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# Xylem tension relaxation under water does not lead to refilling in embolized grapevine petioles

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## Abstract

In recent years, the validity of embolism quantification methods has been questioned, especially for long-vesseled plants. Some studies have suggested that cutting xylem while under tension, even under water, might generate artificial cavitation Wheeler et al. (2013). Accordingly, a rehydration procedure prior to hydraulic measurements has been recommended to avoid this artefact. However, concerns have been raised that xylem refilling might occur when samples are rehydrated.

**Fig. 1 (a)** The relation between PLC and  $\Psi_x$  in grapevine petioles under tension (filled circles) or after relaxation in which the shoots were submerged under water for 10 min (open circles). The sigmoidal regression suggest

that the relaxation shifted the

 $\Psi_{50}$  from -1.35 to -1.44 MPa.

(b) Statistical comparison of

the methods (with or without

relaxation) to generate xylem

vulnerability curves in (a). The

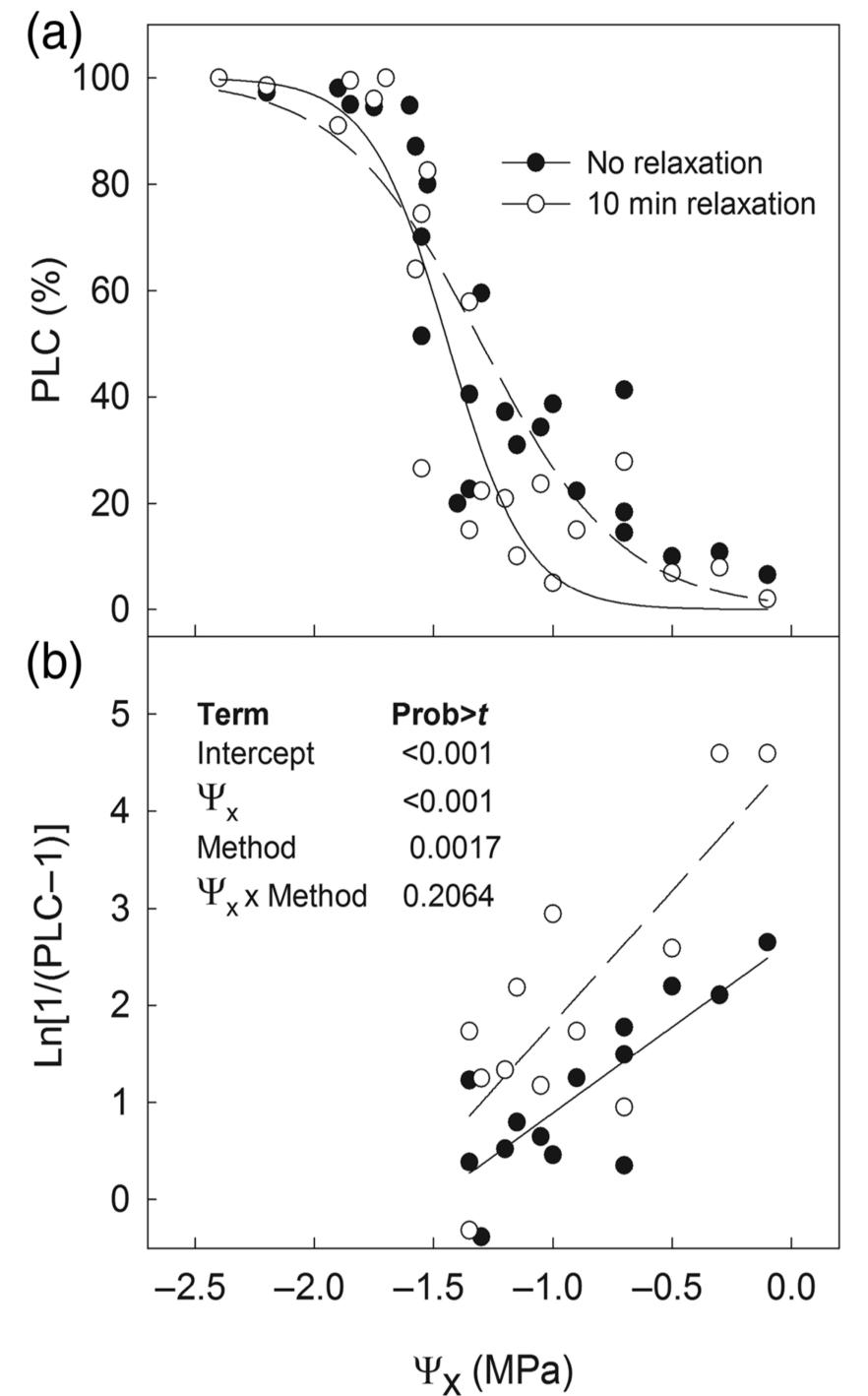
linearized and compared using

a regression model with  $\Psi_{x}$ ,

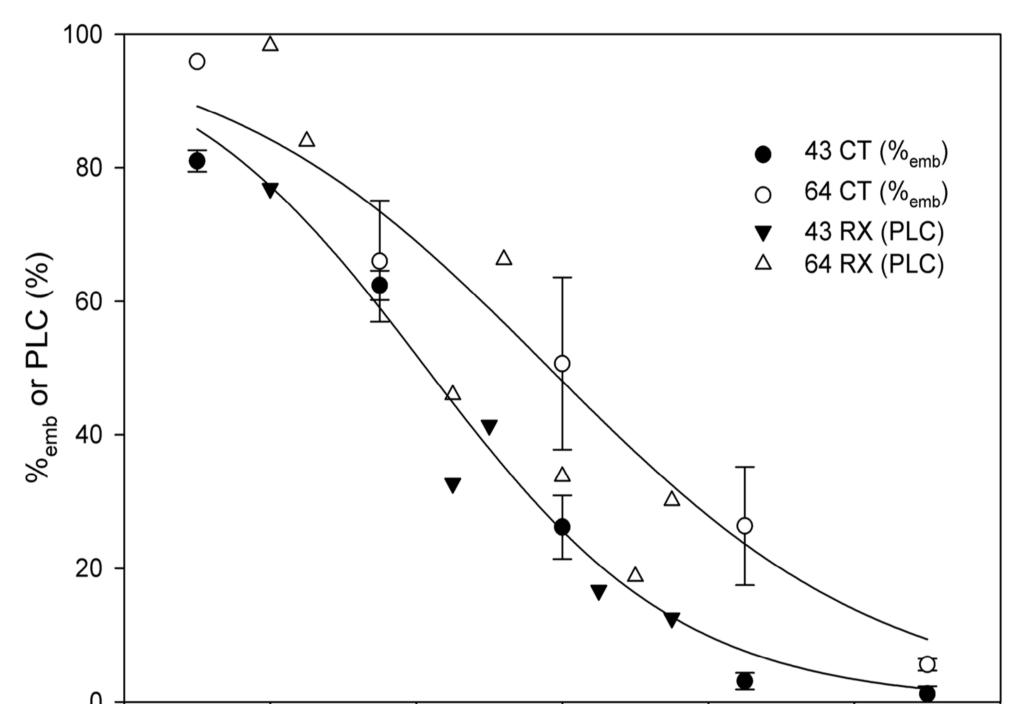
interaction as model effects.

sigmoidal curves were

the method, and their



Here, we explored the potential biases affecting embolism quantification for grapevine (*Vitis vinifera* L.) petioles harvested under tension or after xylem relaxation. We employed direct visualization of embolism through X-ray micro-computed tomography (microCT) to test for the occurrence of fast refilling (artifactually low percent loss of conductivity (PLC) due to rehydration prior to sample harvest).



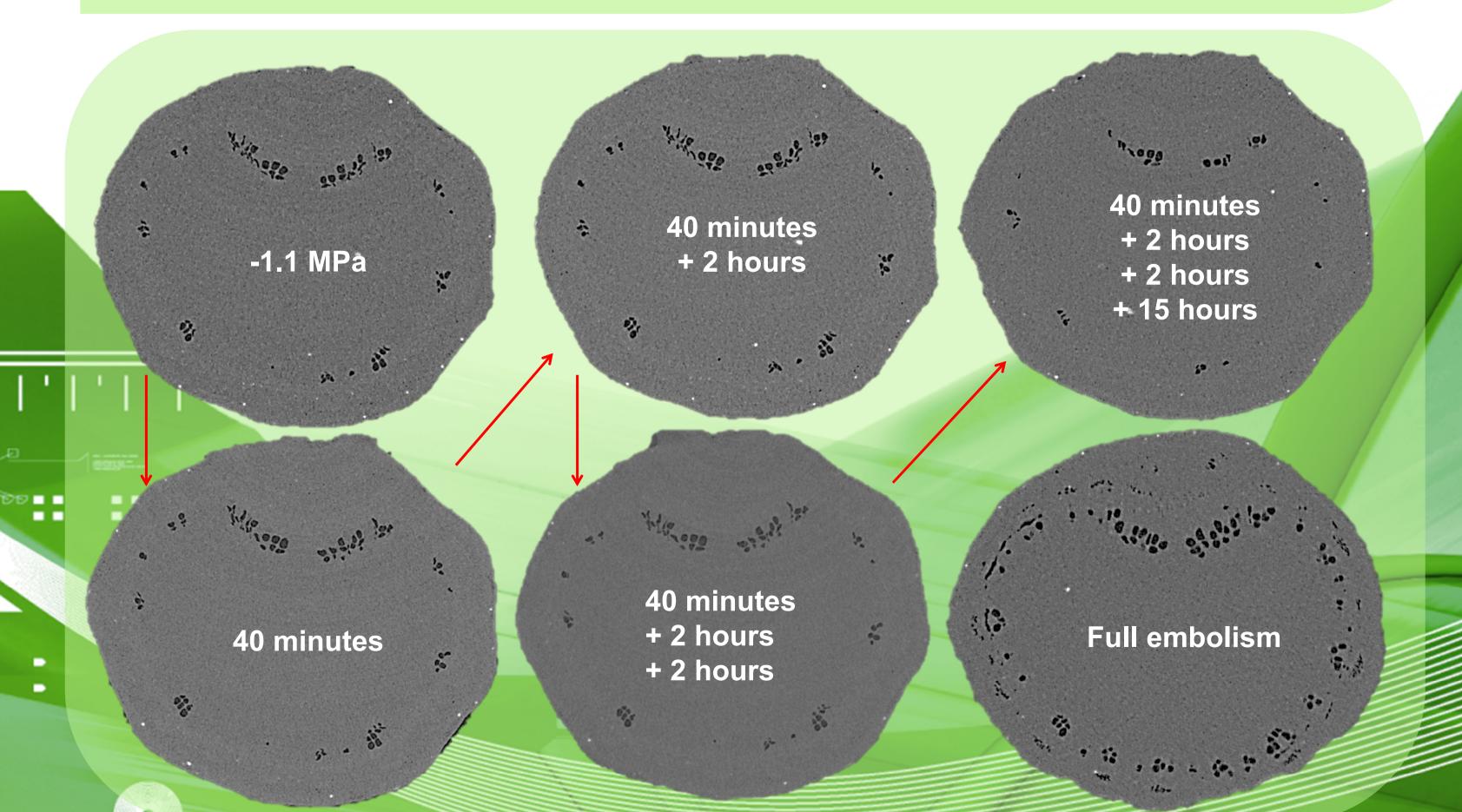
**Fig. 2** Comparison of xylem vulnerability curves built by imaging intact vessels with X-ray microCT (CT—circles) or by using the hydraulic apparatus with relaxed segments (RX—triangles). The relationship between the  $\Psi_x$  and the PLC or  $\%_{emb}$  was assessed in grapevine petioles of two grapevine genotypes: 043 (filled symbols) and 064 (open symbols). X-ray microCT data are averages of four different plants ±SE, while data points of the hydraulic apparatus represent averages of three petioles excised from the same shoot. The lines are the sigmoidal regressions of the X-ray microCT data.

## -1.6 -1.4 -1.2 -1.0 -0.8 -0.6 -0.4

 $\Psi_x$  (MPa)

## **Key results**

- Comparison of VC measured with 10 min relaxation or without prior relaxation, showed a significantly lower PLC for the relaxed treatment (P < 0.05) for Ψ<sub>x</sub> values higher than -1.4 MPa (Fig. 1).
- VC constructed using hydraulic apparatus for relaxed samples was similar to VC established using microCT (Fig. 2).
- ✓ Our results suggest that segments could be submerged under water for several hours without any risk of refilling (Fig. 3).
- Relaxation incorporation into hydraulic measurements of grapevines would result in reliable evaluations of PLC.



#### Methods

We have performed three experiments (more details in **Hochberg et al. 2016**):

- i. Test whether short-time xylem relaxation results in a reliable measurement of embolism
- ii. Compare xylem vulnerability curves (VC) of petioles from shoots under tension or relaxed
- iii. Investigate (using microCT) the time required for refilling vessels when placed under slight (4 cm) positive pressure
- Plant material consisted on 1-year-old V. vinifera (Syrah × Grenache progeny) grafted on R110 rootstock, grown in 10 L pots in a controlled environment during the 2015 winter in INRA Clermont-Ferrand (France).
- ➢ For hydraulic measurements we used long shoot segments (1.5 m) that were bench dehydrated to a range of pressures between −0.5 and −2 MPa before they were excised under water and connected to the Xyl'em apparatus (Bronkhorst, France) (i.e., cut under tension treatment). The cut surface of the remaining shoot was held under water for 10 min and then 3 additional petioles were cut under water and connected to the Xyl'em apparatus (relaxed treatment).

**Fig 3.** MicroCT observation of grapevine petiole embolism refilling. No significant refilling was observed after 4 hours and 40 minutes under water. Many vessels were refilled after 20 hours under water. Full embolism was achieved by cutting the segment before microCT imaging.

For embolism evaluation using the X-ray microtomograph (Nanotom 180 XS, GE, Wunstorf, Germany) we used a plant segment containing a leaf with 3 internode beneath the remaining petiole insertion. We validated that such size excludes open vessels from the petiole.

#### References

Hochberg U, Herrera JC, Cochard H, Badel E (2016) Short-time xylem relaxation results in reliable quantification of embolism in grapevine petioles and sheds new light on their hydraulic strategy. Tree Physiology 36:748–755. Wheeler JK, Huggett BA, Tofte AN, Rockwell FE, Holbrook NM (2013) Cutting xylem under tension or supersaturated with gas can generate PLC and the appearance of rapid recovery from embolism. Plant Cell Environ 36:1938–49.

A short movie demonstrating the protocol is freely available at: https://www.youtube.com%20/watch?v=YzpDN7laepo

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