

Use of Positive Pressures to Establish Vulnerability Curves¹

Further Support for the Air-Seeding Hypothesis and Implications for Pressure-Volume Analysis

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ABSTRACT

Loss of hydraulic conductivity occurs in stems when the water in xylem conduits is subjected to sufficiently negative pressure. According to the air-seeding hypothesis, this loss of conductivity occurs when air bubbles are sucked into water-filled conduits through micropores adjacent to air spaces in the stem. Results in this study showed that loss of hydraulic conductivity occurred in stem segments pressurized in a pressure chamber while the xylem water was under positive pressure. Vulnerability curves can be defined as a plot of percentage loss of hydraulic conductivity versus the pressure difference between xylem water and the outside air inducing the loss of conductivity. Vulnerability curves were similar whether loss of conductivity was induced by lowering the xylem water pressure or by raising the external air pressure. These results are consistent with the air-seeding hypothesis of how embolisms are nucleated, but not with the nucleation of embolisms at hydrophobic cracks because the latter requires negative xylem water pressure. The results also call into question some basic underlying assumptions used in the determination of components of tissue water potential using "pressure-volume" analysis.

Long distance transport of water from the soil through the plant occurs via xylem conduits (vessels or tracheids). Since the introduction of the cohesion theory of ascent of sap in plants (6) it has been recognized that water in conduits is under tension (negative or subatmospheric pressures, typically of -1 to -3 MPa). This means that water must remain liquid at pressure well below its vapor pressure. In this metastable state, vaporization or cavitation can occur in plants subjected to even moderately low negative pressure associated with mild water stress (21). Immediately after cavitation, the xylem conduit begins to embolize, i.e. fill with atmospheric gases, which diffuse from the surrounding tissue and come out of solution from the xylem sap into the water vapor void. The main consequence of embolism is the reduction of the hydraulic conductivity of the xylem conduits.

Methods now exist to quantify the effect of embolism on

hydraulic conductivity (10, 11, 13, 16). In essence, these methods consist of measuring, under steady-state conditions, the flux of water perfused under moderate pressure differences (2–6 kPa) across an isolated stem segment that has been subjected to a given degree of water stress. This conductivity is then expressed as a percentage of the maximum conductivity obtained after removal of emboli by "flushing" water at high-pressure difference (100–150 kPa) through the same sample. By repeating this procedure with different samples at different Ψ^2 , a "vulnerability curve" (VC) can be established. A VC expresses the percentage of loss of hydraulic conductivity versus the minimum Ψ experienced by the stems.

So far, these VCs have been obtained either from excised branches dehydrated on the laboratory bench (12, 14, 16) or from whole tree seedlings grown and dehydrated in pots (15). Theoretically, a pressure chamber can be used to induce embolism without xylem tension if, as many studies have shown (5, 12–14), the "air seeding" hypothesis is correct. According to the hypothesis, the "positive pressure needed to blow air through the largest water-filled pores should be the same in magnitude but opposite in sign to that needed to cause embolism [during drought stress]" (21). However, the question arises as to whether cavitation is induced only during the release of pressure when xylem tension arises or also during the compression phase when there is no xylem tension.

This paper has a double purpose: (a) to test the possibility of measuring VCs from samples dehydrated to a given Ψ within a pressure chamber, and (b) to test the requirement of tension for observing decreased hydraulic conductivity by embolisms.

MATERIALS AND METHODS

Current-year branches of willow (*Salix alba* L.) and eastern cottonwood (*Populus deltoides* Bartr. ex Marsch.) <6 mm

² Abbreviations: Ψ , water potential; F , water flux (kg s^{-1}); K , hydraulic conductivity ($\text{kg m s}^{-1} \text{MPa}^{-1}$); PLC, percentage loss of hydraulic conductivity; PV, pressure-volume; VC, vulnerability curve; ΔP , applied pressure difference across a stem segment (MPa).

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diameter were excised from field-grown trees in September and recut under water and allowed to rehydrate for >1 h. Enough stem was cut from the base of the branches to ensure that the study samples included no air bubbles sucked into the stems after the initial cut. VCs were established by three different methods:

(I) The traditional method in which excised branches are dehydrated on a laboratory bench.

(II) A method in which the pressure chamber is used to dehydrate branches to desired Ψ s. Branches were enclosed in a pressure chamber with the cut end protruding outside the chamber. The pressure was increased at a rate of $<5 \text{ kPa s}^{-1}$ until reaching a pressure ranging from 0.75 to 2.75 MPa and maintained at the desired constant pressure until sap no longer came out (which means the branch had reached a balance pressure). Pressure was then released at $<2 \text{ kPa s}^{-1}$ to atmospheric pressure. Dehydrated branches were kept overnight in an air-tight bag (to prevent further dehydration) and embolism measured via its effect on loss of hydraulic conductivity on 2 to 3 cm stem segments excised from the main axis under water.

(III) The last method, which utilized the pressure chamber in a different way. The aim here was to progressively compress a branch, as in the previous method, but without producing tension in the xylem conduits while releasing the pressure. One-year-old branches, about 80 cm long, were excised under water, bent, and enclosed in a 20-cm diameter pressure chamber with both ends protruding out of the chamber (Fig. 1). Willow and eastern cottonwood branches were flexible enough to be bent as in Figure 1 without breaking. One cut end was connected to a 1.65-m water-filled glass tube large enough in diameter to allow the free escape of the air bubbles coming out of the cut end. This system enabled the continuous measurement of water flux

through the branch segment while the air pressure inside the chamber was gradually increased. The water flux was measured gravimetrically by collecting, over a period of 3 to 5 min, the water coming out of the free end onto a dry, preweighed, cotton collector enclosed in a plastic tube. The initial flux was measured at atmospheric pressure, and then the pressure was increased step-wise by increments up to a maximum pressure of 2 MPa. At each pressure, the flux was monitored until a constant flux was attained (usually after 1–3 h). After this compression phase, the free end of the branch was connected to a 5-cm long water-filled tube and the pressure was released slowly ($<2 \text{ kPa s}^{-1}$) in a step-wise fashion in steps of 0.3 to 0.8 MPa. The 5-cm water-filled tube permitted the rehydration of the branch from both sides while minimizing the development of xylem tension during the decompression. At the end of each step-wise decrease, the water flux was again measured through the branch segment. By repeating this procedure, the pressure within the chamber could be reduced to atmospheric pressure without developing tensions in the branch. Hydraulic conductivity, K , was calculated from flux, F , and pressure drop, ΔP , from $K = F/\Delta P$. The extent of embolism in the xylem was calculated using the maximum K , K_{max} , which usually occurred when the branch was initially placed in the pressure chamber or after the first pressure increase in the chamber. At progressively higher pressures, K decreased. The percentage loss of hydraulic conductivity, PLC , was calculated from

$$PLC = 100(K_{\text{max}} - K)/K_{\text{max}}$$

At the end of each pressure-chamber experiment, the extent of embolism in the xylem was measured via its effect on loss of hydraulic conductivity (11) on 2- to 3-cm long samples excised under water from the main axis cut at least 15 cm from each end.

RESULTS

In Figure 2 we show the VCs obtained by all three methods on eastern cottonwood. The open circles are the vulnerability data obtained by air dehydration on excised branches (method I, reproduced from ref. 15). The open squares were obtained by air dehydration of rooted cuttings in pots (reproduced from ref. 15). Open inverted triangles were obtained by pressure-chamber dehydration in this study by method II (pressure-chamber dehydration with xylem tension following pressure release); error bars are sds of means of five to six samples. The solid circles connected by straight lines were obtained on one branch by method III (pressure-chamber dehydration without xylem tension following pressure release); the direction of the arrows indicate the progression of pressure increase and decrease.

In Figure 3 we show the VCs obtained by all three methods on willow. Data are presented as means and sd of 5 to 10 samples. Open circles were obtained by method I. Open inverted triangles were obtained by method II. Closed symbols connected by straight lines were obtained by method III on three excised branches.

In all experiments by method III, air continuously flowed from both ends of the stem segments whenever the bomb

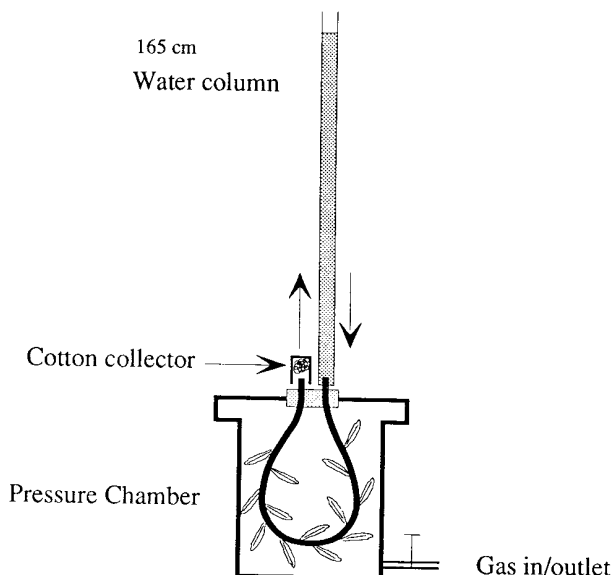


Figure 1. Diagram of equipment used to induce xylem embolism without developing xylem tension. See text for details.

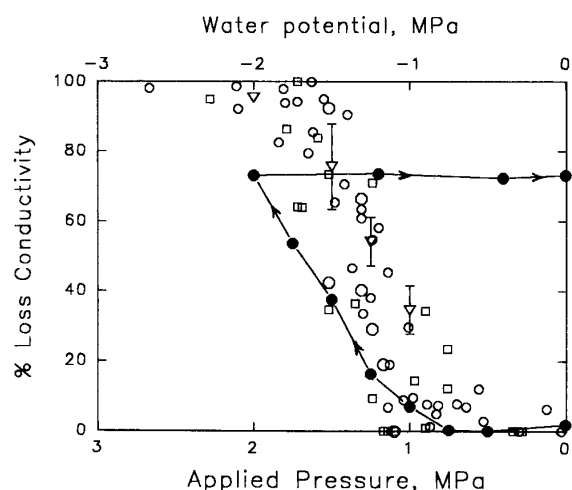


Figure 2. Vulnerability curves of cottonwood obtained by three different methods. The open circles are the vulnerability data obtained by air dehydration on excised branches (method I; ref. 13). The open squares were obtained from air dehydration of rooted cuttings dehydrated in pots (reproduced from ref. 13). The open triangles were obtained by pressure-chamber dehydration in this study by method II (pressure-chamber dehydration with xylem tension following pressure release). The solid circles connected by straight lines were obtained on one branch by method III (pressure-chamber dehydration without xylem tension following pressure release); the direction of the arrows indicate the progression of pressure increase and decrease. See text for details on the methods.

pressure was above 0.1 MPa, and the rate of air flow increased with air pressure in the bomb. This indicated that there were pathways for air to enter stem tissue. Whenever air pressure exceeded about 1 MPa, *K* decreased in the stems; this shows that air can enter vessels from the bomb while the pressure of the xylem fluid is positive (above atmospheric).

These two riparian trees are very vulnerable species. Embolism significantly developed when water stress in the xylem was less than approximately -1 MPa (or when applied pressure in methods II or III was above 1 MPa), and branches were fully embolized at Ψ values of -2 MPa. Branches treated as described for method III always exhibited the same water flux pattern. During the compression phase, *PLC* was nearly constant and near zero until the applied pressure reached a threshold value around 1 MPa. Then any increase of pressure in the pressure chamber produced significant decrease of *K*. When pressure was released, no further loss of *K* was observed. Thus, loss of *K* was induced only during the compression phase of the experiment. The embolism extent, measured on 2- to 3-cm segments excised from the central part of these branches, was comparable to the overall loss of conductivity (data not shown). Consequently, the loss of *K* was due to dissolvable embolism induced in intact vessels rather than a more permanent blockage. When compared with methods I and II, method III produced similar results for both species, although cottonwood's VC obtained by method III tended to

be shifted left on the graph (i.e. was a less vulnerable profile).

DISCUSSION

There are now known to be three species of trees that are extremely vulnerable to loss of stem conductivity by cavitation events: *P. deltoides* (this study and ref. 15), *S. alba* (this study), and *Schefflera morototoni* (Aublet) Maguire, Steyermark, Frodin (20). All three species start to cavitate at -1.0 MPa and have lost all conductivity in their stems or petioles by -2.0 MPa. Because many other species of trees routinely experience Ψ s of -1.5 to -4 MPa, this suggests that drought resistance in the above species may be determined in part by their vulnerability to cavitation. We would not be surprised to find many more species as vulnerable as those listed above. The role of cavitation in drought resistance and habitat preference of trees deserves more study.

Comparison between VCs obtained from branches dehydrated by pressure (method II) or by air (method I) gave similar results. One of us (H.C.) already obtained such VCs using method II with conifers and both diffuse and ring-porous hardwood species (2, 3) and came to the same conclusions. Therefore, we think that this method is appropriate to establish VCs for a large variety of woody species. This method has the advantage over others of being quicker and providing the possibility to dehydrate branches accurately to given values regularly spaced over the experimental range of pressures. One can expect a good agreement between method III and the other two methods only if the native level of embolism in experimental branches is very low. In other words, the branch should not have experienced Ψ s that could have induced xylem cavitation. If this is the case, the applied pressure in the pressure chamber has to be higher to induce any detectable loss of *K*. This would lead to an underesti-

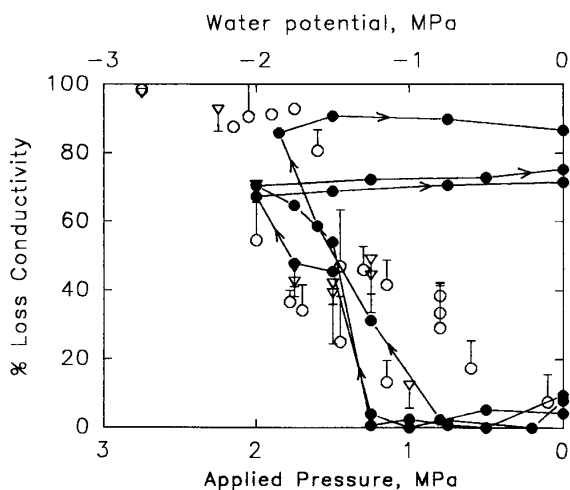


Figure 3. Same as Figure 2 for willow. Open circles were obtained by method I; open triangles were obtained by method II; closed symbols connected by straight lines were obtained by method III on three excised branches.

mation of the vulnerability of the branch. A native embolism of about 20% is enough to explain the discrepancy between methods III and I for cottonwood (Fig. 2). With this restriction, this method has the advantage over the others of enabling the establishment of an entire VC on a single branch without the necessity of resaturating the xylem to estimate the extent of embolism.

Our results have shown that xylem tension is not needed to induce loss of stem conductivity. In method III, conductivity is lost while xylem fluid is at or slightly above atmospheric pressure. Embolism was introduced into the vessels when the air-water menisci were pushed into the vessels by positive pressure. In method I (the traditional method), embolism was induced by xylem tension (negative xylem pressure); in this case, according to the air-seeding hypothesis (17, 21, 22), emboli were sucked into the vessels through micropores in the pit membranes separating embolized from water-filled vessels. Because the VCs were similar for methods I, II, and III, we feel that it is unlikely that many emboli were induced in willow and eastern cottonwood by homogeneous nucleation or by nucleation at hydrophobic cracks (9, 17). This follows because homogeneous nucleation and nucleation at hydrophobic cracks require xylem tension.

This work also has potentially serious implications for the underlying assumption in PV analysis (18, 19). The pressure chamber is frequently used to measure the dehydration isotherm of leaves and shoots, from which information can be obtained about the components of tissue Ψ , e.g. turgor potential, solute potential, cell wall elastic modulus, symplasmic and apoplastic water contents. A necessary underlying assumption is that apoplastic water content is constant during the dehydration of leaves or shoots. Because water in xylem conduits contributes to apoplastic water content, and because hydraulic conductivity can change in our experiments only if xylem water content is replaced by air, it follows that the basic assumption that apoplastic water content is constant during the dehydration of leaves and shoots is false.

Already, several authors (1, 4, 7) using different approaches came to the conclusion that the apoplastic water fraction decreases with dehydration during the generation of PV curves. However, cavitation is not the only factor involved in that change, and, furthermore, its exact role and importance is still a matter of discussion (7, 8). The importance of the error will depend on several factors, among them: the relative sizes of the apoplastic volume compared with the symplastic volume, the possibility that nonconducting dead wood fibers may also embolize during dehydration isotherms of plant tissue, and, obviously, the VC of the sample. If the apoplastic volume is large and the xylem very vulnerable, then pressure-induced embolism could introduce serious deviations. This error will be most in PV curves measured on whole shoots where apoplastic water content is one-third or more of the total water content.

Embolisms in minor leaf veins could also block water flow from certain regions of a leaf blade, thus "removing" (by hydraulic isolation) progressively more symplastic water volume from the system in the course of the dehydration of the leaf. Xylem embolism naturally occurs in air-dried branches, but we have demonstrated that this was also the case for the pressure-chamber technique, so only minor differences be-

tween these methods are to be expected. More work needs to be done to determine how much error is introduced into the estimation of the other components of tissue given the ability of the pressure chamber to displace apoplastic water and to isolate some symplastic volume by embolism of interconnecting vessels during the generation of PV curves. A complete reevaluation of the pressure-chamber technique is advisable in light of our results.

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