Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*

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ABSTRACT

A modified version of a method that uses positive air pressures to determine the complete cavitation response of a single axis is presented. Application of the method to *Betula occidentalis* Hook. gave a cavitation response indistinguishable from that obtained by dehydration, thus verifying the technique and providing additional evidence that cavitation under tension occurs by air entry through interconduit pits. Incidentally, this also verified pressure-bomb estimates of xylem tension and confirmed the existence of large (i.e. >0·4 MPa) tensions in xylem, which have been questioned in recent pressure-probe studies. The air injection method was used to investigate variation within and amongst individuals of *B. occidentalis*. Within an individual, the average cavitation tension increased from 0·66 ± 0·27 MPa in roots (3·9 to 10·7 mm diameter), to 1·17 ± 0·10 MPa in trunks (12 to 16 mm diameter), to 1·36 ± 0·04 MPa in twigs (3·9 to 5 mm diameter). Cavitation tension was negatively correlated with the hydraulically weighted mean of the vessel diameter, and was negatively correlated with the conductance of the xylem per xylem area. Native cavitation was within the range predicted from the measured cavitation response and *in situ* maximum xylem tensions: roots were significantly cavitated compared with minimal cavitation in trunks and twigs. Leaf turgor pressure declined to zero at the xylem tensions predicted to initiate cavitation in petiole xylem (1·5 MPa). Amongst individuals within *B. occidentalis*, average cavitation tension in the main axis varied from 0·90 to 1·90 MPa and showed no correlation with vessel diameter. The main axes of juveniles (2–3 years old) had significantly narrower vessel diameters than those of adults, but there was no difference in the average cavitation tension. However, juvenile xylem retained hydraulic conductance to a much higher xylem tension (3·25 MPa) than did adult xylem (2·25 MPa), which could facilitate drought survival during establishment.

Key-words: Betula occidentalis; Betulaceae; birch; hydraulic conductance; water relations; water stress; xylem cavitation; xylem structure.

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INTRODUCTION

Xylem cavitation is becoming recognized as an important stress response that places unambiguous limitations on water transport. Evidence from a variety of sources has demonstrated that high xylem tension causes cavitation because air is aspirated through interconduit pits (Crombie, Hipkins & Milburn 1985; Sperry & Tyree 1990; Cochard, Cruiziat & Tyree 1992). Plants that cavitate at higher tensions have interconduit pit membranes that are less permeable to an air–water interface.

A direct measure of cavitation in xylem conduits is the extent to which it reduces hydraulic conductance (Sperry, Donnelly & Tyree 1988). 'Vulnerability curves' show how this loss in conductance increases with increasing xylem tension. These curves have provided insights into the stress adaptation of plants. However, the cavitation response measured in this way is often extremely variable. A recently published curve for *Betula occidentalis* indicated that a tension of 1·5 MPa induced anywhere from 0 to 80% loss in conductance (Sperry *et al.*, 1994). It is important to understand the basis for this variation because it is potentially related to how plants can respond to stress.

The usual method for obtaining these curves invites variation and limits its analysis. Typically, each data point represents a separate axis that has been dehydrated and then measured for loss of conductance. Usually more than 30 data points are required and therefore several individuals must be sampled. Although the curves are representative of a population they may also contain multiple samples from single individuals. The amount of variation occurring within versus between individuals is difficult to determine because the methodology requires extensive and destructive sampling.

There are alternative methods for measuring these curves that take advantage of the fact that cavitation occurs when air crosses interconduit pit membranes. Vulnerability curves showing the loss of conductance in air-injected stems that have xylem tensions near zero are indistinguishable from curves for cavitation induced by elevated xylem tension (Sperry & Tyree 1990). As demonstrated by Cochard *et al.* (1992), this makes it possible to measure the progressive loss of hydraulic conductance on *single stems* progressively embolized by air injection.

In the work reported here we used the air-injection method of obtaining vulnerability curves from single

stems to investigate the nature of variation in cavitation tension within and between individuals of a single species, *Betula occidentalis*. Based on previous work (Salleo & LoGullo 1989; Sperry & Tyree 1990) we hypothesized that intra-plant variation would be correlated with vessel diameter: the narrower the diameter, the higher the cavitation tension. However, this would not necessarily be the case for inter-plant variation because previous work has conclusively shown that, at least across taxa, there is no correlation between vessel size and cavitation caused by xylem tension (Tyree & Dixon 1986; Sperry & Sullivan 1992). We also considered how intra-plant variation was related to the conducting efficiency of the xylem, *in situ* xylem tensions and embolism, and the tissue water relations of the leaves. Inter-plant variation was also considered relative to juvenile versus adult developmental stage.

A somewhat *ad hoc* objective of the present paper was to provide a more direct demonstration of the existence of significant xylem tensions (i.e. >1 MPa) than is possible with the pressure bomb or psychrometer. Although normally this would be superfluous, recent work by Zimmermann and co-workers (e.g. Balling & Zimmermann 1990; Zimmermann *et al.* 1993) has questioned the existence of xylem tensions greater than *ca.* 0·4 MPa (relative to atmospheric) in plants as measured with the pressure bomb.

MATERIALS AND METHODS

Plant Material

Betula occidentalis Hook. is a small (<15 m) diffuse-porous tree with more than 85% of its branch vessels shorter than 50 mm (Sperry & Sullivan 1992). It is strictly a riparian species in its native habitat in the western United States. Consistent with its constant and favourable water supply, pre-dawn and midday xylem tensions remain nearly constant throughout the year (*ca.* 0·2–0·3 and 1·2–1·4 MPa, respectively; Sperry, Alder & Eastlack 1993). It develops multiple stems that facilitate repeated sampling from the same individual. In some experiments we compared 'juvenile' trees to 'adults'. Juveniles were defined as being between 2 and 3 years old as measured from ring counts of the main axis; they were typically less than 1 m tall. Adults used were more than 5 years old and reached heights of 13·5 m.

All material was collected from the Red Butte Canyon Research Preserve (*ca.* 1370 m) located about 8 km from our laboratory at the University of Utah in Salt Lake City, Utah. Collections were tightly bagged during transport to minimize dehydration. Lateral roots were relatively easy to excavate because they were less than 200 mm deep, and unbranched for lengths of 1 m or more.

Vulnerability curves; air-injection method

The principle of the method was to measure the reduction in the hydraulic conductance of a single axis while cavitation was induced by forcing air across inter-vessel pits into the intact vascular system using elevated air pressure. Conductance should decrease with increasing air pressure in the same manner as it decreases with increasing xylem tension if cavitation occurs by air penetration of the vascular system.

Shoot or root segments between 0·15 and 0·25 m in length were mounted in a double-ended pressure bomb with both ends protruding. The morphologically proximal end was attached to a tubing system designed to measure the hydraulic conductance of the segment (Fig. 1). This bomb is similar in principle to the device described by Salleo *et al.* (1992). Hydraulic conductance (*k*) was defined as the mass flow rate through the segment divided by the pressure difference. We used filtered (0·2 μm) water adjusted to a pH of *ca.* 2 with HCl to prevent microbial growth. This solution was superior to the oxalic acid solutions we have used in the past (e.g. Sperry *et al.* 1988) for two reasons; being inorganic it further minimized microbial growth, and it avoided precipitation of calcium oxalate within the stems which was occasionally a problem with oxalic acid. The solution was stored in a plastic bag attached to the stem with clear tubing (Fig. 1, 'solution source'); the bag could be raised or lowered to control the pressure difference across the stem. An additional, open-ended tube served to vent air bubbles emerging from the stem during bomb pressurization. This minimized their disturbance of the pressure difference (Fig. 1, 'air-vent'). To measure conductance, a hydraulic pressure difference of about 0·01 MPa was induced, and effluent from the stem
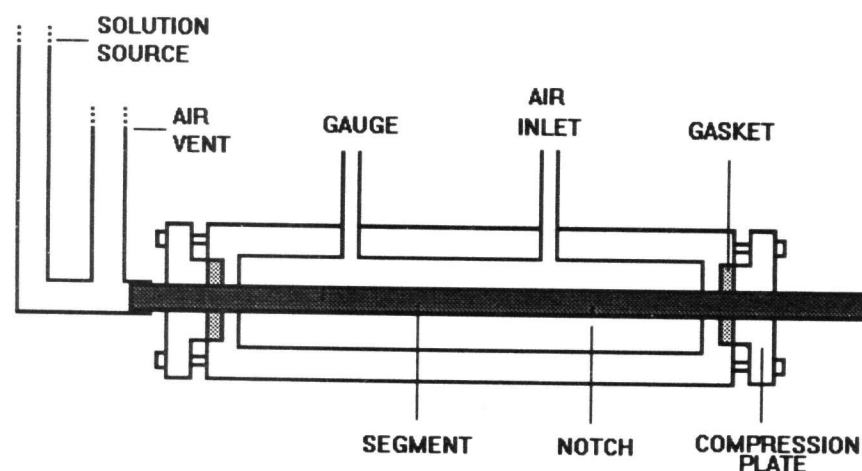
Figure 1. Apparatus for measuring vulnerability curves on single hydrated stems using air pressure. The stem (SEGMENT) was sealed in a double-ended steel bomb with both ends protruding. Rubber gaskets were fitted around the stem and held in recessed wells. An air-tight seal was made by screwing on compression plates and squeezing the gasket around the stem. Although we used a variety of bomb designs, the one shown was the latest and the most efficient. Air pressure in the bomb was controlled with a regulator and measured with a test gauge via a separate port. The separate measurement port avoided errors resulting from the pressure drop along the inlet flow route. An intravenous bag was used as a solution source fitted to the stem with clear tubing (SOLUTION SOURCE); elevation of the bag forced solution through the segment under gravity during conductance measurements. Effluent was collected using pre-weighed vials containing cotton wool. An open-ended tube allowed escape of air during pressurization (AIR VENT).

was collected over 1 min intervals in pre-weighed vials filled with cotton wool.

The central portion of each segment was notched with overlapping cuts about 50 mm apart. This directly exposed the xylem to the air while allowing water to flow laterally around the cuts in intact vessels (Fig. 1, 'notch'). Leaves or side-branches were removed flush with the main axis to provide additional air entry points. It was important to insure direct access of air to the vascular system to minimize the time required to embolize fully the xylem at a given air pressure.

To obtain a vulnerability curve, the bomb was first pressurized to 0·1 MPa (maintained with a regulator) and allowed to equilibrate for 10 min. Flow through the segment was stopped during this time by lowering the bag. This minimized reductions in conductivity resulting from clogging by any particles accumulating in the solution over time; the solution was also changed every few days. After 10 min, conductance was measured until it stabilized. The pressure of 0·1 MPa in the bomb kept all vessels severed by the transverse cuts and other air entry points filled with air, and prevented leakage of solution through them into the bomb. At the same time, this low pressure was not sufficient to force air through pit membranes and into intact vessels. Following this first measurement, the flow was again reduced, and the bomb pressure was raised by a specified amount (usually 0·25 or 0·5 MPa) and held for 10 min. The bomb was then depressurized to 0·10 MPa and this pressure was held for 3 min before flow was induced and the conductivity remeasured. This measurement was expressed as percentage below the initial measurement and represented the drop in conductance caused by vessels embolizing during the pressurization phase. This procedure was repeated using progressively higher bomb pressures until the conductance dropped by 95% or more from its original value. The vulnerability curve was established as the cumulative percentage loss in conductance as a function of the bomb (injection) pressure (e.g. Fig. 2, solid symbols).

The reason for lowering the bomb pressure to 0·1 MPa for each conductance measurement rather than maintaining the higher pressure was that the air coming from the cut stem at higher pressures tended to spray water about, preventing accurate collection of effluent. Lowering of the air pressure to 0·1 MPa eliminated this problem but also created the possibility of dissolving the air that had been injected into the vessels. To minimize this we only induced positive hydraulic pressure immediately before the actual conductance measurement; conductances rarely increased with time suggesting no refilling. We found no effect of the rate of depressurization on the embolism induced by a given injection pressure. This suggested that embolism was not occurring as a result of air coming out of solution.

We conducted several tests to determine whether there was a difference between the curve for the HCl solution and that for tap or treated water alone, but could detect none. In some cases, in measurements taken after prolonged (overnight) soaking in tap water followed by perfusion
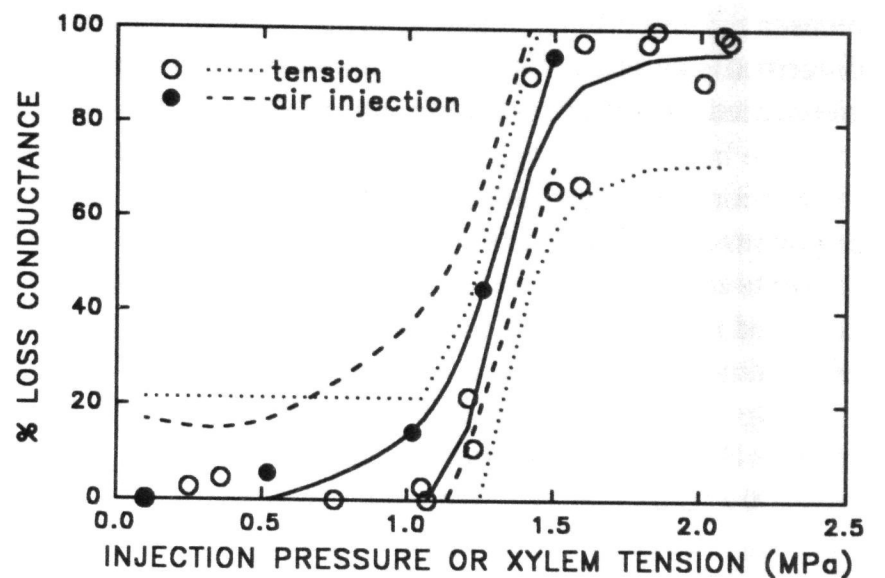
Figure 2. Percentage loss of hydraulic conductance versus bomb air pressure for hydrated stems (solid circles; means for 5 stems) and versus xylem tension for dehydrated stems (open circles; individual observations). Curves (solid lines) were fitted using the Weibull function, which assumes monotonic change in the dependent variable as the independent variable increases (Rawlings & Cure 1985; Neufeld *et al.* 1992). The dashed and dotted lines are the 95% confidence limits for the air-injection and tension curves, respectively. Stems were of similar diameters and from one (multiple-stemmed) individual of *Betula occidentalis*. Each curve lies within the other's 95% confidence interval indicating an identical embolism response to air pressure or tension.

with the HCl solution the xylem was dramatically more resistant (i.e. cavitation at higher pressures) than in measurements carried out immediately. To avoid this we soaked stems for less than an hour prior to measurement.

We tested whether 10 min of air injection was sufficient to maximally embolize the xylem by repeatedly pressurizing stems for up to 40 min after an initial 10 min treatment. The loss of conductance remained approximately constant, indicating that 10 min of air injection saturated the embolism response. When stems were not notched and contained no cut side-branches or exposed leaf traces, time intervals of several hours were required, and in some cases we could never saturate the response at a given pressure.

It was useful for us to represent vulnerability curves for a given stem by a single average cavitation pressure. This was achieved by first considering the vulnerability curve as a cumulative distribution of loss of conductance with pressure, and then replotting it as a frequency distribution over the same pressure classes. The mean pressure for this distribution was calculated using the mid-points of each pressure class. In previous work, the cavitation pressure inducing a 50% loss of conductance was used to compare vulnerability curves (e.g. Sperry *et al.* 1994); this will only equal the mean cavitation pressure for perfectly symmetric distributions.

Vulnerability curves; dehydration method

To assess the air-injection technique, we compared it with the dehydration method which is more commonly used to

measure vulnerability curves. The method has been described elsewhere (Sperry & Sullivan 1992). Branches were dried to a range of xylem tensions (measured with a pressure bomb) and bagged for an hour to equilibrate, and the tension was remeasured on 3 to 5 shoot tips per branch. Segments from the branch were excised under water (to prevent air entry) and mounted on a tubing apparatus designed to measure the hydraulic conductance before and after removal of embolism by high-pressure (175 kPa) flushing of solution through the xylem (Sperry *et al.* 1988). The percentage loss of conductance was plotted versus the xylem tension to obtain a vulnerability curve.

Native embolism, xylem tension and leaf turgor

Native embolism was measured by harvesting branches or roots, cutting segments from them under water, and measuring the percentage reduction in conductance using the flushing technique described above. Care was taken to insure that branches or roots collected in the field were long enough (>1·5 m for roots, >0·5 m for stems) that no vessels embolized at the cut ends during harvest were included in the central conductivity segment.

In situ xylem tensions in an axis were estimated by pressure bomb measurement of the tension of covered leaves or small side-branches. Leaves or branches were sealed with reflective aluminum tape the day before to prevent transpiration and to promote equilibration with the parent axis. Root tensions were estimated by covering small shoots emerging from the root crown.

Leaf turgor as a function of leaf xylem tension was estimated from pressure–volume curves for individual leaves using the bench dehydration method. Curves were analysed using the methods of Schulte & Hinckley (1985) and a computer program generously provided by the former author.

Anatomical measurements

When vulnerability curves had been completed for a segment, a transverse section through its mid-section was cut for the measurement of vessel diameters. All vessels in a given sector delimited by rays and covering the most recent two growth rings were measured; four sectors at 90° intervals were measured per section. Sectors were chosen to include at least 50 vessels, giving a minimum of 200 vessels per stem. Measurements were made using a drawing tube and a bit pad (Donsanto microplan II).

The diameter was calculated as the equivalent circle diameter for the measured cross-sectional area of the lumen. The frequency distribution of vessels versus diameter classes was determined, as was the percentage each diameter class contributed to the total predicted hydraulic conductance of the sample. The predicted hydraulic conductance was assumed to be proportional to the sum of all radii to the fourth power (Σr^4) according to Poisieulle's law; the contribution of each diameter class was expressed as a percentage of the total Σr^4. From this latter distribution of conductance across diameter classes, a hydraulically weighted mean vessel radius was calculated. Mathematically, this mean equals the sum of the radii to the fifth power divided by the sum to the fourth power ($\Sigma r^5 / \Sigma r^4$).

For some experiments, the hydraulic conductance of the segment was expressed per transverse functional xylem area (specific conductance, or k_s). Functional xylem was identified by perfusing the segment with filtered (0·2 μm) basic fuchsin (0·1% w/v) siphoned through the stem (*ca.* 7 kPa below atmospheric pressure at the low-pressure end) after the conductance measurement. Three transverse sections were cut from the segment, one at the mid-point and two about 10 mm from either end. The average dye-stained area for each segment was determined with the microscope-bit pad apparatus.

RESULTS

Vulnerability curves obtained using the air-injection technique (Fig. 2, solid symbols) were indistinguishable from those obtained using the dehydration technique (Fig. 2, open symbols). The comparison in Fig. 2 is for stem segments of restricted diameter range (12–16 mm) from the same individual. This was consistent with cavitation occurring by air entry through conduit walls. This result gave us confidence in the technique which was employed exclusively to predict cavitation tensions in the remaining experiments.

Variation in vulnerability curves within an individual was extensive. Figure 3 shows results from a single, open-grown individual (maximum height 5 m) where the variation was related to the position of the xylem in roots versus that in shoots (Fig. 3a). Lateral roots between 3·9 and 10·7 mm in diameter ($n = 7$, circles) had a mean cavitation tension of 0·66 ± 0·27 (mean ± SD), which was significantly lower ($p < 0.01$) than the mean tension of 1·17 ± 0·10 MPa for trunks sampled at *ca.* 1 m height and between 12 and 16 mm in diameter (n=5, triangles). Twigs between 3·9 and 5 mm in diameter sampled from the tops of the same trunks (*ca.* 4·5 m above-ground) were the most resistant, with a mean cavitation tension of 1·36 ± 0·04 MPa ($n = 5$, squares). All axis types showed a 100% loss in conductance near the same tension of 1·75 MPa.

This increase in cavitation resistance with distance along the axis was consistent with the increase in *in situ* xylem tension along the flow path. Midday xylem tensions and cavitation values were measured on the same individual and are plotted using open symbols in Fig. 3a. Measurements were made under conditions of maximum transpiration; maximum tensions varied little over the season because of consistent pre-dawn xylem tensions resulting from the riparian habit of this species. As expected, roots had the lowest tensions (0·61 ± 0·05 MPa, $n = 5$), twigs the highest (0·92 ± 0·12 MPa, $n = 5$), and trunks intermediate tensions (0·67 ± 0·12 MPa). From these tensions and the vulnerability curves in Fig. 3a, roots were predicted to have a negative safety margin from
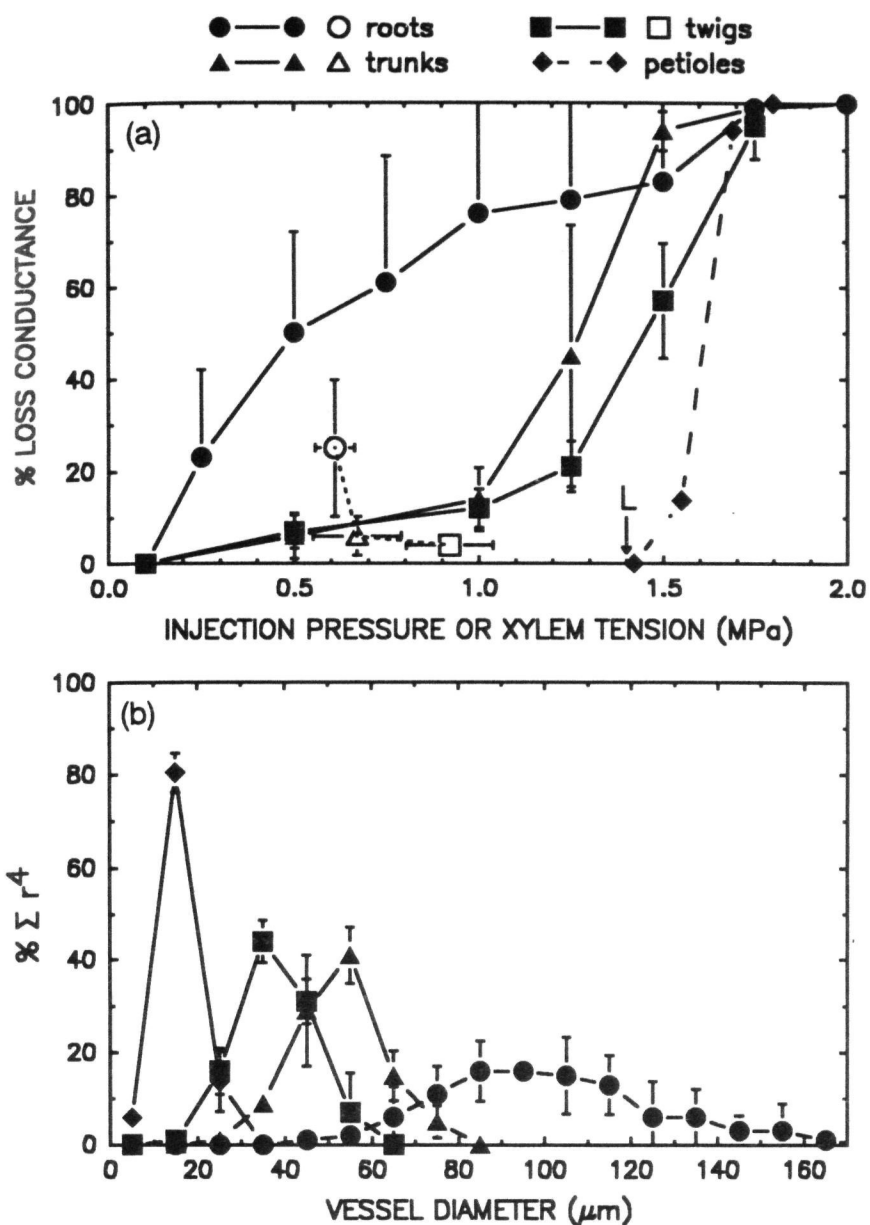
Figure 3. (a) Vulnerability curves for root (solid circles, $n = 7$), trunk, (solid triangles, $n = 5$) and twig (solid squares, $n = 5$) segments collected from a single individual of *Betula occidentalis* (mean ± SD). Curves are for the air-injection technique. The vulnerability curve for petiole xylem (solid diamond) was predicted from vessel diameters (Fig. 3b) and the relationship between diameter and vulnerability in Fig. 4 (solid circles). Maximum field tensions for roots (open circle, $n = 5$), trunks (open triangle, $n = 5$) and twigs (open square, $n = 5$) are plotted versus the *in situ* embolism values for each axis type ($n = 5$). The xylem tension of transpiring leaves is indicated by the arrow (L) on the x-axis. (b) Percentage Σr^4 contributed by vessels in $10 \mu m$ diameter classes for the same segments as in (a) (mean ± SD).

cavitation, while both trunks and twigs would have a 0·3 to 0·4 MPa buffer between maximum field tension and the onset of significant cavitation. This suggested that at least some refilling had occurred prior to the determination of the root vulnerability curve; otherwise, no cavitation would be expected until air pressures greater than 0·6 MPa were reached. Refilling could have occurred *in situ* (i.e. overnight) or during preparation of the material.

Consistent with these predicted safety margins, there was significant native embolism in roots at midday (Fig. 3a, open circle, 25 ± 14%, $n = 5$) but very little in trunks and twigs (Fig. 3a, open triangle and square at 6 ± 4 and 4 ± 2%, respectively; $n = 5$). Although the mean native cavitation in roots was lower than predicted from the vul-

nerability curve (Fig. 3a; compare open circle with root curve), the difference was not significant owing to the extensive variation in root vulnerability curves and in the native cavitation measurements.

The within-tree variation in vulnerability shown in Fig. 3a was correlated with the vessel diameters of the same axes (Fig. 3b). Vessels were widest in roots, narrowest in twigs, and intermediate in trunks (Fig. 3b; circles, triangles and squares, respectively). Furthermore, the breadth of the distributions corresponded to the shape of the vulnerability curves. Cavitation in roots occurred over a wide range of tensions, and the vessels showed a similarly wide range of diameters. Vulnerability curves in trunks and twigs were steep and associated with relatively narrow vessel diameter distributions. Finally, the correlation between mean cavitation tension and mean hydraulic diameter was highly significant with an r^2 of 0·87 (Fig. 4, solid circles).

The hydraulic efficiency of the xylem, measured as k_s, showed a progressive drop from roots to twigs, in contrast to the increase in cavitation tension (Fig. 4, open circles). This was expected from the trend in vessel diameter, and suggested a trade-off between efficiency and safety for within-tree variation in vessel diameter.

The correlation in Fig. 4 allowed us to estimate the vulnerability curve for petiole xylem from its vessel diameter distribution (Fig. 3b, diamonds). The predicted cavitation tension for the lower end of each diameter class was calculated from the relationship in Fig. 4, and the associated percentage loss in conductance was assumed to be equal to the contribution of that size class to the total hydraulic conductance (i.e. its $\%\Sigma r^4$; Fig. 3b). The predicted vulnerability curve (Fig. 3a, diamonds) indicated that very little cavitation would occur in petioles because midday leaf xylem tension (mean = 1·39 ± 0·10 MPa, $n = 6$) was about 0·15 MPa below the tension required to initiate cavitation (1·55 MPa). The slope of the petiole vulnerability curve was the steepest of all the curves, with 90% of the conduction lost between 1·55 and 1·70 MPa.
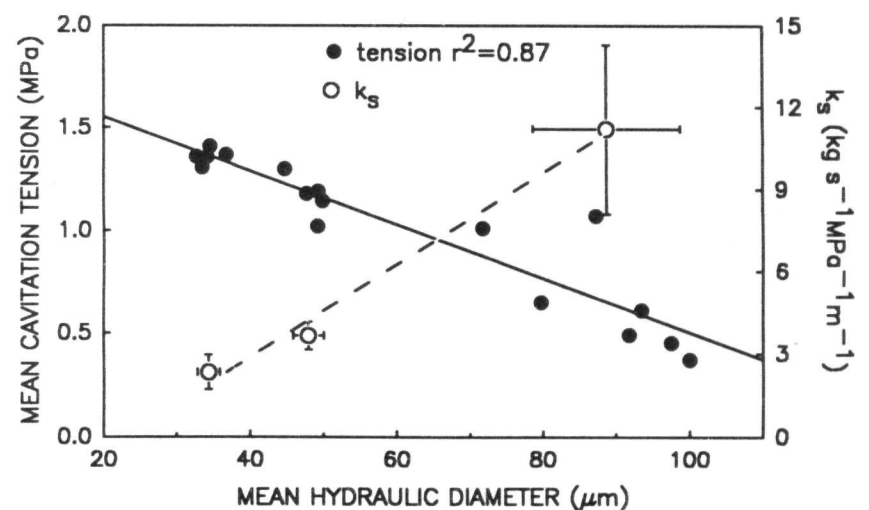
Figure 4. The correlation between mean cavitation tension and mean hydraulic vessel diameter for individual root, trunk and twig segments from Fig. 3 (solid circles), and the correlation between mean k_s (hydraulic conductance per xylem area, $n = 5$) and mean hydraulic diameter for root, trunk and twig segments from the same individual (open circles). Error bars are standard deviations.
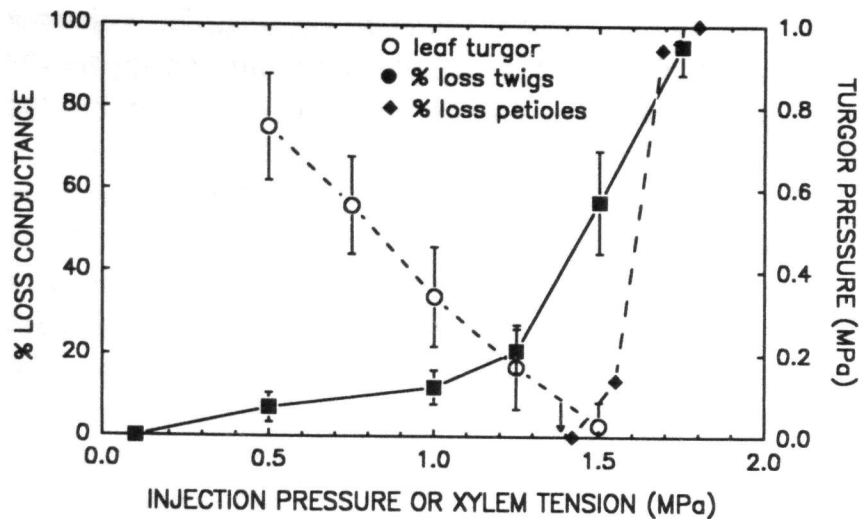
Figure 5. Vulnerability curves for twigs (solid squares) and petioles (solid diamonds) from Fig. 3a shown relative to the loss of turgor as a function of xylem tension estimated from pressure–volume curves for leaves from the same individual (open circle, $n = 5$, mean ± SD). The mean midday xylem tension is indicated by the arrow on the X-axis.

The bulk-averaged turgor pressure in the leaves (Fig. 5, open circles) dropped to zero at the same tension as was predicted to initiate cavitation in the petiole (solid diamonds). Actual leaf tensions (mean = 1·39 MPa, Fig. 3a) suggested that midday turgor pressures would be near 0·1 MPa. The coincidence of turgor loss with cavitation induction was probably critical for the prevention of cavitation-inducing tensions *in situ* via the presumed influence of turgor loss on stomatal closure.

The strong correlation between vessel diameter and cavitation tension found within an individual (Fig. 4) became insignificant when we compared shoot axes amongst individuals for both juveniles and adults (Fig. 6; compare closed and open symbols). This was also true within each age category (Fig. 6, open squares or open triangles). Although adults and juveniles did not differ significantly in their average cavitation tension (1·39 ± 0·13 versus 1·65 ± 0·29 MPa), the shapes of their vulnerability curves were significantly different (Fig. 7a). At tensions above 1·75 MPa, the average cavitation in juveniles was significantly less than that for adults, and juveniles did not completely cavitate until tensions reached 3·25 MPa. The hydraulic mean vessel diameter was significantly narrower in juveniles than in adults (Fig. 7b, 31 ± 1 versus 37 ± 4 μm, respectively; $p < 0.01$). The midday xylem tensions of the main axis for trees in the same riparian meadow were similar in juveniles and adults (0·51 ± 0·11 versus 0·57 ± 0·12 MPa, respectively; $n = 7$), as were native embolism values in the main axis (1·8 ± 0·29 versus 2·3 ± 1·1%, respectively).

DISCUSSION

The results verified the air-injection technique, making it possible to measure the entire cavitation response on a single axis (Fig. 2) and facilitating the measurement of cavitation tension within individuals and species. It is

essentially the same method as that employed by Cochard *et al.* (1992) with the added step of introducing air entry points to insure rapid and complete cavitation at each applied pressure. The technique is simple and relatively quick, and should find wide use in cavitation studies. In addition to evaluating the technique in *B. occidentalis*, we have found it to work in several other species, including *Abies concolour*, *Glycine max* and *Salix exigua* (N. N. Alder, S. E. Eastlack, W. T. Pockman & J. S. Sperry, University of Utah, unpublished results). This technique, like the dehydration method, gives the maximum possible cavitation response at a given applied pressure because air entry into the vascular system is facilitated by cut branch ends, side-branches, and notches. *In situ* cavitation may be less than predicted from these curves if air entry is restricted and tensions are not sustained for long periods.

The existence of xylem tensions of the magnitude predicted from the pressure bomb measurements in Fig. 2 (tension curve) has recently been questioned (e.g. Zimmermann *et al.* 1993). As in previous assaults on the cohesion theory for xylem transport (i.e. Plumb & Bridgeman 1972), the dissent arises from the difficulty of making direct measurements of xylem tension. Direct measurements require the insertion of a manometer or pressure probe into the vascular system. The result is an extreme manifestation of the uncertainty principle wherein making a measurement alters the measured property. At tensions above *ca.* 0·4 MPa (relative to atmospheric; Steudle & Heydt 1988), cavitation occurs either during probe insertion or shortly thereafter, and apparent tension immediately drops to values below 0·1 MPa (i.e. corresponding to pressures between atmospheric pressure and the vapour pressure of water; Balling & Zimmermann 1990). The cavitation arises from air-seeding at the insertion point, or from lack of adhesion between water and the surfaces of the probe (Heydt & Steudle 1991). This limitation of the
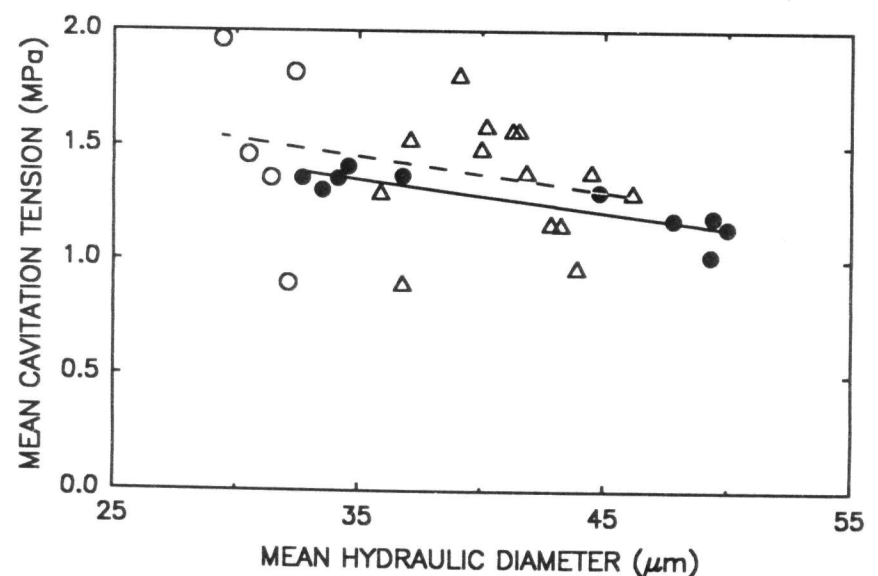
Figure 6. Mean cavitation tension versus mean hydraulic diameter for shoot xylem. Within-plant data (solid circles) for shoot segments (trunk and twig) from Fig. 4 show a significant correlation ($r^2 = 0.75$, $p < 0.01$). However, the between-plant correlation for adults (open triangles) and juveniles (open circles) was not significant ($r^2 = 0.07$).
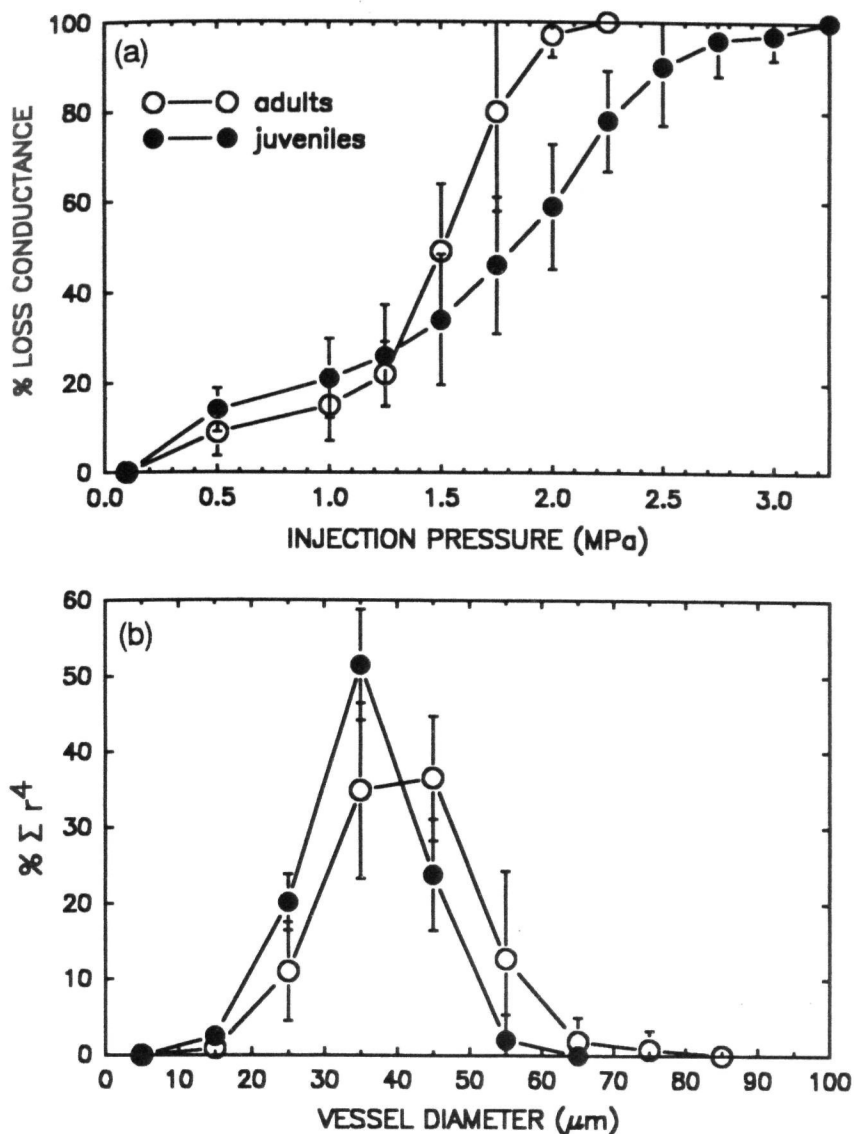
Figure 7. (a) Vulnerability curves for adults (open circles, $n = 9$; mean ± SD) and juveniles (solid circles, $n = 5$) for similar-diameter segments (3·9 to 10·8 mm). (b) Percentage Σr^4 contributed by vessels in 10-μm-diameter classes for adult and juvenile segments from (a) (mean ± SD).

pressure probe has led Zimmermann and co-workers to question the existence of tensions above its measurement range (e.g. Balling & Zimmermann 1990), although others using the technique have been more cautious in their interpretation (Heydt & Steudle 1991).

The correspondence between cavitation induced by air injection and that induced by dehydration (Fig. 2) provides a more direct demonstration of significant xylem tensions than does the pressure bomb. Pressure differences between air and water-filled xylem conduits of between *ca.* 1·0 and 1·5 MPa were required to embolize the entire vascular system based on the air-injection procedure (xylem tension = 0; Fig. 2, air injection curve). Therefore, when air pressure is zero, the same amount of embolism would occur when xylem tensions increase from 1·0 to 1·5 MPa. As shown in Fig. 2, the correspondence between the embolisms induced by the two methods of manipulating the pressure difference (i.e. air pressure versus tension) at once confirms the existence of xylem tensions in this range, and the accuracy of the pressure bomb in estimating these tensions. At water potentials below those that cause *complete* cavitation, xylem tensions essentially no longer exist and the pressure bomb cannot be used to estimate

water potential. The existence of tensions between 1·0 and 1·5 MPa in the field in *B. occidentalis* has been similarly demonstrated by the fact that air-injection pressures of less than 0·4 MPa at midday were required to induce > 90% cavitation; injection pressures of 1·5 MPa or more were required at night when xylem tensions were near zero (Sperry & Pockman 1993).

There is a pervasive tendency to assume that larger volume conduits are more vulnerable to cavitation (e.g. Mauseth 1991). Vessel diameter and length are correlated (Zimmermann & Potter 1982; Ewers, Fisher & Chiu 1990), so vessel diameters are approximate indicators of their volumes. Evidence confirms the relationship between conduit volume and vulnerability for cavitation induced by freeze–thaw cycles via bubble formation (Ewers 1985; Sperry & Sullivan 1992; LoGullo & Salleo 1993), but such a relationship was not found for water-stress-induced cavitation when different taxa were compared (Tyree & Dixon 1986; Sperry & Sullivan 1992). This means that across taxa the permeability of the pit membranes to air is independent of conduit size. Our results extend this finding to within-species comparisons of different individuals (Fig. 6, open symbols).

Only within a genotype were we able to demonstrate a significant correlation between vessel diameter and vulnerability (Figs 4 and 6, closed circles). Importantly, this was not because of any inherent causal link between conduit diameter (and by implication, volume) and vulnerability. It arose because the larger conduits were more readily injected with air. Within a genotype, this relationship may arise because the primary wall of a large vessel is less tightly woven than that of a smaller one owing to greater expansion of the cell. Pit membranes develop from the primary wall, and a more loosely woven wall would probably be more permeable to air and result in a lower cavitation tension. Amongst genotypes, the relationship between pit membrane permeability and overall conduit size was not consistent (Fig. 6, open symbols).

The permeability of inter-vessel pits to an air–water interface is probably related to pore sizes in the pit membrane. These can be predicted from the cavitation tension using the capillary equation (see Zimmermann 1983; Sperry & Tyree 1990). The mean cavitation tensions of the root and twig xylem of the individual tree shown in Fig. 4 (0·66 and 1·36 MPa, respectively) correspond to circular pore diameters ranging from 0·44 to 0·21 μm in roots and twigs, respectively. Although we have not attempted to measure the actual pit membrane pore sizes, these are within the range observed in other plants. Inter-vessel pit membranes in *Medicago sativa* stems had an average pore diameter of 0·27 μm with maximum diameters of *ca.* 0·45 μm (Van Alfen *et al.* 1983).

An important qualification for the data shown in Fig. 4 is that they were obtained by sampling the trunks and twigs of shoots of equivalent height and exposure. For this reason, the shoots were in similar environmental and morphological conditions when the xylem was being produced. Preliminary data suggest that the within-genotype correla-

tion between diameter and vulnerability can become insignificant if conditions during development are altered; for example, we found no relationship between diameter and vulnerability when shaded sprouts less than 2 m tall were compared with the tops of exposed shoots 13·5 m tall from the same individual. If confirmed, this suggests that the processes underlying vessel expansion versus pit membrane structure are not coupled in any simple way even within a genotype.

Variation in cavitation tension is only significant if it influences plant growth and survival. In adult *B. occidentalis*, the predicted onset of cavitation in the leaf occurred at tensions only slightly greater than those maintained in the leaf by stomatal regulation (mean = 1·39 MPa; Fig. 5, arrow). From the data in Fig. 3a, and assuming that soil–plant hydraulic conductance and pre-dawn xylem tension (0·30 MPa) were constant, if stomatal opening allowed the steady-state flow through the plant to increase by as little as 10%, the leaf xylem tension would increase from 1·40 to 1·50 MPa, which is within the cavitation range for the petiole. The strict regulation of leaf xylem tension by stomatal control in this species is necessary to avoid a positive feedback between tension and cavitation causing complete loss of water transport (Milburn 1979; Tyree & Sperry 1988).

We are currently investigating the stomatal response to water status in this species. The correspondence of turgor loss with the onset of cavitation (Fig. 5) is suggestive of a leaf-level feedback between turgor pressure and stomatal conductance. The link is perhaps via accelerated abscisic acid (ABA) release as turgor approaches zero (e.g. Pierce & Raschke 1980). The resulting stomatal closure and reduced transpiration would cause turgor pressures to increase and stomata to re-open. Oscillations in stomatal conductance and tissue water potential would occur locally within the leaf but be obscured at the whole-leaf level. For this reason, it is premature to assume that a leaf-level feedback loop cannot account for the maintenance of constant bulk-leaf water potential (or turgor). This is a critical flaw in the logic motivating root-signalling research (Davies & Zhang 1991). Recent experimental data strongly support a leaf-level response of stomata to reduced hydraulic conductance (Sperry *et al.* 1993), soil drought, and atmospheric drought (Saliendra, Sperry & Comstock, submitted to *Planta*).

Although the control of leaf xylem tension was apparently adaptive in preventing substantial cavitation in the shoot, root xylem was surprisingly vulnerable to cavitation and was significantly cavitated under normal conditions (Fig. 3a). Is this partial cavitation adaptive? Although we know that the shoot xylem of this species does not refill overnight (Sperry *et al.* 1993), even weak root pressures could refill root xylem, making root cavitation reversible. If this were the case, the most vulnerable and efficient xylem would function over the first part of each day before cavitating. With or without refilling, some cavitation in the roots (or anywhere in the plant) would result in less rapid extraction of soil water if the stomata maintain constant

leaf water potential throughout the day as is the case in *B. occidentalis* (Sperry *et al.* 1993). This would prolong soil water availability and could also maximize the total conductance of the plant and soil in series.

These considerations provide a hypothesis for the adaptive significance of the different vulnerability curves seen in juveniles and adults (Fig. 7). Although we did not observe any differences in native xylem tension between these categories, the root systems of juveniles are probably shallower than those of adults and could make them more vulnerable to drought. The presence of more resistant xylem in the juveniles for injection pressures above *ca.* 1·3 MPa would allow them in theory to survive higher xylem tensions than adults. Paradoxically, at tensions lower than 1·3 MPa, juveniles tended to cavitate *more* than adults; this was more pronounced in some juveniles than in others. Cavitation at modest tensions could reduce the rates of water extraction in juveniles compared with those in adults and prolong water availability during drought. To make the implications of these two curves more intuitively obvious, if two plants, differing only in whether they had juvenile or adult xylem, were placed in equal-sized pots with the same initial water supply and then droughted, we would predict that the plant with the juvenile xylem would survive longer and to lower soil water potentials than the plant with adult xylem.

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