



## Use of centrifugal force in the study of xylem cavitation

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### Abstract

Two methods were evaluated for using centrifugal force to measure the occurrence of cavitation as a function of negative pressures in xylem. The general protocol was to measure the hydraulic conductivity of xylem segments (stem or root pieces) before and after centring them on a centrifuge rotor and spinning them about their long axis to generate negative xylem pressure. The percentage decrease in conductivity from the initial to final measurement was used to quantify the embolism resulting from cavitation during spinning. In one approach, segments were spun with their ends exposed to air. This method could only be used when xylem conduits were much shorter than the segment. Results from an angiosperm (*Betula occidentalis*) and a gymnosperm (*Abies lasiocarpa*) corresponded to previous observations of embolism caused by air dehydration where negative pressure was measured with the pressure chamber. Results also agreed with embolism caused by injection of air into the xylem, in support of the air-seeding hypothesis for cavitation. In a second approach, segments were spun in a rotor designed to keep the segment ends immersed in water during spinning. This gave the same results as for non-immersed segments. Immersing the segment ends allowed measurements on any material, regardless of conduit length, as demonstrated for roots of *B. occidentalis*. The chief advantage of the centrifugal force method is the rapidity and precision with which any desired xylem pressure can be imposed.

Key words: Cavitation, embolism, drought stress, water relations, water transport.

### Introduction

The controversy surrounding the validity of the cohesion-tension theory for water transport (Passioura, 1991;

Zimmermann *et al.*, 1994; Canny, 1995) has forced the re-evaluation of methods for studying this aspect of plant water relations. In part this has been achieved through the development of new techniques. One of these involves using centrifugal force to create known negative pressure in xylem water of excised plant parts (Holbrook *et al.*, 1995; Pockman *et al.*, 1995). Pressures generated in this manner agreed with pressure bomb measurements, thus validating this widely-used instrument (Holbrook *et al.*, 1995). Spinning stems also demonstrated that significant negative pressures (e.g.  $< -1$  MPa) could be sustained by xylem, and that cavitation occurred at species-specific pressures in agreement with results from other methods (Pockman *et al.*, 1995). Both observations add to the already considerable evidence in favour of the cohesion-tension theory (Sperry *et al.*, 1996).

The purpose of this paper is to present a more detailed evaluation of the use of centrifugal force for studying xylem cavitation than was possible in the earlier study (Pockman *et al.*, 1995), and to describe significant improvements in the method. Cavitation is an important parameter in the response of plants to water stress, because it defines a limit to negative pressure, and because this limiting pressure is often within the physiological range (Tyree and Sperry, 1989). One of the more useful ways to characterize a plant's cavitation response is to generate a 'vulnerability curve' the relationship between the decrease in hydraulic conductivity of xylem and xylem pressure (Tyree and Sperry, 1989).

Two methods of measuring these curves have been used prior to the advent of the centrifugal force approach. In the 'dehydration method' (Tyree and Sperry, 1989), plant material is dried to various xylem pressures (measured psychrometrically or with the pressure chamber) and xylem segments (stem or root pieces, usually) are excised from the plant underwater (to avoid air-blocking the severed conduits). The hydraulic conductivity of the xylem segment is measured before and after any air in the conduit system has been dissolved, thus giving the

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decrease in hydraulic conductivity associated with the minimum xylem pressure during the dehydration. A vulnerability curve is determined by repeating this procedure over a range of xylem pressures. Although this method has been used successfully on a number of species, it has disadvantages. It requires a considerable number of segments, the resulting curve is therefore usually representative of a population of plants rather than a single plant or genotype, and it is tedious and time-consuming. Furthermore, cavitation that occurred *in vivo* prior to the experiment obscures the relationship between conductivity and xylem pressure over the *in vivo* pressure range.

The 'air injection' method (Cochard *et al.*, 1992; Sperry and Saliendra, 1994) avoids some of these disadvantages. In this approach, a stem or root segment is inserted through a double-ended pressure bomb and the hydraulic conductivity of the xylem is measured alternately with exposure of the segment to progressively higher air pressures inside the chamber (xylem pressure is always near atmospheric). Usually any embolized conduits in the segment are intentionally re-filled prior to the experiment, reversing any *in vivo* cavitation. When the air pressure is sufficient to force air into the xylem conduits and displace their water, the hydraulic conductivity decreases. The cumulative decrease in hydraulic conductivity versus injection pressure generally agrees well in comparison with the dehydration method: the air pressure required to cause a given loss of conductivity is equal and opposite to the xylem pressure causing the same loss of conductivity in the dehydration experiment (Sperry *et al.*, 1996). The apparent reason for this agreement is that cavitation in the dehydrated stem is caused by the aspiration of air into the conduits (the 'air-seeding' hypothesis, Zimmermann, 1983; Crombie *et al.*, 1985). It takes the same pressure difference to pull air into the conduit by negative xylem pressure as it does to push it in by increasing air pressure. The air injection method has the advantage of allowing the entire vulnerability curve to be obtained on a single stem or root. However, it is also fairly time-consuming, and only provides an estimate of the vulnerability curve. Ideally it should be tested against the dehydration method for each species.

The use of centrifugal force for determining the vulnerability curve (Pockman *et al.*, 1995) has several advantages. By centring a xylem segment on the appropriate centrifuge rotor and spinning it, any desired xylem pressure can be obtained within minutes, and the xylem pressure is known with considerable accuracy. The physics of the situation are unambiguous (see 'theoretical considerations and rotor design' below), and have been used by Briggs and others in empirical tests of the stability of water under negative pressure (Briggs, 1950; Smith, 1991). In these experiments, the water was held in glass capillary tubes with the ends bent back at an acute angle with

respect to the middle of the tube. This 'Z' shape kept the water centred in the tube as it was rotated; at least until cavitation occurred. While stability of water in these 'Z' tubes was extremely variable, with cavitation occurring at any tension from a few tenths of a megapascal to over 25 MPa, experiments with plant xylem indicated very consistent cavitation pressures within a species (Pockman *et al.*, 1995).

The disadvantages of the centrifugal force method, at least as used previously (Pockman *et al.*, 1995), are that several xylem segments are required to complete the vulnerability curve, and only species with relatively short (< 50 mm) xylem conduits can be measured. The protocol was to measure the hydraulic conductivity of a segment before and after spinning to a desired speed and negative pressure. During rotation, the severed xylem conduits at either end of the segment were open to the air which allowed at least some of the water in them to evaporate and/or to be thrown out by the rotational motion. It was necessary to re-cut the segments underwater (after spinning) to remove the air-blocked ends before remeasuring the hydraulic conductivity (a length-specific measure). This re-cutting step meant that a segment could only be spun to one pressure, and many segments had to be used to determine the full cavitation response. Most xylem conduits had to be fairly short so that after the blocked conduits at the ends were removed there was still enough of a segment left for measuring conductivity.

In principle, these important disadvantages can be overcome if the segment ends are immersed in water during rotation. Water would be held in the conduits at the ends of the segment and the re-cutting step could be avoided. The hydraulic conductivity of a single segment could be remeasured as many times as necessary to complete the entire vulnerability curve and the length of the conduits would be immaterial. If desired, it should be possible to refill the segment and re-use it again.

In this paper an evaluation of the centrifugal force method for measuring vulnerability curves of plant xylem is provided. A rotor design is included that allows the ends of xylem segments to be immersed during rotation, and this modification is tested on root and stem material. The importance of a number of variables in the protocol have also been considered including duration of spinning, period between spinning and conductivity measurement, and the composition of the measuring solution. Two species were used whose cavitation response was familiar, based on use of the dehydration and/or air-injection methods: *Betula occidentalis* Hook. (hereafter referred to as 'water birch,' Sperry and Saliendra, 1994) and *Abies lasiocarpa* (Hook.) Nutt. ('subalpine fir,' Sperry and Sullivan, 1992). These species also allowed us to evaluate the method on both vessel-based and tracheid-based conducting systems.

## Materials and methods

### Theoretical considerations and rotor design

It is most intuitive to consider the forces on a rotating object from its rotational reference frame. From this perspective, the object experiences an outwardly directed 'centrifugal force' that is balanced by an inwardly directed tensile force. (In the external reference frame there is no centrifugal force and only the latter centripetal force exists.) As a column of water is spun about the centre of its long axis, the tensile force holding the column in place is manifest as a pressure gradient along the column from atmospheric pressure at the outer meniscus to a minimum negative pressure ( $P_{min}$ , relative to atmospheric pressure) at the column's centre. The gradient ( $dP/dr$ ) is given by the following equation:

$$dP/dr = \rho\omega^2 r \quad (1)$$

where  $r$  is the distance from the axis of rotation,  $\omega$  is the angular velocity, and  $\rho$  is the density of water (Trevena, 1975). If  $r_{max}$  is the distance from the axis of rotation to the outer meniscus, integrating equation 1 from this point to any lesser distance  $r$  gives the negative pressure at  $r$  ( $P_r$ ):

$$P_r = 0.5\rho\omega^2(r^2 - r_{max}^2) \quad (2)$$

This equation, graphed in Fig. 1, shows that pressures decrease to within 11% of the minimum ( $r=0$ ;  $P_{min} = -0.5\rho\omega^2 r_{max}^2$ ) in the central third of the column. The average pressure of the gradient is  $2/3P_{min}$ .

The column of water can only be spun in this way if it is perfectly centred or otherwise held in place, because an off-centre column will be displaced by rotational motion. In a segment of xylem whose ends are open to air, the water column can only be held in place if the xylem conduits are shorter than the segment (Fig. 2a). The capillary forces at inter-conduit pit membranes retain the water in the intact conduits inside the segment as the stem is rotated and allow negative pressure to develop. As mentioned, water may tend to drain from the severed conduits at the segment ends. For a segment whose ends are open to air, the distance  $r_{max}$  from the axis of rotation to the end of the outer meniscus will be closely approximated by half the segment length. This is because at least some intact conduits out of the thousands in the segment will extend close to the segment ends (Fig. 2a). As conduits cavitate during spinning,  $r_{max}$  will not change as long as the segment continues to be able to conduct water, because this requires a continuous

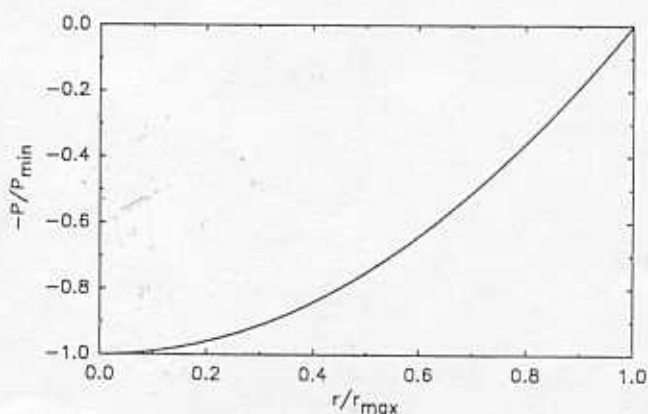


Fig. 1. Water column pressure ( $P$ ) relative to minimum ( $P_{min}$ ) versus distance from the column centre ( $r$ ) relative to maximum distance ( $r_{max}$ ) for a water column rotating about its long axis.

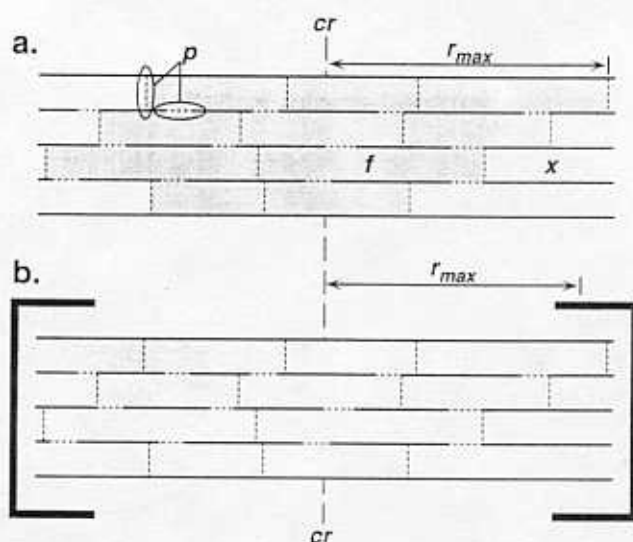


Fig. 2. The water column in spinning xylem segments. (a) A non-immersed segment with ends exposed to air. (b) A segment with ends immersed in water. The vertical dashed line  $cr$  is the centre of rotation. Each segment consists of several conduits connected to one another via pits represented by dashed regions ( $p$ ) in the conduit walls. In the non-immersed segment (a), severed conduits at the segment ends (e.g.  $x$ ) may be partially or fully (as shown) drained during spinning. Intact conduits in the segment (e.g.  $f$ ) will hold water because of capillary forces in the pits between air- and water-filled conduits (e.g. between conduits  $f$  and  $x$ ). The  $r_{max}$  (equations 1, 2) will be approximated by half the segment length because some of the intact conduits will extend to each end of the segment. In the immersed segment (b), all conduits remain water-filled, and  $r_{max}$  is the distance between  $cr$  and the free surface of the reservoir. The pressure gradient in Fig. 1 applies to the entire water column (the shaded region) between  $cr$  and  $r_{max}$  regardless of how many conduits comprise it.

water column through the segment. It should be emphasized that the pressure profile in Fig. 1 is only dependent on the length of the continuous water column (i.e.  $r$  in equations 1 and 2). It does not matter how many conduits are containing the column or how this varies with  $r$  or pressure.

To develop negative pressure, it is not necessary to have the segment pass through the axis of rotation as long as most conduits are shorter than the segment. Rather than modifying rotors to hold centred segments, the segments could be inserted in the centrifuge tube slots of a standard rotor. In this case, equation 2 would still apply, and the xylem pressure would be lowest at the end of the segment closest to the rotational axis (at minimum  $r$ ). The problem with this orientation is that the minimum pressure is at the location where the segment must be re-cut to remove severed and air-blocked conduits at the segment ends. This procedure will inevitably remove many intact conduits that were cavitated during spinning. The impossibility of separating the contribution of severed versus intact and cavitated conduits on the hydraulic conductivity of the segment eliminates this option for segment orientation. One might also be tempted to position segments on a rotor such that they are centred with respect to length, but pass to one side of the axis of rotation. Equation 2 does not apply to this situation and no attempt was made to analyse the forces. However, preliminary experiments with water birch showed this configuration produced more cavitation than for centred segments of the same length (257 mm) and spun at the same  $\omega$  ( $392 \text{ rad s}^{-1}$ ; Student's  $t$  test,  $P < 0.01$ ,  $n = 7-8$ ).

The rotor design shown in Fig. 3 allowed centred xylem

segments to be rotated while keeping their ends in water. As illustrated in Fig. 2b, this configuration will hold water in all xylem conduits of a segment regardless of their length or location in the segment. Segments were held at their middle with their ends unrestrained, but within 'L' shaped plexiglas reservoirs (Fig. 3, 'a'). These reservoirs fit in slots in the rotor floor and were held by the outer lip of the rotor. The 'foot' of the L was filled with water prior to spinning the segment. At modest angular velocities (e.g.  $\omega = 54 \text{ rad s}^{-1}$  for a 257 mm long segment), the water was pushed up against the 'leg' of the L and immersed the segment end (Fig. 3, insert 'e'). Under these conditions,  $r_{\text{max}}$  in equation 2 was the distance from the segment centre to the free surface of the water in the leg of the L-shaped reservoir (Fig. 3, dotted line in insert 'e,' see also Fig. 2b). This

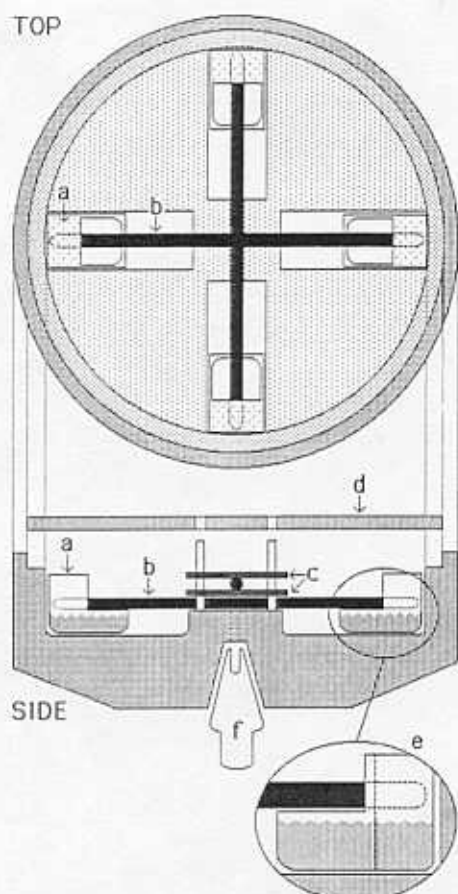


Fig. 3. Rotor for spinning xylem segments with their ends in water. The rotor was machined from a single piece of 7075 T6 aluminum. Slots in the main body retained plexiglass 'L' shaped reservoirs (a) containing water. Segments (b) are held by thin aluminium plates (c) secured by nuts on the bolts that also hold the lid (d). A maximum of two segments can be spun at once, crossing in the middle and held by a sandwich of two retaining plates. Segment attachment plates and lid bolts are absent in the top view for clarity. The rotor is attached to the centrifuge shaft (f) by a bolt (not shown). During rotation, centrifugal force moves the water in the reservoirs to the position indicated by the dotted line in the insert (e), and immerses the segment ends. Finite element analysis indicated a safety margin of  $>2.8$  for  $\omega < 1361 \text{ rad s}^{-1}$  for a new rotor; this margin declines with fatigue. The peak stresses develop in the bolt holes (for lid bolts) and inner corners of the reservoir slots (M. Perry, personal communication). An  $\omega$  of  $1361 \text{ rad s}^{-1}$  corresponds to a minimum xylem pressure of  $-15.3 \text{ MPa}$  for  $r_{\text{max}} = 0.129 \text{ m}$ , the longest segment the rotor could accommodate (equation 2).

distance was determined prior to spinning by placing the reservoir on its back and dipping the segment to the appropriate depth.

The pressure in the segments after spinning has ceased may have implications for the stability of the emboli formed by the cavitation. Post-spin pressure in immersed segments will return to atmospheric. This may result in rapid dissolution of the emboli if they consist mostly of water vapour after a relatively brief spinning period (with time after cavitation, conduits become increasingly air-filled by diffusion of gases into the vapour void; Tyree and Sperry, 1989). For non-immersed segments, the post-spin pressure is more ambiguous. The pressure after spinning will equilibrate to the average pressure of the gradient in Fig. 1 ( $=0.667P_{\text{min}}$ ) under the following conditions: (a) symplast water potential equilibrates with xylem pressure during spinning (along the gradient in Fig. 1), (b) water removed from the symplast is spun out of the segment along with all water in severed conduits at segment ends, (c) the relationship between water content of the cells and their water potential is linear and constant for all cells. The last two of these assumptions are very unlikely and, as a consequence, the pressure may well rise close to atmospheric even in non-immersed stems after spinning.

#### Collection of plant material

Stem segments of water birch were harvested from natural stands along a perennial stream in the Red Butte Canyon Research Natural Area (c.  $111^{\circ} 47' \text{ W}$ ,  $40^{\circ} 47' \text{ N}$ ; elevation 1770 m). Birch root segments were excavated from a site further up the canyon (elevation c. 1890 m) described in Sperry and Saliendra (1994). Collected roots and stems ranged from 5.3 to 11.9 mm and from 4.2 to 8.8 mm in diameter, respectively. Branch segments of subalpine fir were collected from the Wasatch Mountains near Brighton (c.  $111^{\circ} 35' \text{ W}$ ,  $40^{\circ} 36' \text{ N}$ ; elevation 2825 m) at the same site as that studied by Sperry and Sullivan (1992). Collected stems ranged from 5.9 to 15.9 mm in diameter. Upon cutting, branches and roots were immediately wrapped tightly in plastic bags to prevent dehydration and brought to the laboratory.

#### Centrifugal force method: non-immersed segments

Only stem segments were used for the non-immersed protocol because it was known that their xylem conduits were short enough (tracheids in subalpine fir, 95% of the vessels shorter than 50 mm in water birch; Sperry *et al.*, 1994). Many vessels in birch roots exceeded the maximum length of segments able to be accommodated by the centrifuge (257 mm). Stem segments were cut from larger branch systems under water to lengths of 200 mm for birch and 150 mm in fir. These segments were free of side branches, and otherwise uniform in appearance. All segments were shaved at the ends with fresh razor blades; the bark and phloem from the ends (c. 10 mm) of fir segments were also shaved off to reduce resin clogging of the tracheids.

Segments were attached to a tubing manifold filled with filtered ( $0.2 \mu\text{m}$ ) HCl solution (pH=2). The acidity of this solution prevents microbial growth which otherwise populates the tubing and can rapidly clog xylem. All water birch stems were 'flushed' with solution for 20 min at high pressure (c. 100 kPa) to remove air emboli formed *in vivo* and during harvesting. Segments of subalpine fir were not flushed because native embolism was shown to be low in this species during an earlier study (Sperry and Sullivan, 1992). The initial hydraulic conductivity ( $k_i$ ) of the segments was then measured:  $k$  was calculated as the quotient of the mass flow rate of solution through the segment (measured gravimetrically) and the pressure

gradient along the segment (induced by 3–7 kPa hydraulic head).  $k$  was measured continuously in 15 s intervals and calculated from a running mean of the previous five readings. The value chosen was the first running mean to deviate from a previous trend (of two or more means; Sperry *et al.*, 1988).

Immediately following the  $k_i$  measurement, segments were individually spun in a Sorvall RC-5C Centrifuge (DuPont Instruments, Wilmington, Delaware, USA). Each was attached to the centre of rotation of the rotor (DuPont, SS-34) using teflon cable ties and a steel hose clamp. The standard spin period (at target angular velocity) was 3 min. Segments were then detached from the rotor, and held in a plastic bag for a standard time of 1 h to promote diffusion of air into any conduits cavitated by spinning.

Segments were recut underwater to remove the presumably air-blocked conduits at each end, and the 'final'  $k$  was measured ( $k_f$ ). The  $k$  value was length-specific (i.e. flow rate was divided by pressure gradient rather than pressure difference) and thus independent of segment length. The percentage that  $k_f$  was below  $k_i$  gave the percentage loss of conductivity (% loss  $k$  in figures) induced by cavitation during spinning. The length removed from each end of the segment was 10 mm in subalpine fir (longer than any tracheid in this species). In water birch it was determined experimentally by spinning stems to a modest negative pressure (–0.1 or –0.2 MPa) that was expected to drain severed vessels but not cause cavitation in intact ones. The loss of conductivity was then measured as a function of stem length removed from each end and compared with non-spun controls. It was found that removing 25 mm from either end was sufficient to return the percentage loss of conductivity in treated stems to control values that were not significantly different from zero (Student's 't' test,  $P > 0.05$ ,  $n = 7-8$ ).

Vulnerability curves using this protocol were compared with those obtained using the dehydration and air-injection methods for water birch (Sperry and Saliendra, 1994; see below) and the dehydration method for subalpine fir (Sperry and Sullivan, 1992).

#### Variations on the protocol for non-immersed segments

Various aspects of the standard protocol were varied to determine their effect on the loss of conductivity caused by spinning. These tests were only performed with water birch stem segments. All tests were performed using negative pressures in the range known to cause cavitation based on results with the standard protocol (e.g. between c. –1.0 and –1.25 MPa, Fig. 4a).

The effect of spin period on segments from water birch was tested. Longer spins could cause a greater loss of conductivity if there was significant hysteresis between the achievement of minimum negative pressure and cavitation, and if longer spins allowed more air to diffuse into the emboli in cavitated conduits. The loss of conductivity caused by spin periods of 3, 25 and 60 min was compared. To reduce evaporation during spinning, segments were wrapped tightly in plastic-wrap prior to centrifuging.

The loss of conductivity for segments held in plastic bags for different periods after spinning was also compared. This variable could influence the loss of conductivity in two ways. If emboli were initially mostly vapor-filled after spinning, and if xylem pressures after spinning remained low enough to prevent dissolving of the gas, longer bagging periods would facilitate air diffusion into the emboli and perhaps increase the loss of conductivity. In contrast, if xylem pressures were high enough to allow emboli to dissolve, longer bag times could decrease the loss of conductivity. Periods of 0 h (immediate remeasuring

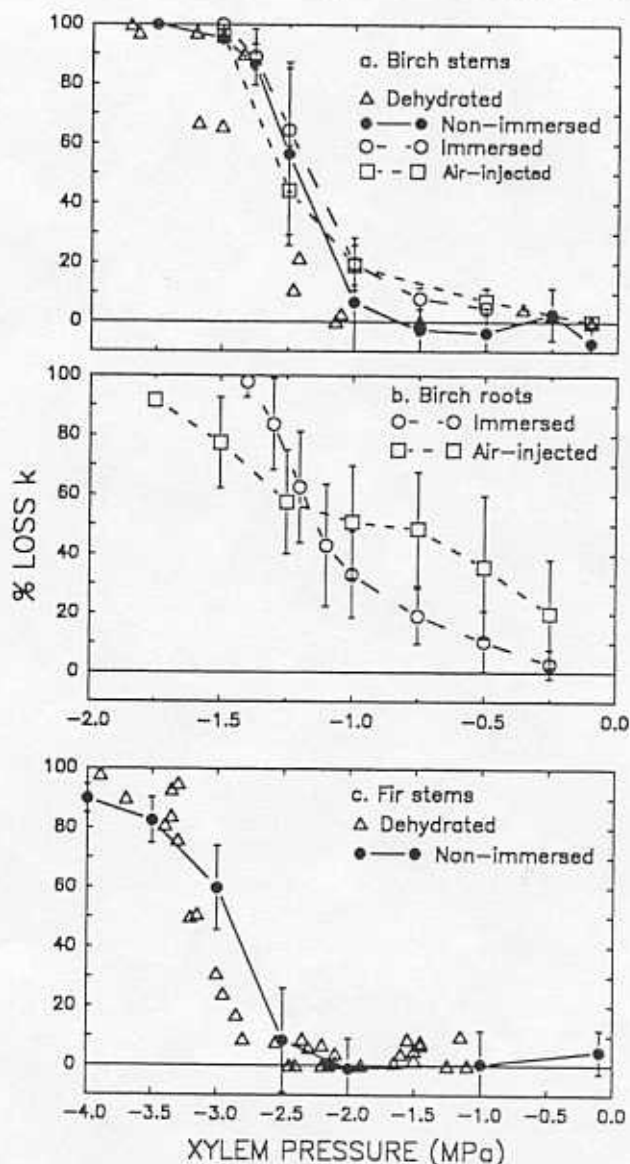


Fig. 4. Percentage loss of conductivity (% loss  $k$ ) versus minimum xylem pressure for *Betula occidentalis* (birch) stems (a) and roots (b) and *Abies lasiocarpa* (fir) stems (c). Note differences in pressure scale for birch versus fir. Dehydration data are from Sperry and Saliendra (1994, birch) and Sperry and Sullivan (1992, fir). Air pressure for injection experiments is shown as its negative for comparison with xylem pressure. Error bars are standard deviations.  $n = 7-8$  except for air-injection experiments where  $n = 5$ .

of  $k_f$ ), 0.5, 1, 2, and 3 h were tested. The water potential of stems after spinning to determine their water potential was also measured. Water potential was measured with a stem hygrometer (Dixon Instruments, Guelph Ontario) attached to the stem centre immediately after spinning.

To evaluate any influence which the HCl solution might have on cavitation vulnerability, a comparison was made with purified water (filtered to 0.2  $\mu\text{m}$ , electrical resistivity  $> 140$  Mohm mm). To test for pH effects, a KCl solution was also tested (0.02 M). Some of these tests were also performed on immersed stems.

*Centrifugal force method: immersed segments*

The rotor shown in Fig. 3 was used to evaluate the possibility of spinning segments with their cut ends immersed in water. Stem and root segments of water birch were used in these tests. Segments were cut to 257 mm in length to insure immersion in the water-filled plexiglas reservoirs given the dimensions of this rotor. All segments were flushed and  $k_i$  measurements were made as described above. After centrifuging, segments were immediately attached to the tubing apparatus for measuring  $k_f$  without bagging the segments or re-cutting them. In measuring  $k$  for roots a 3 kPa hydraulic head was used because higher pressures were sufficient to displace air from embolized vessels running completely through the segment (Sperry and Saliendra, 1994). Vulnerability curves for stems were compared with those obtained from non-immersed stems, the air-injection method (see below), and the dehydration method (Sperry and Saliendra, 1994). Vulnerability curves for roots were compared with the air-injection method (see below). The repeatability of this technique was also tested, generating vulnerability curves for a set of six water birch stems, flushing them to restore the initial  $k$  values, and repeating the vulnerability curves.

In immersed segments, there was the possibility that air could enter the severed conduit ends before and after spinning because the segment ends were not under water at this time. However, the hydraulic conductivity of a hydrated segment was not changed even after exposing its ends to air for 15 min (J.S. Sperry, personal observations). In addition, the vulnerability curves obtained by the method generally show no change in conductivity until a threshold pressure is reached (Fig. 4a, open circles) indicating that the exposure of the segment ends to air before or after spinning does not in and of itself cause a drop in conductivity.

*Air injection experiments*

For roots and stems of water birch, vulnerability curves were determined using the air-injection method (Sperry and Saliendra, 1994). Root segments ranging from 5.4 to 7.2 mm in diameter, and 0.21 to 0.23 m in length, and stem segments ranging from 9.6 to 11.8 mm in diameter and 0.20 m in length were flushed (to reverse any embolism) and inserted through a double-ended pressure bomb. Tubing filled with HCl solution was attached to the proximal end. The distal end was open to air. Flow through the segment was induced by a hydraulic head of 3 kPa for roots (see previous paragraph) and 7 kPa for stems. Mass flow rate of solution was measured by collecting effluent from the distal end with tared vials filled with cotton wool over 1 min intervals. Hydraulic conductivity was calculated as above. Hydraulic conductivity measurements were alternated with 10 min exposure of the segment inside the chamber to progressively higher air pressures. Pressure in the chamber during conductivity measurements was always lowered to 0.1 MPa. Measurements were continued until hydraulic conductivity had decreased by more than 95% from its initial value because of air entering intact xylem conduits. The cumulative per cent loss in conductivity was plotted versus maximum air pressure to give an estimated vulnerability curve for the segment. This assumes that cavitation in dehydrated stems was triggered by air seeding (Zimmermann, 1983; Sperry *et al.*, 1996).

**Results***Non-immersed segments*

The vulnerability curve obtained by centrifugal force in non-immersed stems of water birch (Fig. 4a, solid circles)

corresponded closely with the curve obtained by Sperry and Saliendra (1994) using the dehydration method (open triangles), and with the curve obtained in this laboratory using the air-injection technique (open squares). The same close correspondence between the centrifugal and dehydration methods was seen for stems of subalpine fir (Fig. 4c, solid circles versus open triangles) where the dehydration curve was obtained from trees at the same site by Sperry and Sullivan (1992). No air-injection curve was attempted for subalpine fir; however, experiments with other conifers show reasonable agreement between air-injection and dehydration methods (Sperry and Tyree, 1990).

There was no effect on the loss of conductivity from either varying the spin time from 3–60 min (Fig. 5a) or bagging time from 0–3 h after a 3 min spin (Fig. 5b) for birch stems. Although bagged stems tended to have a lower loss of conductivity than those not bagged, these differences were not significant as indicated by the lack of correlation between time and conductivity (Fig. 5b;  $P > 0.05$ ). Stem water potential after 3–60 min spins to pressures as low as  $-2$  MPa were not different from 0 MPa. These observations indicated that 3 min at the

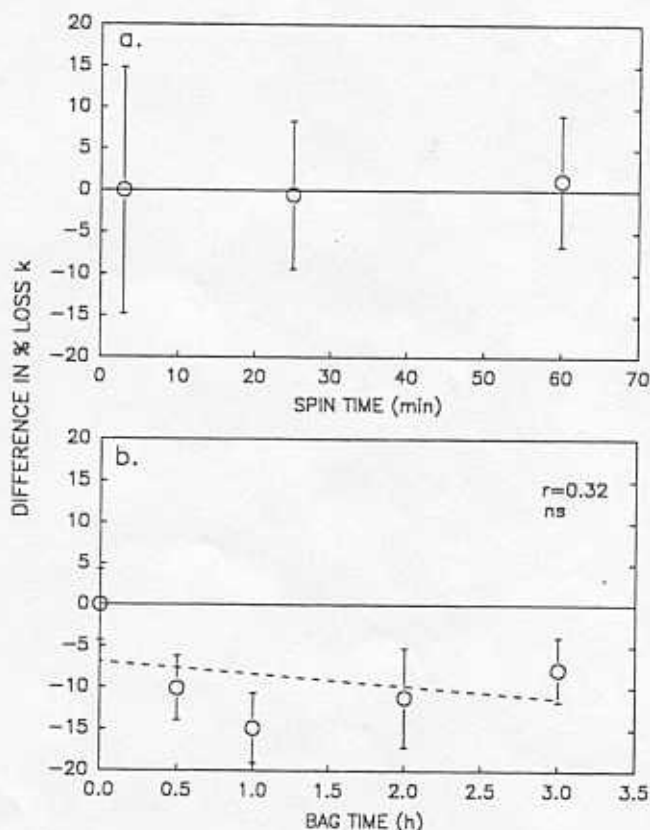


Fig. 5. Difference in percentage loss of conductivity (% loss  $k$  minus % loss  $k$  at earliest time) versus time of spinning at a target pressure of  $-1.0$  MPa (a;  $n=4-5$ ) or time in plastic bag after spinning 3 min at  $-1.25$  MPa (b;  $n=5$ ). All data for birch stems. Neither relationship was significant ( $P > 0.05$ ). Error bars are standard deviations.

minimum negative pressure was sufficient to saturate the embolism response, and that emboli formed during a 3 min spin were stable at atmospheric pressure for up to 3 h.

Birch stems perfused with water versus HCl solution or water versus KCl solution showed no difference in the loss of conductivity induced by spinning to  $-1.25$  MPa (Student's  $t$  test,  $P > 0.05$ ,  $n = 5$ ).

#### Immersed segments

A major concern with the immersed segment experiments was that emboli would dissolve too rapidly for stable and accurate  $k_f$  measurements to be obtained. The initial results confirmed these fears because  $k_f$  measurements made on stems spun to pressures inducing severe cavitation (below  $-1.25$  MPa) showed a continual increase unlike the stable values for non-immersed segments (Fig. 6, compare curve 2 with curve 1, per cent loss  $k$  plotted as proxy for  $k_f$ ). Instead, an initial abrupt increase in  $k$  (lasting  $< 1$  min) was followed by a more gradual but steady increase from appropriately low (embolized) values. This is shown in Fig. 6 in terms of a continual decline in percentage loss of  $k$  for curve 2. The increase in  $k_f$  was such that the running-mean criterion for choosing a value was never met. It was reasoned that the initial uptake represented the final stages of the equilibration of the symplast with zero xylem water potential, because segments were attached to the conductivity apparatus within minutes of the conclusion of spinning. The slower increase was felt to result from

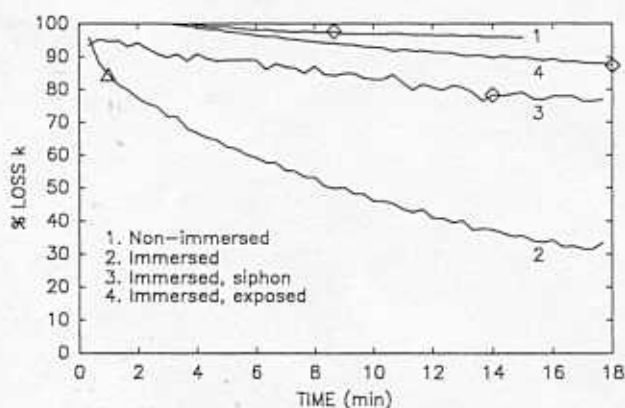


Fig. 6. Percentage loss in conductivity based on individual  $k_f$  measurements (15 s intervals) versus cumulative time for birch stems spun to  $-1.38$  MPa. Curve 1 is from a non-immersed stem, curve 2 is from an immersed stem. Note that an increase in  $k_f$  with time corresponds to a decrease in the percentage loss  $k$ . Curve 3 is an immersed stem in the siphoning configuration where xylem pressures were below  $-14.9$  kPa. Curve 4 is an immersed stem from which large side-branches had been removed, exposing the xylem to air. Diamonds in curves 1, 3, and 4 indicate the first  $k_f$  running mean that deviated from the previous trend of running means ( $n = 5$ )—our selection criterion for the  $k_f$  value. Means from curve 4 never satisfied this criterion and the triangle indicates the  $k_f$  value selected by the first slope less than  $10^{-7}$  kg m s $^{-2}$  MPa $^{-1}$  (based on regression through the previous five  $k_f$  readings).

gradual dissolution of the emboli in the stem. Xylem pressures in the stem during these measurements were between 7 kPa (influx end) and 1 kPa (efflux end) above atmospheric.

In support of this conclusion, it was found that the gradual increase in  $k_f$  in immersed stems could be eliminated by reducing the xylem pressure in the stem during the  $k_f$  measurement. Theory predicts that as long as xylem pressure is low enough to oppose the surface tension forces acting to compress the air bubbles, dissolution of the bubbles will be avoided (Yang and Tyree, 1992). These surface tension forces equal  $2T/l$  where  $T$  is the surface tension of water and  $l$  the radius of curvature of the bubble. Therefore xylem pressure for stable  $k_f$  measurements would have to be below  $-2T/l$  where  $l$  was equal to the vessel radius (assuming the emboli were large enough to span the vessels). For  $l = 10$   $\mu$ m (actual mean vessel radius =  $13.5$   $\mu$ m, data from Sperry and Saliendra, 1994), xylem pressures during the  $k$  measurement would have to be below  $-14.6$  kPa to avoid embolism dissolution. These pressures were achieved by elevating stems more than 1.49 m above the solution source and siphoning the solution through the stems rather than pushing it through as before. As predicted, this resulted in stable  $k_f$  values (Fig. 6, curve 3) that corresponded to a percentage loss of conductivity within the range seen for non-immersed stems (compare selected running means indicated by diamonds, and data in Fig. 4). Stable readings were also obtained when the solution was pulled through the stems using vacuum pressure (data not shown).

Given that the psychrometer measurements indicated that non-immersed stems were at atmospheric pressure after the spin, and yet these stems showed stable  $k_f$  readings (Fig. 6, curve 1), the greater instability of the emboli in immersed stems could not be attributed to their having a higher post-spin xylem pressure. Instead, it was reasoned that the uptake of air into the embolized vessels in immersed stems was less than that in non-immersed stems. This would result in lower volumes of air in the embolized vessels of immersed stems and thus a greater effect of the dissolving of this air on  $k_f$ . To test if the exposure of the xylem to air during spinning allowed more air uptake in the non-immersed segments, stems were spun with their ends immersed, but with the xylem exposed to air where large side-branches had been removed. In support of this suspicion, these immersed stems gave stable  $k_f$  readings when measured with the usual positive xylem pressures (Fig. 6, curve 4). Apparently exposure of the xylem to air enhanced the uptake of air into the cavitated vessels.

It proved impractical routinely to measure immersed stems in the siphoning configuration. Rather than eliminating the gradual re-filling stage of the  $k_f$  measurement, the standard hydraulic head arrangement was used and

$k_f$  was chosen at the earliest point where the slope of  $k$  versus time (based on the previous five 10 s readings) dropped below a threshold value of  $10^{-7} \text{ kg m s}^{-2} \text{ MPa}^{-1}$  (Fig. 6, curve 2, triangle). This gave a percentage loss of conductivity within the range obtained in immersed stems when refilling was avoided by either siphoning or exposure of xylem (Fig. 6, diamonds on curves 3, 4). Using this protocol it was possible to generate complete vulnerability curves for single stems. The average curve for seven immersed stems coincided closely with that made with non-immersed stems (Fig. 4a, compare open and solid circles). When stems were re-filled after completion of a vulnerability curve ( $k_f$  values within 2.5% of each other) and the process was repeated, the same vulnerability curve was obtained (Fig. 7).

The immersion method allowed us to attempt a vulnerability curve on roots (Fig. 4b, circles), whose longer vessels made the non-immersion approach impossible. In agreement with previous results using the air-injection technique (Sperry and Saliendra, 1994), roots of birch were more vulnerable to cavitation than stems (Fig. 4a, b; compare open circles); however, the differences were not as great as in this earlier study. Interestingly, the  $k_f$  measurements on roots showed none of the instability seen with stems. The larger vessel diameters in roots versus stems (mean diameter = 69 versus 27  $\mu\text{m}$ , respectively; data from Sperry and Saliendra, 1994) would increase the radius of curvature for emboli and decrease the surface tension force on the bubbles, thus inhibiting refilling.

Although the air-injection data for birch roots and stems also indicated that roots were more vulnerable to cavitation than stems, the correspondence between the air-injection and centrifugal-force methods was not as close for roots as for stems (Fig. 4b, compare squares and circles). At moderate test pressures (e.g.  $\geq -0.75$  MPa on the xylem pressure scale in Fig. 4b),

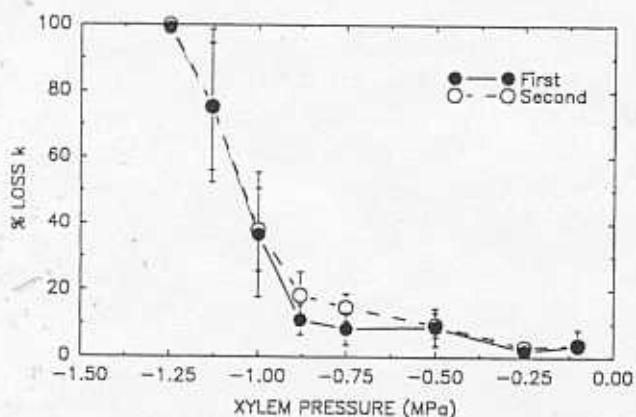


Fig. 7. Repeated vulnerability curves for birch stems. The initial curve (solid circles) was indistinguishable from the second curve (open circles) generated after refilling the cavitated xylem. Error bars are standard deviations,  $n=6$ .

air-injection caused a greater loss of conductance (Student's  $t$  test,  $P < 0.05$ ). At the more extreme pressures ( $\leq -1.5$  MPa in Fig. 4b), air injection caused less loss of conductance than the centrifugal force technique.

## Discussion

The results with the non-immersed stems were identical to those of Pockman *et al.* (1995) using the same procedure: centrifugal force produced the same vulnerability curve as the dehydration or air-injection method for both the vessel-bearing water birch and the tracheid-bearing subalpine fir (Fig. 4a, c). Spinning periods of 3 min were sufficient to saturate the response (Fig. 5a) and the data indicated that no bagging time was necessary following the minimum pressure treatment (Fig. 5b). Furthermore, the measuring solutions tested gave the same result.

The most important result was that it was possible to measure vulnerability curves on single stem or root segments regardless of vessel length by immersing the segment ends in water during spinning (Fig. 4a, b). This method is more accurate than the non-immersion one because it avoids re-cutting the segment. Although hydraulic conductivity is a length-specific measure, re-cutting can cause variation in this value independent of embolism if conductivity is not constant along the segment. Furthermore, the immersion method allowed a full vulnerability curve to be completed on a single segment. The rotor design developed in this work (Fig. 3) was a simple solution to the practical problem of keeping the segment ends immersed during rotation. The only difficulty that was encountered was an instability of the  $k_f$  measurements in the birch stems. Although this problem could be solved by either reducing the xylem pressure during measurement or exposing the xylem to air during spinning (i.e. at cut side-branches), it was more practical simply to take the first  $k$  reading after the initial rapid hydration phase (Fig. 6). As expected from theory the problem was related to vessel diameter: the larger vessels in roots were associated with stable  $k_f$  readings. However, recent work on other species in this laboratory (e.g. *Artemisia tridentata*; *Larrea tridentata*) has indicated that the instability problem is not widespread even for plants with narrow vessels. Perhaps the scalariform perforation plates characteristic of *Betula* xylem contributed to the problem by breaking up the air in embolized conduits into small bubbles.

The mechanism of cavitation in spinning stems is apparently the same as that proposed for dehydrated stems: air-seeding (Zimmermann, 1983; Crombie *et al.*, 1985). This is supported by the generally good agreement between the air-injection, dehydration, and centrifugal force results (Fig. 4a; see also Pockman, *et al.*, 1995). It is usually assumed that the air-seeding occurs at inter-conduit pit membranes between air- and water-filled



conduits because these are the weakest and/or most porous interface between xylem water and air. This is supported by correlations between porosity of these membranes and the cavitation pressure (Jarbeau *et al.*, 1995). However, air seeding could occur at other sites in the vessel wall, although, in general, the wall proper has very minute pores. Whatever the air seeding sites, they are apparently well distributed throughout the xylem because in the centrifuge experiments the minimum xylem pressure was only experienced at the exact centre of the stem (Fig. 1), and yet the loss of conductance caused by that pressure generally agreed well with dehydrated stems where the xylem pressure would be uniform. The fact that the same vulnerability curve was obtained from twice-cavitated stems of water birch (Fig. 7) indicates that the air-seeding threshold is fixed (at least for the short term) and confirms that re-filling whether in the laboratory or in nature (e.g. by root pressure) can effectively recycle embolized vessels in this species.

The results for roots showed deviations between the air-injection and centrifugal force methods (Fig. 4b). The tendency at low pressures (e.g.  $\leq -1.5$  MPa in Fig. 4b) for air-injection to give a lower percentage loss of conductivity may result from air pressures at the site of air-seeding inside the xylem being less than those measured at the chamber wall by the pressure gauge because of the continuous flow of air exiting the stem through already embolized vessels. The bigger vessels of roots may increase that flow relative to stems, therefore increasing the pressure discrepancy for roots. The reason for air-injection to over-estimate the percentage loss of conductivity at moderate pressures (e.g.  $\geq -0.75$  MPa, Fig. 4b) is less obvious. Perhaps it results from the fact that air-injection will completely fill an embolizing vessel with air whereas an embolus caused by negative pressure will be a mix of air and water vapour. Thus, at atmospheric xylem pressures, the embolus formed by air-injection would tend to have a greater volume than one formed by negative pressure. For relatively small vessels of stems, both types of emboli may have sufficient volume to block flow equally and cause the same percentage loss of conductivity. For larger vessels of roots, it could be that a smaller embolus formed by dehydration does not block flow as completely as the one formed by air-injection. The discrepancy would disappear at more extreme pressures if the more resistant vessels were also the smaller diameter ones, as is often the case within a plant (Sperry and Saliendra, 1994). More experience with the methods is necessary to learn the extent of the discrepancy and its cause.

The advantages of the centrifugal force method, particularly the immersed segment protocol, have already been emphasized. However, which of the three methods (centrifugal force, air-injection, dehydration) is most accurate? The dehydration method is probably least

accurate because the measure of the xylem pressure is most indirect, relying on pressure chamber measurements or psychrometer readings. Furthermore, the usual necessity of constructing a single curve by sampling different individuals increases the difficulty of interpreting any variation in the results. If the speculations in the previous paragraph are correct, the air-injection method may be inaccurate at more extreme pressures—giving the impression the xylem is more resistant to cavitation than in reality. At modest pressures, however, the air-injection method could actually be more accurate than the centrifugal force technique because it insures that embolized vessels are fully blocked with gas. However, it is not possible to be confident about this with the little information in hand. Certainly the control of the xylem pressure is most precise with the centrifugal force method; any ambiguity results from interaction between the emboli formed during spinning and the  $k_f$  measurement. This degree of control, together with the efficiency with which vulnerability curves can be determined by this approach, should facilitate the study of how cavitation influences plant water relations.

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