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ORIGINAL PAPER

Improvement to the air-injection technique to estimate xylem vulnerability to cavitation

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Abstract Several techniques have been developed to quantify the degree of embolism of the xylem using hydraulic conductance. Although there have been several improvements to these techniques, their reliability is still questionable and many technical pitfalls persist. We are proposing here a manometric approach to improve the accuracy of xylem cavitation measurement by the original air-injection technique which uses twigs exposed to pressurized air to cause cavitation. The measured parameter is air bubble production (P_b) caused by xylem cavitation in birch (*Betula pendula* Roth) twigs from which the percent increase in bubble production is calculated to quantify xylem cavitation. Data produced by three different methods (bench-drying, air-injection, and manometric approach) are compared. Xylem vulnerability curves (VCs) constructed by the reference and reliable bench-drying technique and the manometric approach show similar sigmoid "S" shape, but a small anomaly appeared in the VC constructed by the original air-injection technique. The xylem pressure inducing 50% of embolism (P_{50}) was the same with the three techniques. Furthermore, there was a strong positive correlation between the estimators of xylem cavitation measured by the three different methods. For its reliability, precision and ease we recommend the manometric technique as an improved version of the original hydraulic air-injection method.

Keywords Bubble production · Embolism · Manometric technique · Reference hydraulic technique · Vulnerability curves

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Introduction

The xylem is the main element conducting water in the plant, from the soil to the evapotranspiration sites. Structural and functional integrity of this tissue is crucial for maintaining plant productivity and survival (Brodribb and Cochard 2009). Because xylem sap is transported under tension, the risk of rupture of hydraulic continuum increases under drought conditions; such rupture leads to cavitation, i.e., the obstruction of xylem vessels following the introduction of air bubbles into the water column (Tyree and Zimmermann 2002). This phenomenon can cause substantial damage to the conductive system leading to the death of some parts or even the entire tree (Cochard and Granier 1999).

The functional significance of tree hydraulics has become increasingly clear and experimental evidence shows that these traits may affect leaf gas exchange (Cochard 2002; Hacke and Sperry 2001; Lemoine et al.

2002), tree growth (Cochard et al. 2007; Daudet et al. 2005; Tyree 2003), mortality (Brodribb and Cochard 2009), and species distribution (Ewers et al. 1997; Kursar et al. 2009). Cavitation resistance is now considered as one of the most significant physiological processes involved in plant drought resistance. It can be predicted that cavitation analysis will be more and more incorporated in ecological studies and, even, in breeding programs. Substantial variations in cavitation resistance have indeed been found between genotypes within the same species (Cochard et al. 2008; Ennajeh et al. 2008; Feng et al. 2008). Obviously, the chance of success of these investigations is partly determined by the reliability and the accuracy of the techniques used to detect cavitation.

The susceptibility of plant species to cavitation events, therefore to severe drought, can be estimated from their vulnerability curves (VCs) (Ennajeh et al. 2008; Hacke and Sperry 2001). These curves are plots of xylem pressures versus an estimator of the degree of cavitation in the xylem conduits. Several techniques are used to build VCs. The technique developed by Sperry et al. (1988) estimates the extent of embolism based on a measurement of the loss of hydraulic conductance in the xylem. This hydraulic technique is considered the most reliable and the VCs it produced are considered the "reference", but it is time consuming. VCs obtained with this technique have typically a sigmoidal shape. We will call 'S' this type of curves thereafter. For 'S' type curves, cavitation forms only when the xylem pressure drops below a critical threshold. This defines a 'safe' range of xylem pressures where cavitation does not occur or is very limited. This physiological range has been found to correspond closely to the range of pressures species typically experience in their natural habitats (Hacke and Sperry 2001). Therefore, efforts have been put in the development of more efficient methods for constructing VCs. Presently, two methods are frequently used to induce cavitation: centrifugation and air-injection. The centrifugation technique (Alder et al. 1997; Cochard 2002; Cochard et al. 2005; Pockman et al. 1995) uses the centrifugal force to lower the xylem pressure in the conduits of a cut shoot segment. Again, the variation of conductance with pressure yields a VC (Cochard et al. 2005). The air-injection technique (Cochard et al. 1992; Salleo et al. 1992) consists in injecting compressed air in a pressure sleeve covering a portion of a cut xylem segment, which forces air into the xylem conduits. The variation of tissue conductance with increasing air pressure yields a VC (Cochard et al. 1992).

The suitability of these ecophysiological approaches depends on the reliability of the technique used. No cavitation should be formed or dissolved during shoot sample collection or hydraulic conductance measurements, if not the value would be artifactual. Despite the intermittent improvement of all hydraulic techniques, practice-dependant anomalies persisted. These difficulties can be systemic pitfalls of the technique or are in relation with the plant species under study.

The present work is an attempt to perfect the hydraulic air-injection technique (Cochard et al. 1992) by using a manometric measurement method. We compared birch VCs obtained by the air-injection method with and without the proposed manometric improvement approach to a VC obtained by the reference bench-drying technique.

Materials and methods

Plant material

The experiments were performed on stems of birch (*Betula pendula* Roth), a diffuse–porous species with short xylem vessels (0.16 m SD = 0.04, $n = 6$), from the INRA-Crouël campus in Clermont-Ferrand (central France). One meter long shoots, longer than maximum vessel length, were cut in the morning, placed in black plastic bags and brought to the laboratory where they were analysed the same day.

Air-injection technique

First, the hydraulic conductivity (K) was determined by the air-injection method (Cochard et al. 1992). Non-branched terminal shoots less than 3 years old having the same length (approx. 0.37 m) were used. They were flushed with filtered (0.2 µm) perfusion solution (20 mM KCl and 1 mM $CaCl_2$) for more than 30 min to refill any xylem conduits that were embolized in situ (native embolism) or during collection. The stems were inserted through a double-ended pressure chamber. One cut end was connected to a 1.65-m tube large enough in diameter to allow the free escape of air bubbles coming out of the cut end. This system enables the continuous measurement of water flux through the branch segment while the air pressure inside the chamber was being gradually increased.

Flow through the segment was induced by a hydraulic head pushing the perfusion solution typically at 0.06 bar. The water flux was measured gravimetrically by collecting effluent from the distal end with pre-weighed cotton collector enclosed in a plastic tube over 1-min intervals.

Hydraulic conductivity (K) was calculated as the rate of mass flow divided by the pressure difference. The degree of embolism in the xylem was calculated using the maximum K, K_m, which corresponds to the initial hydraulic conductivity at 1 bar in the pressure chamber. After that, at progressively high pressures, increased by 5 bar intervals, K decreased. At each pressure level, 1-min measurements were continued until flow rate remained constant. Hydraulic

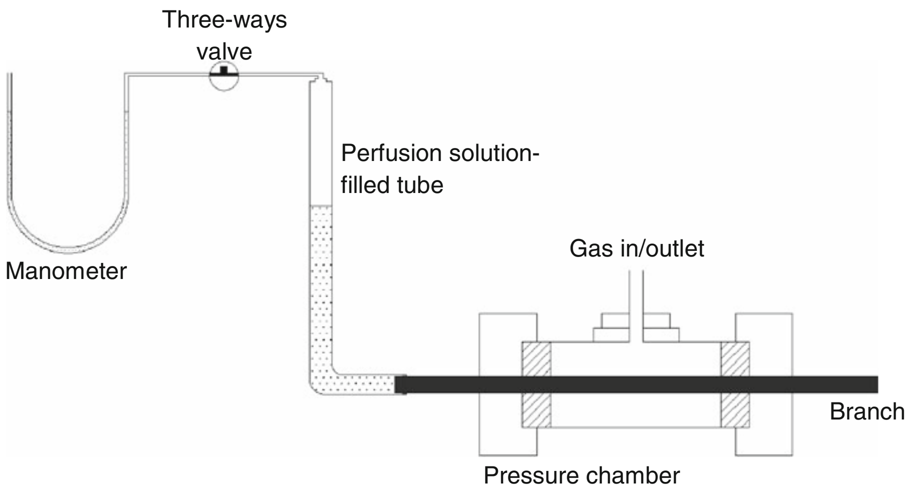

Fig. 1 Apparatus for measuring xylem vulnerability to cavitation. It is the same apparatus used in the case of the original air-injection technique (Cochard et al. 1992) with the addition of a manometer: U-tube containing colored manometric fluid. Bubbles produced at the proximal branch end during air pressurization were collected with an hermetic tube system installed at the end of the large perfusion solution-filled tube. A three-way valve was used to release accumulated air between measurements. The manometer was used to quantify air bubbles as they form

conductivity was the average of at least three 1-min readings during steady flow. The percentage loss of hydraulic conductivity, PLC, was calculated as:

$$\mathrm{PLC} = (1 - K/K_{\mathrm{m}}) \times 100$$

Manometric approach

A manometric approach based on the air-injection technique was used to characterize tree vulnerability to cavitation. Experiments were conducted on the same branches of birch used for the original air-injection technique. The manometric approach was inspired from the observation that air bubble production increased with increasing xylem vessel cavitation. Thus, air bubble production will be proportional to the number of cavitated xylem vessels.

The same measurement apparatus of the air-injection method was used with an addition of a small instrumental part essentially a manometer constructed by a thin glass U-tube containing colored manometric fluid (Fig. 1). This part allowed us to quantify the air bubbles coming out from the proximal shoot cut end inserted into the 1.65-m perfusion solution-filled tube. Air bubbles were recuperated at the end of the large tube with a hermetic canal system connected to the manometer through a three-way valve. Bubble production (P_{b}) at the proximal shoot-end was detected by displacement of manometric fluid in the thin glass U-tube. A direct reading gave the rate of change of P_{b}. P_{b} was determined at each applied pressure level in the double-ended pressure chamber. When a steady hydraulic flow through the distal shoot-end was reached and maintained for a few minutes, applied pressure in the chamber was relaxed to 1 bar. The system was left to equilibrate for at least 10 min. Then bubble production (P_{b}) was determined as the average of at least three 1-min readings on the manometer during the bubble steady production period. Maximum bubble production (P_{bm}) was measured for the corresponding applied pressure inducing total xylem embolism (PLC = 100%) (no water flow at the distal shoot-end). The percentage increase of bubble production, PIB, was calculated as:

$$\mathrm{PIB} = (1 - P_{\mathrm{b}}/P_{\mathrm{bm}}) \times 100$$

Xylem vulnerability curves

A reference VC based on the original bench-drying technique (Sperry et al. 1988) for *Betula pendula* was obtained in the past by the group of *Lab'eau* (Cochard et al. 2005). One meter long shoots were cut from birch tree early in the morning and dehydrated on a bench to obtain a range of xylem pressures from −0.5 to −3 MPa. Xylem pressure was measured with a pressure chamber on bagged leaves. The reference percent loss of xylem conductance (PLCr) due to embolism was measured on five 2-cm long shoot segments cut under water at regular intervals on the shoots as described before by Sperry et al. (1988). A total of 27 different shoots were used to construct this reference VC.

Generally, VCs were plots of xylem pressures versus an estimator of the degree of cavitation in the xylem conduits. Commonly, the most used estimator of cavitation is the degree of 'embolism'. It is, generally, quantified by measuring hydraulic conductivity, like the cases of the original bench-drying and air-injection techniques. In the case of the manometric approach, however, the estimator of cavitation is the increase of bubble production by the stem under pressure. Therefore, the experimental VCs were the evolution of PLC and PIB as a function of the positive applied pressure. Xylem pressure provoking 50% loss of hydraulic conductivity (PLC, PLCr) (P_{50}) and 50% increase in bubble production (PIB) were computed graphically.

Constructed VCs were compared and correlations between various xylem cavitation estimators as measured by the three methods (PLC, PLCr and PIB) were computed.

Results

Xylem vulnerability curves

Figure 2 shows the experimental VCs established with the air-injection technique and its improved manometric version and a reference VC constructed with the original bench-drying technique. Presumably, all curves should have an 'S' shape. However, there was a subtle departure from this form in the case of the original air-injection VC. All VCs showed that the degree of cavitation (PLCr, PLC and PIB) was low under moderate dehydration. When dehydration became severe, PLCr, PLC and PIB increased indicating a rise in xylem cavitation. However, this induction was more precocious in the case of the original air-injection method (1.2 MPa) compared with the two others techniques (1.4 MPa $< P$ or $P_{\text{xylem}} < -1.4$ MPa).

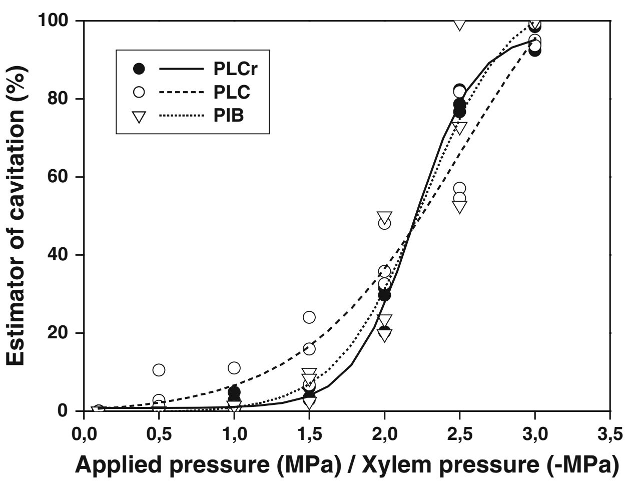

Fig. 2 A reference curve of xylem vulnerability to cavitation of birch species (*Betula pendula* Roth) (*circle black symbols* and *solid lines*) represented as the percentage loss of hydraulic conductivity (*PLCr*, determined by the original bench-drying technique) as a function of xylem pressure is superimposed onto two experimental vulnerability curves represented as the percentage loss of hydraulic conductivity (*PLC*, determined by the air-injection technique) (*circle open symbols* and *short dashed lines*) or the percentage increase of bubble production (*PIB*, determined by the manometric method) (*triangle open symbols* and *dotted lines*) as a function of applied pressure

Xylem pressure inducing 50% of cavitation (P_{50}) had, approximately, the same value (2.2 or −2.2 MPa) with different methods. Hence, the technique type had only a slight impact on the *Betula* VCs, essentially, on the incipient (P_{incp}) and the lethal (P_{leth}) xylem pressure, but not on the P_{50}.

Correlation between cavitation estimators

The superimposition of the three estimator parameters of cavitation demonstrate that they were highly correlated (Fig. 3). A strong positive correlation was found between the increase in PLCr and PLC ($R^2 = 0.98$; $P < 0.05$), PLCr and PIB ($R^2 = 0.99$; $P < 0.001$) and PLC and PIB values ($R^2 = 0.99$; $P < 0.001$).

Discussion

The manometric method produced VCs similar in shape to the one produced with the reference bench-drying technique. This finding brings insights on the reliability of the manometric improvement approach. Thus, the estimator of the degree of cavitation (PIB) as determined by the first method may be reliable and have physiological significance for the tree. P_{incp} and P_{leth} estimated from VC developed manometrically are, approximately, the same as those estimated from the reference VC, but are slightly different from those estimated using the original air-injection technique.

VCs based on the bench-drying technique (Sperry et al. 1988) are considered the reference according to several

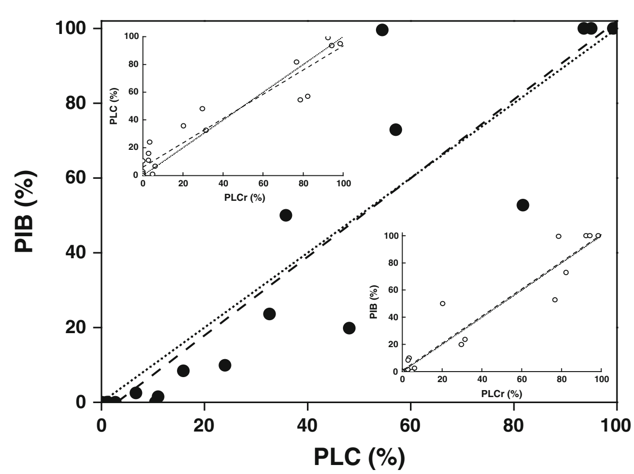

Fig. 3 Correlation between the air-injection percent loss of hydraulic conductivity (*PLC*) and the manometric percent increase of bubble production (*PIB*). The *insert graphs* show the correlation between PLC and PIB in one part and the reference bench-drying percent loss of hydraulic conductivity (*PLCr*) in the other part. The *short dotted lines* indicate the 1:1 line

plant hydraulics experts (Barigah et al. 2006; Cochard et al. 2005; Sperry and Saliendra 1994). The bench-drying technique has proved to be very robust and hydraulic VCs it gives are considered true physiological curves (Bréda et al. 1993; Cochard et al. 1996, 2002). However, it suffers from several practical pitfalls. It presents a risk of overestimating the extent of embolism in the case of ring-porous species (Cochard et al. 1997) as was reported for the Cavitron technique (Cochard et al. 2010; Li et al. 2008). There is also the possibility of accidental dissolution of embolism during sample installation in the hydraulic measurement system hence the risk of underestimating the occurrence of embolism. Indeed, it is easy to displace the air bubbles trapped in the open vessels during this phase. Also, an underestimation of maximal conductance may occur because of the contamination and the impurity of perfusion water (Sperry et al. 1988). Furthermore, the apparatus for measuring hydraulic conductance using this technique (bench-drying) is heavy and hard to use in the field.

The air-injection technique has its pitfalls too. An underestimation of water flow rate can occur if water evaporates from the cotton in the plastic tubing used to recuperate the perfusion solution. The cotton can also be dried by the air flux at the distal end of the twig at the time of water recuperation or when removing the tube for weighting. Xylem vessels (at the proximal end) can be blocked by air bubbles injected in the perfusion solution-filled tube (Sperry and Saliendra 1994). Also some xylem vessels can be closed by the impurities in the perfusion solution. Consequently, embolism may be overestimated by this classic air-injection technique.

The manometric approach can provide solutions to several practical difficulties encountered with the original air-injection technique. With the manometric approach, xylem vessels produce air bubbles only when they are damaged and subjected to cavitation; so there is no risk of overestimating embolism. Similarly, the risk of underestimating embolism due to accidental blockage of xylem vessels is low. Therefore, the manometric approach appears to be more precise than the classic air-injection method. Furthermore, the manometric approach is fast and easy to use. Indeed, water flux at the distal branch end stabilized slowly compared to air flux at the proximal end. This method does not require a precision balance making it easier to construct an apparatus for field measurements.

In conclusion, the high similarity between the VCs constructed using the manometric and the reference methods, the strong positive correlation between the three estimator parameters of cavitation and the numerous practical advantageous of the manometric approach led us to consider the latter technique very favorably. However, more measurements on others plant species will be necessary before recommending it for characterizing plant vulnerability to cavitation.

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