A method for measuring xylem hydraulic conductance and embolism in entire root and shoot systems

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Abstract

Current methods for determining the influence of xylem cavitation on hydraulic conductance are limited to unbranched stem or root segments with hydraulic conductances above c. 2 mmol s⁻¹ MPa⁻¹. Lower conductances and/or highly branched systems are encountered in seedlings, arid-land shrubs, herbs, and distal portions of shoot and root systems of trees. In order to quantify the hydraulic impact of cavitation in such systems, existing techniques have been modified. Branched shoot or root systems were prepared for measurement by removal of leaves, or root tips, respectively. The shoot or root system was enclosed in a vacuum chamber with the proximal end protruding and supplied with perfusing solution. Flow through the xylem was induced by chamber vacuum. Hydraulic conductance was determined from the slope of the flow rate versus pressure relationship. Xylem embolism was quantified from the increase in hydraulic conductance following high pressure (100 kPa) perfusion of solution through the plant. Examples are provided of the application of the method to cavitation studies in the cold desert shrub *Artemisia tridentata*.

Key words: Hydraulic conductance, xylem cavitation, embolism, whole root/shoot system.

Introduction

Water evaporates from a plant while stomata are open and carbon dioxide is being fixed. This water is replaced by uptake through the roots and transported in xylem conduits to the site of evaporation. In order for a plant to retain a favourable leaf water status and positive carbon gain, water transport through the plant must be maintained.

The occurrence of cavitation and subsequent embolism in the xylem decreases hydraulic conductance and may diminish the ability of a plant to maintain water transport and carbon uptake. Cavitation is the vaporizing of water held under negative pressure in the xylem conduits. It can be triggered when air is pulled into a functional xylem conduit from a neighbouring air-filled conduit through the pit membranes of inter-conduit pits (Zimmermann, 1983; Sperry and Tyree, 1988; Cochard *et al.*, 1992). Cavitation results in a vapour-filled xylem conduit which gradually becomes air-filled as air diffuses in from surrounding tissues. These air-filled, or embolized, conduits are incapable of water transport and reduce the overall conductance of the xylem. The xylem pressures causing cavitation limit the water-stress tolerance of a species, and also limit gas exchange because transpiration rates can not exceed those causing 'runaway' cavitation (Tyree and Sperry, 1988; Jones and Sutherland, 1991).

Much of what is known about cavitation comes from measurements made on stems and roots of mature woody plants. In these studies, cavitation is often quantified by how much the hydraulic conductance of the xylem increases following refilling of embolized vessels by a pressure treatment (Sperry *et al.*, 1988). The method consists of measuring gravity-induced flow through excised stem or root segments (pressure head of *c.* 3–10 kPa), and calculating hydraulic conductance as the flow rate through the stem segment for a given pressure. Emboli are removed by flushing the stem with pressurized solution (100–175 kPa) which dissolves and expels emboli from the segment. After removal of emboli, the hydraulic conductance is remeasured and flushing repeated until the hydraulic conductance ceases to increase. Reversible

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embolism is quantified by the percentage that the initial conductance is below the maximum value.

While this technique has been useful, it is limited to unbranched segments of sufficient hydraulic conductance to permit measurable flow rates at pressures of a few kilopascals. In a typical implementation of the method where flow rate is measured gravimetrically with an electronic balance (0.1 mg resolution), the minimum flow that can be reliably measured is approximately 0.02 mmol s^{-1}. This corresponds to a minimum conductance of 2 mmol s^{-1} MPa^{-1} at a pressure of 10 kPa. Although lower conductances could be measured by using higher pressures, this would defeat the purpose of the measurement by causing embolized vessels to refill.

These limitations have precluded studies of hydraulic conductance and cavitation on material with low conductances and/or dense branching. In many cases, it is just such systems that need to be measured. For example, beginning with Zimmermann's hydraulic architecture studies (Zimmermann, 1978), it has been clear that the distal parts of the shoot system exert a disproportionate influence on leaf water status during transpiration. Yet the same traits that make them important in this regard, low conductance and bottlenecks at the many branch points, make them difficult to measure with existing methods. Seedlings of woody plants are another example where hydraulic conductance and cavitation may be at their most limiting for survival, but also difficult to measure because of the seedling's small size and dense branching. A final example is arid-land shrubs. These plants are highly branched and generally have low conductances. However, their adaptation to extreme drought makes them interesting subjects for study.

The purpose of this paper is to describe and evaluate a modification of the original technique of Sperry *et al.* (1988) that was designed to facilitate hydraulic studies of highly branched and/or low conductance systems. The branching problem was eliminated by measuring the intact (or nearly so) branched system rather than attempting to measure its component parts separately. The low conductance problem was minimized in two ways: (1) the distal parts of the flow path with the limiting conductances (branchlets/petioles or rootlets) were measured in parallel, because flow is directed through the entire branch or root system; and (2) vacuum pressure was used rather than above-atmospheric pressure to drive flow. The use of vacuum pressures allowed for a larger pressure difference (and increased flow) with less danger of refilling embolized conduits in the process.

The method was developed to measure the hydraulic conductance of branches from mature *Artemisia tridentata* Nutt. (Asteraceae), a densely-branched dominant shrub in the Great Basin region of the United States. The application of this method has been specifically focused to this species, although it has also been applied to several other species including *Betula occidentalis* (Saliendra *et al.*, 1995), *Glycine max*, *Helianthus annuus*, *Hakea polyanthema*, and *H. smilacifolia* (NZ Saliendra, J Sperry, B Lamont, unpublished data).

Materials and methods

Plant material

The way that plant material was prepared for measurement depended on whether seedlings or mature plants were used. Seedlings were prepared for measurement by carefully excavating the root system from the soil, and tightly bagging the whole plant in plastic for transport to the laboratory. The root system was separated from the shoot by cutting the stem near the root–shoot junction under water. The bases of the shoot and root systems were fitted via tubing to a supply of 0.1% (v/v) concentrated HCl (37.7%) in distilled water (*c.* pH = 2) filtered to 0.22 µm. This low pH solution inhibits microbial growth within the tubing system that can lead to clogging of the xylem during the conductance measurement (Sperry *et al.*, 1988). Tests have shown no influence of this solution on hydraulic conductance compared with distilled water or organic acid solutions (Sperry and Saliendra, 1994).

Shoots were defoliated by removing the lamina at its junction with the petiole with a razor blade. Where short shoots were present, the entire short shoot was removed. By removing the leaves, the xylem from shoot base to petiole was cut at both ends making it possible to measure the hydraulic conductance of this pathway to all leaves of the shoot in parallel.

Roots were washed free of soil, and the root tips cut off. Removing the root tips achieved the same purpose as removing leaves from the shoot: it exposed the xylem at both ends of the flow path from rootlet to root collar. This allowed measurement of the conductance of this xylem pathway in parallel to all severed root tips.

While it was possible to remove all the leaves, it is impractical with most root systems to cut every root tip at the same relative position from the tip. Inevitably, roots were cut at different positions, and many root tips were not removed. The difficulty of reproducibly severing the roots means that comparisons of hydraulic conductance itself between root systems may not always be possible. However, embolism measurements were, in principle, accurate in roots because they were based on paired conductance measurements on the same root system (before and after embolism removal, see below). Root systems were wrapped loosely with damp cloth (i.e. cheesecloth) to prevent the root surface from drying out.

Shoot and root systems of mature plants were, in most cases, too large to be measured in their entirety. However, distal units of these systems were sub-sampled. First, material was collected from the field by removing a large enough portion of the root or branch system so that the distal sample to be measured contained none of the conduits embolized by the original excision. The plant material was tightly bagged in plastic and transported to the laboratory for measurement. The plant material was then prepared in the same manner as described for seedlings after removing the distal sample from the larger branch by cutting it off underwater.

Measurement apparatus

The shoot or root system (still wrapped in damp cloth) was inserted into a vacuum canister (Fig. 1). The canister was made from eight-inch diameter PVC pipe sealed at one end by a PVC
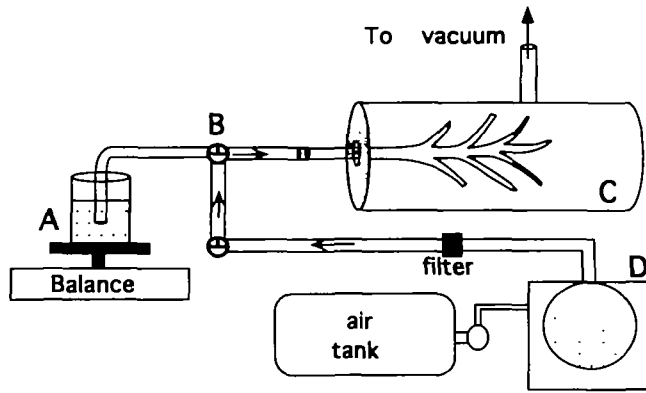
Fig. 1. The apparatus used for hydraulic conductance measurements. Solution is supplied to the root or shoot from a reservoir (A) located on a balance (which was interfaced with a computer). With the exception of the proximal portion, the plant material is enclosed within the canister (C) and vacuum pressure applied. A three-way valve (B) controls the supply of solution either from the reservoir or from a pressurized source (D). This source contains solution in a bladder surrounded by compressed air.

cap and at the other by a removable Plexiglas lid. The lid had an opening for the proximal end of the sample to protrude. An air-tight seal between the stem and the Plexiglas lid was achieved by fitting a rubber stopper around the stem, and inserting the stopper into the opening. This system allows for different plant sizes to be used with the same apparatus. The plant was connected by plastic tubing to a reservoir of HCl solution on a balance (Sartorius A200S or BA210S; tenth of a milligram resolution with RS232 interface).

After the canister was sealed, a partial vacuum in the canister pulled water through the xylem from the reservoir on the balance (Fig. 1). The surface of the water in this reservoir was below the vacuum canister to ensure no gravity-induced flow into the shoot. Vacuum pressure relative to atmospheric was measured with a test gauge (PGT-60L-[30V/30], Omega). The pressure difference across the shoot was calculated as the vacuum pressure minus the gravity-induced pressure caused by having the canister above the surface of the balance reservoir. For shoots, the vacuum-induced xylem flow was in the same direction as during transpiration. In the roots, however, the flow was opposite of normal. However, no directional effect on hydraulic conductance has been observed in xylem (Sperry *et al.*, 1988). The flow rate at a given vacuum pressure was measured automatically via the computer link with the balance; the computer program also calculated hydraulic conductance from flow and pressure data (program available on request).

Principle and protocol of measurement

Measuring hydraulic conductance on relatively large samples, as opposed to short stem or root segments, required a modification in the calculation of hydraulic conductance from that used by Sperry *et al.* (1988). Hydraulic conductance (*k*) of a flow path is defined as follows:

$$k = \Delta V / \Delta P$$

where ΔV is the change in volume flow rate through the pathway and ΔP is the change in pressure difference across the pathway. Alternatively, if hydraulic conductivity is calculated, a pressure gradient (*P*/length) is substituted for *P*. Measurements have confirmed that *k* of non-embolized xylem is constant over a wide range of pressure (Sperry and Tyree, 1990). In the

established method of Sperry *et al.* (1988), *k* is determined from *V* measured at a single *P*. This is justified because the linear relationship between *P* and *V* has a zero intercept for the usually short (50–200 mm) stem or root segments used in that technique.

However, it was found that the *V* intercept of the *P* versus *V* relationship was often greater than zero for branch and root systems. Furthermore, it changed with different measurements (Fig. 2). Therefore, the hydraulic conductance was calculated as the slope of the *P* versus *V* relationship. In practice, *V* was measured at five or more *P* values and *k* determined from a linear regression (Fig. 2). It did not matter if high or low vacuum pressures were used initially. If one or more values appeared anomalous (i.e. off the line), they were remeasured.

Following the initial measurement of hydraulic conductance, the plant was flushed with HCl solution supplied from a pressurized captive air tank at 100 kPa for 20 min to remove any air emboli. The period of time required to refill embolized conduits was determined by repeated short flushes and remeasurement of hydraulic conductance until no further increase was noted. This is the same procedure for emboli removal used in the method of Sperry *et al.* (1988). The extent of cavitation was quantified as the percentage the initial conductance was below the final maximum ('% embolism').

Between the end of a flush and the subsequent hydraulic conductance measurement, the plant was resupplied with solution from the balance reservoir and the tubing that was pressurized during the flush was allowed to relax for 10 min to its pre-pressurized state. The time required for the tubing to return to its initial volume after pressurizing was minimized by using rigid tubing where possible and minimizing the length of flexible tubing in the system.

The fact that this method measures hydraulic conductances on branched systems with distal components present in parallel and each with different pathlengths meant that the flow rate per pressure gradient (pressure difference per unit length) could not conveniently be expressed and the hydraulic conductivity calculated. When desired, measurements were standardized for the size of the shoot or root system by dividing its hydraulic
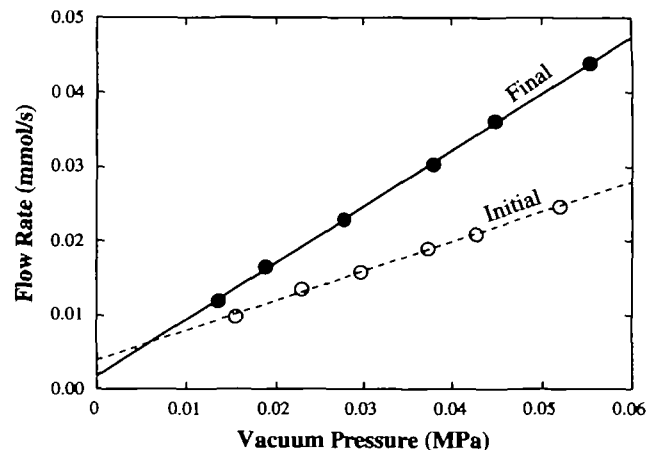

Fig. 2. Representative plot of vacuum pressure and the corresponding flow rate measured on a root system of *Artemisia tridentata*. Each point is a measured flow rate for a given vacuum pressure. The dashed line represents the measurements taken prior to emboli removal, and the solid line represents the measurements after flushing with solution at 100 kPa. The slope is equal to the hydraulic conductance (k_i 0.398 mmol MPa^{-1} s^{-1} and k_f=0.761 mmol MPa^{-1} s^{-1}). The root system shown here was 47.7% embolized.

conductance by the leaf area of the shoot. This gave the leaf-specific hydraulic conductance of the shoot or root system.

Evaluation and application of the method

The method was tested against the previous one of Sperry *et al.* (1988) by comparing hydraulic conductances of the same eight stem segments of *Betula occidentalis* obtained by both techniques. These unbranched stems were *c.* 0.3 m long and had hydraulic conductances above 5 mmol s^{-1} MPa^{-1}.

As an example of the application of the method, it was used to generate a 'vulnerability curve' for branch systems of *A. tridentata*. This is the relationship between the percentage decrease in hydraulic conductance versus xylem pressure. A total of 25 shoots were sampled from 25 mature plants (*c.* 1 shoot/plant) in a single population growing near Tintic Junction, Utah. Measurements were made in the spring when native embolism values were at their minimum, and midday xylem pressures were above -2 MPa (measured with a pressure chamber). Shoots were dried to various xylem pressures and then bagged for a minimum of 12 h to allow pressure equilibration. Pressures were remeasured, and percentage embolism determined as described above.

Results

Figure 2 is a representative plot of the vacuum pressure–flow relationship before and after the removal of emboli. The measurements shown in this figure were taken on a root system of an *A. tridentata* seedling growing in the greenhouse. The initial hydraulic conductance (k_i) was 0.398 mmol MPa^{-1} s^{-1}, and after removal of the emboli the hydraulic conductance (k_f) was 0.761 mmol MPa^{-1} s^{-1}. These values are substantially below the 2 mmol s^{-1} MPa^{-1} resolution of the original method. The difference between the initial and final measurements indicates that initial native hydraulic conductance was 47.7% lower than the maximum because of reversible embolism.

In material that was close to being completely embolized, the slope of the flow versus pressure relationship was occasionally observed to decrease with increasing vacuum pressure. After embolism was removed, the flow versus pressure relationship became linear as usual. Although k_i could be calculated in the usual fashion, it was only an approximate value because of the non-linearity of the data. Non-linearity may have resulted from changes in gas volume within embolized conduits with pressure. When xylem pressures rise to within a few kilopascals of atmospheric, the gas volume in the conduit decreases and water fills a portion of the embolized conduit (Tyree and Yang, 1990; see also micrographs of embolized vessels in Sperry, 1986; Sperry *et al.*, 1988). Under these conditions, dye experiments have demonstrated that limited water (or dye) will move through embolized vessels (Sperry, 1986). If a portion of the flow measured was occurring through embolized conduits, the expansion of bubbles as vacuum pressure was increased may have resulted in a corresponding drop in the

hydraulic conductance. Although this problem was seldom observed, it does represent a potential difficulty for applying the method to severely water-stressed plants that are extensively (i.e. $>90\%$) embolized.

As seen in Fig. 2, the flow rate intercepts of the initial and final pressure-flow regressions were non-zero and different. It was typical for the initial measurement to have a positive intercept, and measurements made after flushing to have a near zero intercept. Three causes of the initial non-zero intercept were suspected: osmotic uptake by the symplast, gradual dissolution of air emboli in xylem conduits, and capillary uptake of water in intercellular spaces (Zimmermann, 1983). In any case, flushing the xylem with solution would eliminate or reduce the intercept. Although transpiration within the canister was a possible cause of a positive intercept, uptake persisted even when the shoot was wrapped in damp cloth and bagged in plastic.

Hydraulic conductances of birch branch segments measured with vacuum versus gravity-induced pressure were statistically identical ($P > 0.08$, $n = 8$). This indicated that, at least for material suited to both techniques, the methods gave the same results.

The vulnerability curve for branches of adult *A. tridentata* at the Tintic Junction site is shown in Fig. 3. As noted, the data were collected during the spring when xylem pressures were above -2 MPa. Native embolism at this time averaged less than 20%. Embolism increased from this percentage to above 90% as shoot xylem pressure dropped to -8 MPa. The minimum leaf water potentials these plants experienced at this site over a two-
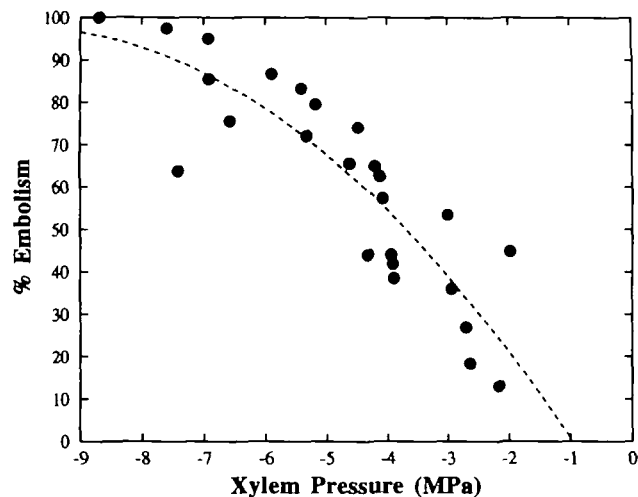

Fig. 3. Loss of hydraulic conductance versus xylem pressure (vulnerability curve) for branches of *Artemisia tridentata*. Each point represents measurements for an individual branch system. As xylem pressures became more negative the percentage loss of hydraulic conductance from embolism increased. *A. tridentata* shoots become 100% embolized at xylem pressures below -7 MPa. At the site where these branches were collected the minimum leaf water potential measured over two growing seasons was -4 MPa (Kolb, unpublished results).

year period was −4 MPa, corresponding to a predicted native embolism value of *c*. 60% (Kolb, unpublished data). Notably, the minimum *in situ* leaf water potential reported for *A. tridentata* was −6 MPa (Evans and Black, 1993), low enough to eliminate over 80% of the hydraulic conductance in the shoot xylem according to Fig. 3. These observations suggest that cavitation is a fairly common occurrence in *A. tridentata*.

Discussion

The results suggest that our modification of the original technique for measuring hydraulic conductance and xylem embolism has successfully extended it to densely-branched and/or low conductance root and shoot systems such as are found in *A. tridentata* seedlings and adults. Initial results suggest cavitation is an important component of the water relations of this shrub.

It was somewhat surprising that the root system of a greenhouse-grown seedling would show a 48% reduction in xylem conductance from cavitation (Fig. 2). Assuming the root xylem had the same vulnerability curve as the adult shoot xylem, this would mean xylem pressures would have had to have reached roughly −3.5 MPa (Fig. 3). Typical pre-dawn xylem pressures in these seedlings were only −1 MPa. While it was possible that the roots of this seedling may have experienced lower pressures, it is perhaps more likely that root xylem in this species is more vulnerable than stem xylem. Similar patterns have been seen in root xylem of tree species (Alder *et al.*, 1996; Sperry and Saliendra, 1994). In principle, it should be possible to test this possibility by using the method to determine a root vulnerability curve.

It addition to *A. tridentata*, the method has also been used successfully with seedlings of *Betula occidentalis* (Saliendra *et al.*, 1995) and *Adenostoma fasciculatum* Hook and Arn. (Williams *et al.*, 1997). In the latter instance, seedling mortality during drought was associated with a more than 80% decrease in hydraulic conductance. Similar investigations are underway on seedlings of *Hakea* species (B Lamont, unpublished data).

The modified method also improves on the original by providing an integrated measure of hydraulic conductance and embolism for nearly entire root and shoot systems. It thus gives a better approximation of the total xylem conductance and its influence on leaf water status during transpiration (Saliendra *et al.*, 1995). Shorter stem or root segments are of limited utility for this purpose because they represent only a fraction of the total xylem conductance.

A similar method has been used by Tyree and colleagues (Yang and Tyree, 1993; Tyree *et al.*, 1994) for measuring intact root and shoot hydraulic conductances. In this method, rather than pulling water through the xylem, it is pushed through under high (0–16 MPa) pressure for extended periods of time (hours) while the flow rate is measured with a high-pressure flow meter at the inlet to the stem or root system. While this technique is useful for measuring maximum hydraulic conductances (especially for *in situ* root systems), it can not be used to measure embolism in the xylem because the pressures used are sufficient to refill any embolized conduits in the system. For this reason, it may over-estimate native conductances in water-stressed material where a significant amount of xylem is embolized.

Acknowledgements

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