (Sperry and Saliendra 1994) extends the segmentation hypothesis of Zimmermann (1983). Zimmermann postulated that during water stress, because xylem pressures are lowest in minor branches and leaves, embolism and dieback would occur first in these peripheral organs. Loss of leaf area and transpiration would moderate xylem pressures and protect the bole from serious embolism. This hypothesis has been supported by modelling studies (Tyree and Sperry 1988) and field studies (e.g., Sperry 1986; Tyree et al. 1993), and is important for embolism induced by dynamic (transpiration-induced) water stress. Our recent observations on root xylem extend the segmentation concept to the underground peripheral network of roots and to possible protection of the plant during static (soil  $\Psi$ -induced) as well as dynamic water stress.

Having documented substantial adjustment in susceptibility to embolism in root xylem between slope and riparian habitats, the obvious question is whether the difference resulted from environmental or genetic influences. We suspect there is a large environmental component because the difference was consistent within a site and populations were close enough (0.25 km) that gene flow via wind and insect pollination was likely. Furthermore, we have recently observed differences in vulnerability of roots to embolism within the same *Pseudotsuga menziesii* root system on a hillside depending on whether roots were sampled upslope (more resistant) or downslope (more vulnerable) from the bole (T. Ikeda and J.S. Sperry, unpublished work). On the other hand, genetic control may be responsible for limiting the plasticity of the environmental response and this aspect of habitat tolerance for the species.

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