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TECHNICAL FOCUS

How reliable is the double-ended pressure sleeve technique for assessing xylem vulnerability to cavitation in woody angiosperms?

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The reliability of a double-ended pressure sleeve technique was evaluated on three woody angiosperm species with contrasting maximum vessel lengths. Vulnerability curves (VCs) were constructed by varying sample length and the size of the pressure sleeves. VCs were compared against curves obtained with reference techniques. For the two diffuse-porous species, *Betula pendula* and *Prunus persica*, VCs built with shoot segments shorter than maximum vessel length strongly overestimated species vulnerability. Furthermore, increasing the size of the pressure sleeve also tended to lead to overestimated VCs. For the ring-porous species *Quercus robur*, the technique strongly overestimated vulnerability to embolism, whatever the sample length or chamber tested. In conclusion, the double-ended pressure sleeve technique only gives reliable VCs on diffuse-porous angiosperms with short pressure sleeves, only when segments are longer than maximum vessel length.

Introduction

In plants, sap is transported under large negative pressures in specialized conduits that form the xylem tissue. Water is a metastable liquid when its pressure is negative but it can suddenly change to a more stable gaseous phase by cavitation. Cavitation provokes embolism, which means it breaks the integrity of the water columns in the xylem pipes and consequently impairs the mechanisms enabling sap to ascend from soil to leaves. Cavitation has major physiological consequences as it can eventually lead to shoot or tree mortality by desiccation (Brodribb and Cochard 2009), prompting a major research push over the last three decades attempting to characterize and understand xylem hydraulics in trees. These efforts have not yet turned toward herbaceous plants (Martre et al. 2000, 2001).

The very peculiar physical state of water in the xylem makes cavitation extremely difficult to observe and quantify. Many different techniques have been proposed for constructing xylem vulnerability curves (VCs). These techniques differ by the way cavitation is induced (air dehydration, air pressurization, centrifugation, etc.) and measured (direct observation, loss of conductance, acoustic emission, etc.). Some techniques are presumed reliable (e.g. the air dehydration and declining conductivity technique of Sperry et al. 1988) whereas many others have proven to be flawed. For instance, Canny (1997) observed the content of frozen xylem vessels under a cryo-SEM and suggested that xylem conduits were extremely vulnerable to cavitation. However, Cochard et al. (2000) showed that the technique was flawed by cavitation build-up during sample preparation, and that the vessels were actually far more resistant

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Abbreviation – VCs, vulnerability curves.

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to cavitation. More recently, Li et al. (2008) used a centrifuge technique to measure cavitation resistance in ring-porous species and found extremely vulnerable xylem conduits, but Cochard et al. (2010) and Choat et al. (2010) have since provided experimental evidence for bias in these centrifuge techniques which greatly overestimate the vulnerability of species with long vessels.

The good agreement between the centrifuge and air-injection techniques has been used as an argument to demonstrate the reliability of both techniques (Li et al. 2008). However, it is equally possible that both techniques are vitiated by the same artefact. The objective of our study was to provide a more extensive test of the reliability of the double-ended pressure sleeve technique (Cochard et al. 1992a, Salleo et al. 1992, Sperry and Saliendra 1994). We tested the hypothesis that sample vulnerability was biased by the presence of vessels cut open inside the pressure sleeve. Therefore, we analyzed VCs obtained with pressure sleeves of different sizes on stem samples of different lengths for three tree species presenting contrasting vessel lengths.

Materials and methods

Plant material

Experiments were conducted on different tree species from the INRA-Crouël campus in Clermont-Ferrand (central France). Three species with contrasting maximum vessel lengths were selected for this study. The air infiltration technique (Ewers and Fisher 1989) was used to measure maximum vessel lengths (L, m). Oak (*Quercus robur* L.), a ring-porous species, had very long vessels ($L = 1.34$ m, SD $= 0.38$, $n = 6$), while birch (*Betula pendula* Roth), a diffuse-porous species, had very short vessels ($L = 0.16$ m, SD $= 0.04$, $n = 6$) and peach (*Prunus persica* (L.) Batsch) offered vessels with intermediate length ($L = 0.42$ m, SD $= 5.8$, $n = 6$). Shoots longer than maximum vessel length were cut in the morning and brought to the laboratory where they were analyzed later the same day. Experiments were conducted on non-ramified terminal shoots less than 3 years old.

Vulnerability curves

The VCs were determined using the double-ended pressure sleeve method according to Cochard et al. (1992a) and Sperry and Saliendra (1994). The principle of the technique is to clamp a special pressure chamber on a branch segment, increase the air pressure in the chamber and measure the effect on branch conductance. Cochard et al. (1992a) demonstrated that for *Populus* and *Salix*, the dependence of xylem conductance on positive air pressure was similar to the dependence of xylem conductance on negative sap pressure. For the purpose of this study, we constructed three pressure sleeves of different lengths. The sleeves were made with 2.5-cm diameter steel tubes. Compression fittings were placed at both tube ends and rubber corks were used to seal the sleeves. The portion of the shoot segment in contact with compressed air inside the chambers was respectively 3.5, 17 and 27.5 cm.

Following Cochard et al. (1992a), hydraulic conductance of the sample was measured by connecting one sample end to vertical plastic tubing filled with a filtered solution (0.2 µm) of 20 m*M* KCl and 1 m*M* $CaCl_2$ in ultrapure water. The water pressure, P, in the tube was adjusted to 6 kPa. The plastic tube was large enough (2 cm diameter) to allow the air bubbles coming out of the cut end to escape freely during pressurization. This system enabled continuous measurement of water flux, F, through the branch segment while the air pressure inside the chamber was gradually increased. Water flow through the sample was measured gravimetrically by collecting the effluent from the distal end in a pre-weighed cotton collector enclosed in a plastic tube over 1-min intervals. Sample conductance, K, was calculated as: $K = F/P$. Before being inserted in the pressure chamber, samples were first flushed with the same solution for 30 min at 0.15 MPa to remove any embolism that may have formed during sample preparation. Preliminary experiments with *Betula* (see the section on Results) indicated that it was necessary to remove sample bark inside the pressure sleeve to induce embolism in the stem.

The VCs were constructed as follows. First, air pressure in the chamber was increased to 0.1 MPa and maximum sample conductance, K_{max}, was measured. Then, the pressure in the chamber was increased in 0.5 MPa steps, and sample conductance, K, was determined at steady state at each step. The loss of hydraulic conductance, PLC, at each pressure was calculated as:

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

Test experiments

To analyze the effect of sample length on VCs obtained using this double-ended pressure sleeve method, we constructed VCs with 17.5-, 27.5- and 37.5-cm long samples for each species. For *Prunus* and *Quercus*, we also ran a test with the 3.5-cm long pressure chamber using shoots longer than their maximum vessel length, i.e. 57.5 cm for *Prunus* and 150 cm for *Quercus*.

To analyze the effect of pressure chamber length, we measured 37.5-cm long shoots of all three species in all three different pressure chambers. For each experiment, we averaged the results of three to five replicates.

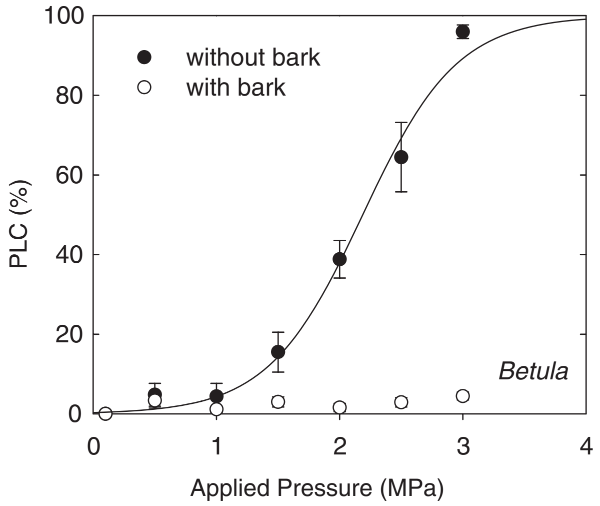

Fig. 1. Effect of bark removal on *Betula* xylem VCs obtained with the double-ended pressure sleeve technique. The wood should be in direct contact with compressed air in the chamber before embolism can be induced. Vertical bars = ± SE.

Reference VCs

Reference VCs using the bench-drying technique (Sperry et al. 1988) have previously been published for *Quercus* (Cochard et al. 1992b), *Betula* (Cochard et al. 2005) and *Prunus* (Cochard et al. 2010). The reference curves for *Betula* and *Prunus* were constructed with the same plant material as that used in this study.

Results

Increasing air pressure in the pressure sleeve decreased stem conductance in all species and all treatments. However, embolism could only be induced after previously removing bark from inside the chamber (Fig. 1).

Segment length influenced the shape of VCs, but the results contrasted strongly across species (Fig. 2). For *Betula*, a short-vessel species, the results were similar across treatments and in good agreement with the reference VC for this species. In contrast, for *Prunus* and *Quercus*, segments appeared less vulnerable at increasingly high lengths. For *Prunus*, VCs obtained with samples longer than maximum vessel length were in agreement with the reference VC, whereas for *Quercus*, the curves established with the longest segments (1.5 m) still strongly overestimated xylem vulnerability.

Length of the pressure chamber also influenced the shape of the VCs (Fig. 3). Again, the effects were small in *Betula* but more substantial in species with longer vessels, where increasing the length of the pressure chamber decreased sample vulnerability. For *Prunus*, VCs constructed with the shortest chamber were in close agreement with the reference VC. For *Quercus*, even the shortest chamber strongly overestimated the xylem vulnerability of this species.

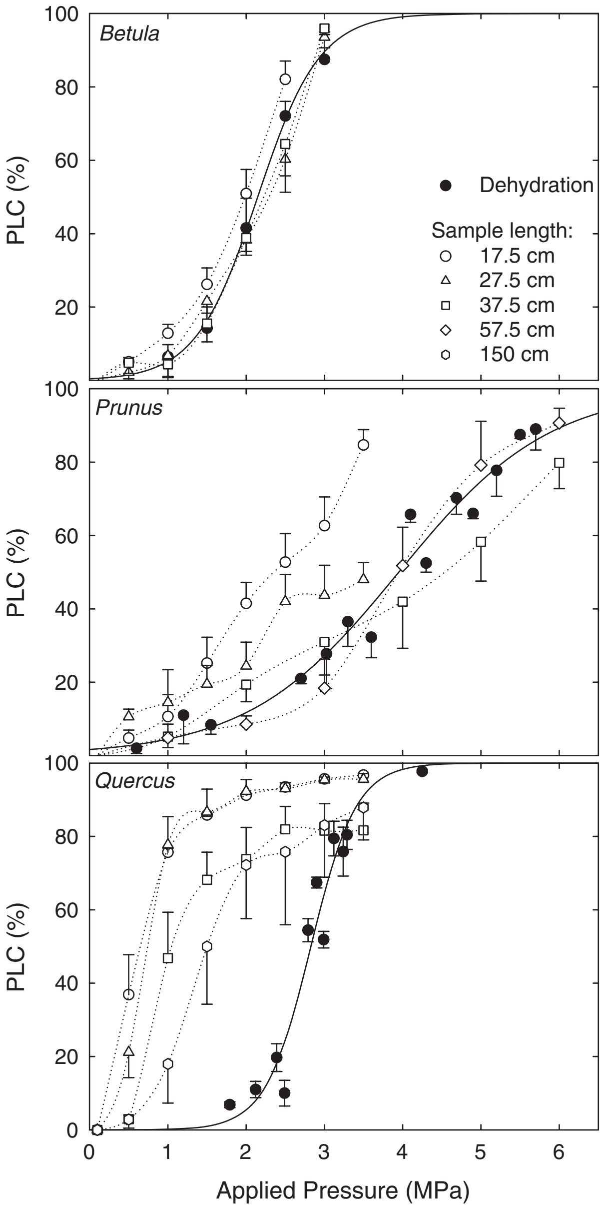

Fig. 2. Effects of sample length on xylem VCs obtained with the double-ended pressure sleeve technique (white symbols) for three woody species (different panels). The length of the pressure sleeve was constant (3.5 cm). VCs obtained with a reference technique are shown with closed symbols. Vertical bars = ± SE.

Discussion

The reliability of the techniques for measuring xylem vulnerability to cavitation has been the subject of much

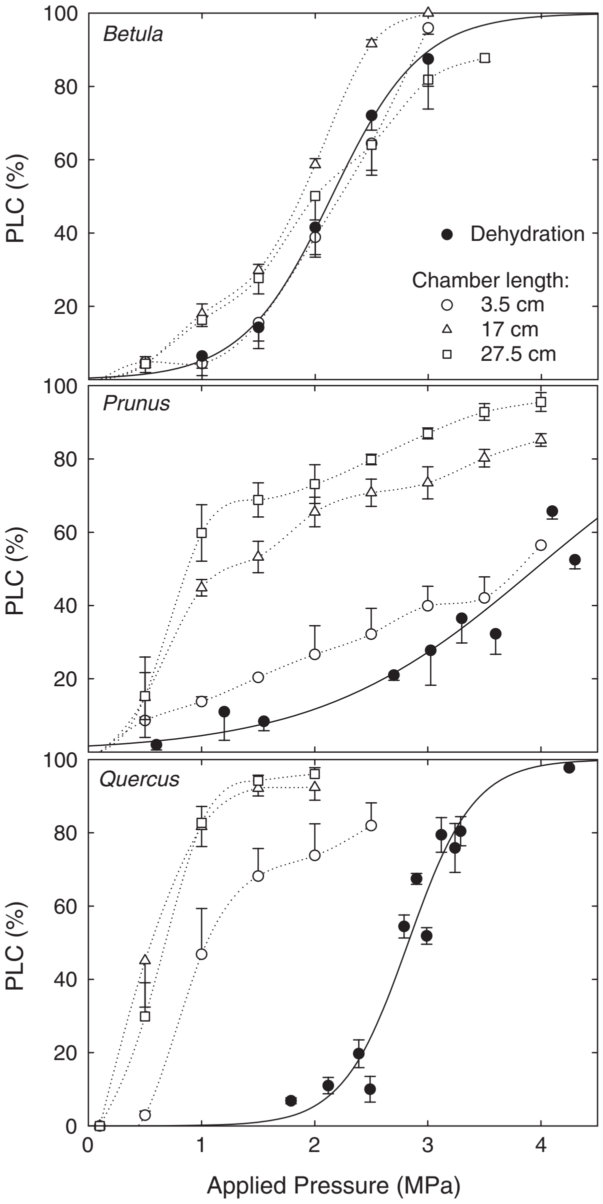

Fig. 3. Effects of pressure sleeve length on xylem VCs obtained with the double-ended pressure sleeve technique (white symbols) for three woody species (different panels). Sample length was constant across species and treatments (37.5 cm). VCs obtained with a reference technique are shown with closed symbols. Vertical bars = ± SE.

debate. A critical aspect in this discussion is to identify a reference methodology, i.e. a method capable of producing VCs similar to the dependence of xylem embolism on xylem pressure measured in situ and in planta. Such 'native' curves have been obtained rarely (Bréda et al. 1993, Cochard et al. 1996, Tyree et al. 1992) because of obvious experimental constraints. Dehydrating cut branches on a bench instead of working with whole plants in situ considerably speeds up the construction of VCs. It has been experimentally demonstrated that this procedure does not alter the reliability of the curves (Bréda et al. 1993, Cochard et al. 1996, Tyree et al. 1992), which also suggests that the faster rate at which plants are dehydrated ex situ has little impact on the cavitation process. Therefore, we can assume the bench-drying method to be reliable. However, this technique remains laborious and time consuming, which explains why other methodologies have been developed.

The double-ended pressure sleeve technique offers an attractive alternative as it enables VCs to be constructed quickly and with limited plant material, and has consequently become a routine technique for cavitation studies in many laboratories worldwide. In a recent literature survey, we estimated that one in four of all the VCs published to date were constructed based on this technique (Choat et al., unpublished data). Our study suggests that this technique has strong limitations and should probably be used more cautiously.

Methodological difficulties associated with air-injection techniques have already been reported in the past. For instance, Sperry and Saliendra (1994) recommended notching the xylem inside the chamber to provoke embolism in *Betula*. Our results on *Betula* confirm that the xylem has to be directly exposed to the pressurized air inside the chamber before cavitation can be induced. We did not test here whether different ways of exposing xylem conduits had an impact on VCs. This finding suggests that bark can form a relatively air-tight barrier isolating xylem conduits from air in the pressure chamber. In this situation, the air pressure at the contact with the xylem vessels equilibrates with the pressure in the chamber, with the result that the VCs are biased. The bark isolation problem appears to be specific to this pressure sleeve technique. Indeed, with bench dehydration or centrifugation techniques, the air pressure inside the xylem is at equilibrium with the atmospheric pressure, whatever the negative pressure in the xylem lumens. We do not know whether bark removal is an appropriate procedure for other species. This is a first point of concern that should be more carefully evaluated whenever this technique is employed.

The second point highlighted in this study is the strong influence of sample length and chamber length on VCs. As a rule, the longer the sample and the shorter the chamber, the closer the curves fit the reference VCs. However, the patterns differed strongly across species, and a dependence on xylem anatomy can be hypothesized. Choat et al. (2010) recently reported that in *Vitis*, a long-vessel species, VCs established on short segments greatly overestimated xylem vulnerability. However, working with segments as long as vessel length gave more reliable curves. Our data confirm this result, and further demonstrate that maximum vessel length is a key parameter for the reliability of the double-ended pressure sleeve technique. For species having very short vessels, like *Betula*, our curves were always in good agreement with the reference VCs, whatever the chamber or sample length. In contrast, all the VCs obtained on *Quercus* strongly overestimated the cavitation resistance of this ring-porous species. For *Prunus*, which has intermediate vessel length, the curves were only in agreement with the reference VC when segments were longer than vessel length, and only with the smallest chamber.

To account for the different effects of sample, chamber and vessel lengths on VCs, we computed the following index I:

$$I = (\text{sample length} - \text{chamber length})/(\text{vessel length})$$

Figure 4 shows how the ratios between sample P50 and reference P50 vary with I. The result of this very simple computation suggests that when (sample length − chamber length) is higher than maximum vessel length, the P50 converges toward the reference P50 value. This condition was sufficient for *Betula, Prunus* and *Vitis*, the species studied by Choat et al. (2010). However, for *Quercus*, this condition was not satisfactory as the P50 were still overestimated. This is possibly because of an underestimation of vessel length for this species, as the pressure chamber was mounted in the middle of the branch where vessels are probably much longer than in the terminal part of the shoot (Cochard and Tyree 1990). Clearly, VCs constructed with this technique using samples shorter than maximum vessel length can greatly overestimate species vulnerability to cavitation. The same conclusion was recently reached with an independent method base on centrifugal forces (Cochard et al. 2010). Therefore, a good agreement between the centrifugation and air-injection techniques does not provide a sound cross-validation of each of the two techniques. Further investigations are required in order to understand why vessels cut open are also experimentally more vulnerable to cavitation with the double-ended pressure sleeve technique.

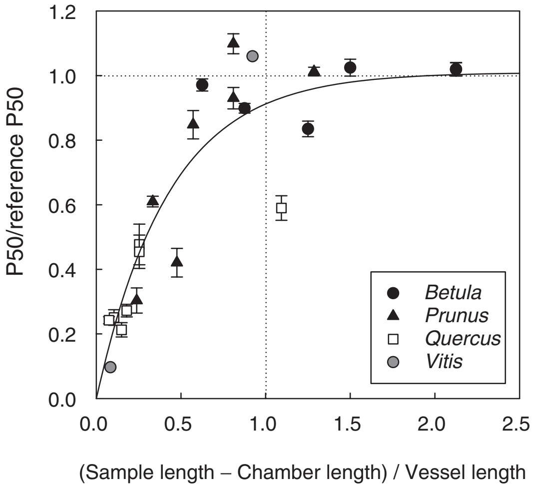

Fig. 4. Combined effect of sample length and pressure sleeve length on xylem pressure provoking a 50% loss of conductance (P50). The x-axis represents the ratio between sample length minus pressure sleeve length and the maximum vessel length of the species. The y-axis is the ratio between P50 and the P50 value of the reference curve for each species. The values for *Vitis* are estimated from the data presented in Choat et al. 2010. Vertical bars = ± SE.

In conclusion, VCs constructed with the double-ended pressure sleeve method can strongly overestimate xylem cavitation when samples are shorter than the species' maximum vessel length. The overestimation is further exacerbated with long pressure sleeves. Within these methodological restrictions, the double-ended pressure sleeve technique can give reliable results for conifers and diffuse-porous angiosperms but probably not with most of the ring-porous angiosperms. The apparent very high vulnerability of ring-porous species reported in recent publications based on these techniques should be reconsidered in light of the findings presented here and in Cochard et al. (2010).

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References

Bréda N, Cochard H, Dreyer E, Granier A (1993) Field comparison of transpiration, stomatal conductance and vulnerability to cavitation of *Quercus petraea* and *Quercus robur* under water stress. Ann Sci For 50: 571–582

Brodribb TJ, Cochard H (2009) Hydraulic failure defines the recovery and point of death in water-stressed conifers. Plant Physiol 149: 575–584

Canny MJ (1997) Vessel contents during transpiration: embolisms and refilling. Am J Bot 84: 1223–1230

Choat B, Drayton WD, Brodersen C, Matthews MA, Shackel KA, Wada H, McElrone AJ (2010) Measurement of vulnerability to water stress-induced cavitation in grapevine: a comparison of four techniques applied to a long-vesseled species. Plant Cell Environ 33: 1502–1512

Cochard H, Tyree MT (1990) Xylem dysfunction in *Quercus*: vessel sizes, tyloses, cavitation and seasonal changes in embolism. Tree Physiol 6: 393–407

Cochard H, Cruiziat P, Tyree MT (1992a) Use of positive pressures to establish vulnerability curves. Further support for the air-seeding hypothesis and implications for pressure-volume analysis. Plant Physiol 100: 205–209

Cochard H, Bréda N, Granier A, Aussenac G (1992b) Vulnerability to air embolism of three European oak species (*Quercus petraea* (Matt) Liebl, *Q. pubescens* Willd, *Q. robur* L). Ann Sci For 49: 225–233

Cochard H, Ridolfi M, Dreyer E (1996) Responses to water stress in an ABA-unresponsive hybrid poplar (*Populus koreana x trichocarpa* cv. Peace) II. Hydraulic properties and xylem embolism. New Phytol 134: 455–461

Cochard H, Bodet C, Ameglio T, Cruiziat P (2000) Cryo-scanning electron microscopy observations of vessel content during transpiration in walnut petioles. Facts or artifacts? Plant Physiol 124: 1191–1202

Cochard H, Damour G, Bodet C, Tharwat I, Poirier M, Améglio T (2005) Evaluation of a new centrifuge technique for rapid generation of xylem vulnerability curves. Physiol Plant 124: 410–418

Cochard H, Herbette S, Barigah T, Badel E, Ennajeh M, Vilagrosa A (2010) Does sample length influence the shape of xylem embolism vulnerability curves? A test with the Cavitron spinning technique. Plant Cell Environ 33: 1543–1552

Ewers FW, Fisher JB (1989) Techniques for measuring vessel lengths and diameters in stems of woody plants. Am J Bot 86: 1077–1081

Li Y, Sperry JS, Taneda H, Bush SE, Hacke UG (2008) Evaluation of centrifugal methods for measuring xylem cavitation in conifers, diffuse- and ring-porous angiosperms. New Phytol 177: 558–568

Martre P, Durand JL, Cochard H (2000) Changes in axial hydraulic conductivity along elongating leaf blades in relation to xylem maturation in tall fescue. New Phytol 146: 235–247

Martre P, Cochard H, Durand JL (2001) Hydraulic architecture and water flows in a growing grass till (*Festuca arundinacea* Schreb.). Plant Cell Environ 24: 65–76

Salleo S, Hinckley TM, Kikuta SB, Lo Gullo MA, Weilgony P, Yoon TM, Richter H (1992) A method for inducing xylem emboli *in situ*: experiments with a field-grown tree. Plant Cell Environ 15: 491–497

Sperry JS, Saliendra NZ (1994) Intra-plant and inter-plant variation in xylem cavitation in *Betula occidentalis*. Plant Cell Environ 17: 1233–1241

Sperry JS, Donnelly JR, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. Plant Cell Environ 11: 35–40

Tyree MT, Alexander J, Machado JL (1992) Loss of hydraulic conductivity due to water stress in intact juveniles of Quercus rubra and Populus deltoides. Tree Physiol 10: 411–415

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