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Deciphering 's'marts from 'r'ubbish VCs: going further the matter of letters?

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Introduction

- ❑ **Vulnerability curve (VC, Figure 1)**, the relationship between the percent loss conductivity (PLC) and the xylem potential, describes plant vulnerability to drought induced cavitation, a crucial trait that correlates with several plant functions and adaptative strategies.
- ❑ Several techniques have been developed to build VC, differing in the way embolism is induced and PLC is assessed (see Mat. & Met.). Among the VC published, an apparent dichotomy exists [1] (*i.e.* Figure 1) :
 - *s-shaped curves* present safety margins consistent with field measurements of xylem water potential.
 - *r-shaped curves* are suspected to be the result of an measurement artifact that would overestimate embolism rate when the sample length is shorter than vessels length; these curves have mostly been reported for long vessels species such as ring porous trees [1] .
- ❑ This possible *vessel length artifact* in the VC estimates is still debated [1,2,3] but may have serious implications. To ensure an accurate measurement of the VC it has been recommended to check the shape of the curve, to cross-validate different methods, and to check if the points of lower xylem potential (or higher pressure) match the points of higher PLC.

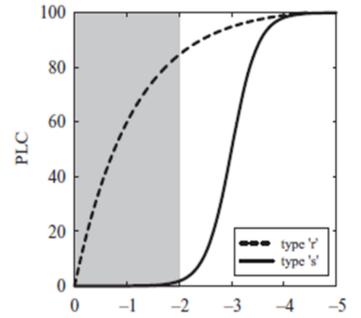


Figure 1 (From Cochard et al., 2010) : r-shaped curves (interrupted line) show an exponential rise and levelling-off. S-shaped (continuous line) have ecologically meaningful safety margin (grey area)

OBJECTIVE : Assessing the validity of 3 methods of VC construction in the long vessel species *Quercus ilex*

Material & Methods

- ❑ **Plant materials size and sampling** : All measurements were performed on current year *Quercus ilex* resprouts sampled at full maturity. Resprouts present 2 advantages :
 - They form long and straight samples (>1.20 m) greater than the maximal vessel length we measured (*ca.* 70 to 90 cm).
 - They experience lower water deficit (<1.5MPa) than adult tree (~-4.5MPa) thus limiting previous embolism and subsequent cavitation fatigue.
- ❑ **VC construction methods** :
 - The **Bench Dehydration-PLC (BD-PLC, Fig. 2A)** consists in letting large sampled branches dehydrate freely in the air to mimic natural cavitation. PLC is assessed by measuring gravimetrically the current conductivity and maximum conductivity of the sample. **This is considered as a standard as it mimics field conditions. It was applied on resprouts exceeding the maximal vessel length (1.20m long).**
 - In the **Pressure Sleeve-PLC (PS-PLC, Fig. 2B)** positive pressures are applied artificially to a branch sample to mimic the negative pressure of the sap. PLC was assessed gravimetrically. Curves were constructed using samples from 15 to 25 cm length.
 - The **Cavitron (CAV, Fig. 2C)** uses centrifugation forces to mimic the negative pressure of the sap and generate embolism. In this method conductivity can be assess during the spinning procedure by creating a pressure gradient that allows a water flow during the centrifugation. Curves were constructed using 2 different rotors (28 & 42 cm length)
 - For **CAV & PS-PLC**, Additional measurements of PLC distribution along the sample at -4MPa were performed.

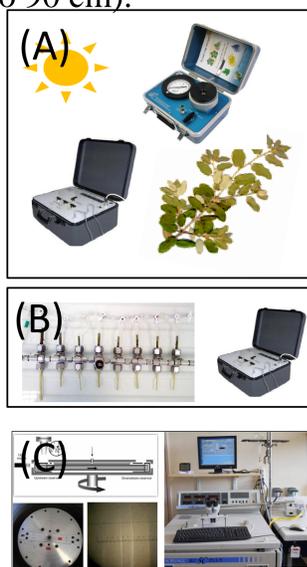


Figure 2: Outline of the 3 methods used to build VCs in this study. (A) The standard bench drying method. (B) The pressure sleeve air injection method. (C) The Cavitron method.

Results & Discussion

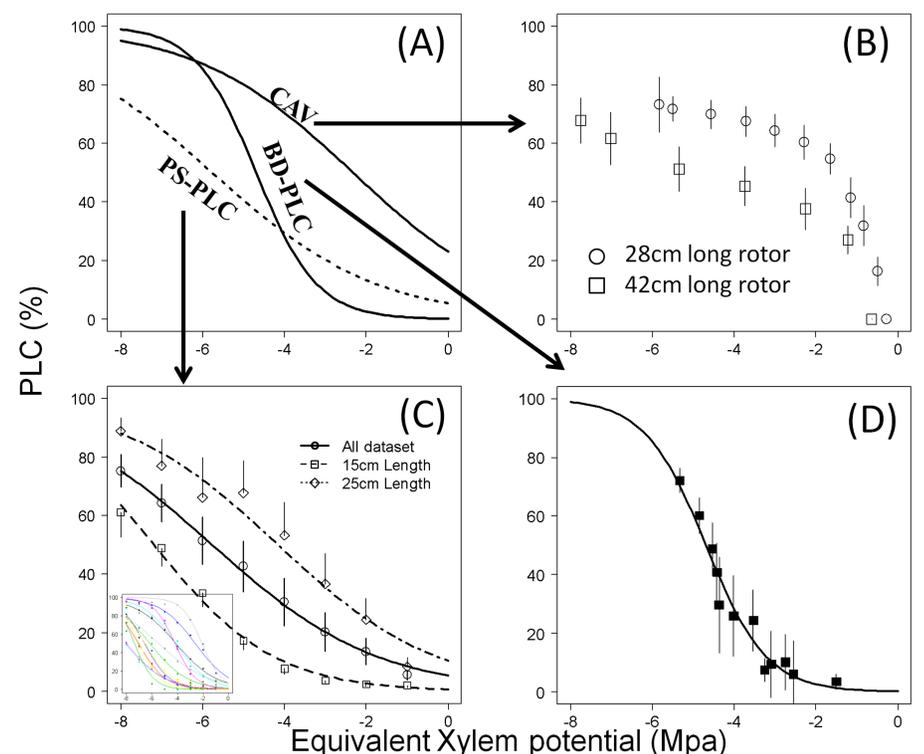


Figure 3: VC build from the 3 different methods using *Q. ilex* resprouts. (A) Fitted sigmoid function using the data points from each method. (B) Cavitron using 2 sample size. (C) Pressure sleeve air injection (PS-PLC) method using 2 sample size, the insert shows curves fitted individually for each sample. (D) Bench drying method (BD-PLC) using samples larger than 1 meter (vessel length did not exceed). Error bars are standard errors.

- BD-PLC produced consistent *s*-shaped VC, with a P50~-4.7 Mpa in agreement with minimum water potential observed in the field [4] (Fig. 3B).
- CAV produced inconsistent *r*-shaped VC, using 2 different sample lengths below maximum vessel length, in accordance with the hypothesis of a *vessel length artifact* (Fig. 3D).
- PS-PLC produced neither '*r*' nor '*s*' shaped VCs but, VC were linear when all points were averaged (Fig3. C). Surprisingly, longer samples (25cm) were less resistant and less S-shaped than shorter ones (15cm). Additionally a very important variability in both the VC' shape and the P50 (-2 → -8MPa) was observed (Fig. 3C). Such variability could be explained by the diameter of the sample (Fig. 4), suggesting that the VC depends on local vessel feature likely in the area of influence of the sleeve.
- Additional supports for a *vessel length artifact* for VCs build using the CAV and the PS-PLC methods come from the distribution of PLC along the sample after decreasing artificially water potential to -4 Mpa (Fig. 5). Whereas PLC should be maximum in the points of minimum pressure, it shows a decreasing pattern. The decrease is steeper for the CAV than for the PS-PLC method.

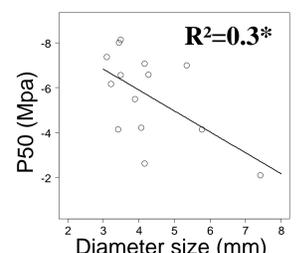


Figure 4: P50 of VC measured using the PS-PLC as a function of the diameter sample.

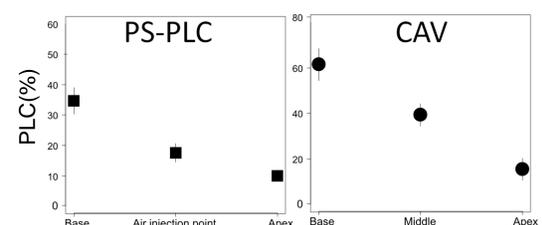


Figure 5: Distribution of PLC of both side of the point where water potential is supposed to be the lowest for a pressure of -4MPa exerted with the PS-PLC or th CAV method. For the Air Injection method, the « air injection point » where the sleeve is placed, corresponds to the point where pressure is maximum. For the cavitron, the middle of the sample match the point of lower potential. Sample length were between 28 and 50 cm. Error bars are standard errors (n=6).

Conclusion

- ✓CAV produced clear vessel length artifact as VC were systematically *r*-shaped.
- ✓PS-PLC produced a gradient of VC shapes from *r* to *s* : The shape of VCs likely depends on the sample feature at the point of air injection.
- ✓Our result suggests that intermediates VC that do not fall into *r* or *s* shapes may also misestimate true vulnerability to cavitation because of length