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USING X-RAY MICROTOMOGRAPHY TO ASSESS REFILLING AFTER DROUGHT-INDUCED EMBOLISM IN PLANTS

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Summary: Plant mortality under extreme drought events has recently been associated with plant vulnerability to xylem cavitation, a phenomenon corresponding to the disruption of water transport in embolized vessels. However, our understanding of fundamental processes involved in embolism repair remains rudimentary. Here, we show a study using synchrotron microtomography to assess refilling processes under tension in xylem plants.

1. Introduction

Plant mortality under extreme drought events has recently been associated with plant vulnerability to xylem cavitation, a phenomenon corresponding to the disruption of water transport in embolized vessels. Despite the recent advances in the field of plant hydraulics, there is still debate as to whether plants routinely face embolism and recover easily from it or are highly resistant to embolism. Some studies have suggest that plants are highly vulnerable to embolism but recover from it on a daily basis. In both branches and leaves, this recovery would involve the refilling of vessels, via transpiration during the daytime or through root pressure. During our first SOLEIL campaign in 2015 [1], we were able to characterize the spread of embolism in the vascular system and to assess vulnerability curves on several species. However, our understanding of fundamental processes involved in embolism repair in plants remains rudimentary. We hypothesize here that embolism repair, as a component of drought recovery following re-watering, only occurs under xylem positive pressure (via root pressure) and initiated by water droplet formation.

2. Experimental Method

The experiments were performed at the Psiché beamline of SOLEIL, the French national synchrotron [2]. The hollow stage allowed us to mount the stem of intact plants utilizing a custom built pot holder and to scan up to 1.2 m above the pot. This special stage (not available in other beamlines to our knowledge) allows to broaden greatly the spectrum of experiments that can be performed on plants (scan on stem at different heights, branches, petioles and leaves). After a drought period, the plants were scanned at different heights and at different times after re-watering. Using stem psychrometers and pressure probes, xylem bulk pressure and PLC were concomitantly measured at different heights of the same plant. Different species were studied (pinus, poplar, laurel, etc). Here, we focus on grapevine.

3. Results

Direct, non-invasive observations of embolism formation and repair reveal a lack of refilling under negative pressure in grapevine, as shown in Figure 1. Indeed, re-watered plants were scanned either in the basal (1 cm above the grafting), or in the distal part (ca. 1m above soil). In the basal part, significant changes in the amount of

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air-filled vessels were observed over a 24 hours period, after the plant was re-watered. Most vessels were dark grey (i.e. air-filled) before re-watering (PLC = 86.8%, Figure 1). After 7.5 hours, evidence of xylem refilling and an increase in the number of functional vessels was observed (Figure 1), even though PLC was barely affected (PLC = 81.2%). After 15.5 hours, many additional vessels had refilled, decreasing the PLC to 57.4%. Refilling of xylem vessels was not observed as long as the pressure remained negative. However, as soon as positive pressure was measured, xylem refilling could be seen. In contrast, in the upper part of re-watered plants, even after more than 48 hours of re-watering, there was no significant change in PLC in the upper part of the plants, even though most living cells remained alive (note that in parallel some studies using fluorescence were used to assess the impact of the x-rays on the plant - not shown here). Refilling was not observed at the apex ($\Delta PLC = 0.02 \pm 0.01\%$), regardless of the initial levels of embolism ($13.7.8\% < PLC < 92.4\%$; Figure 1).
Our findings provide evidence that grapevine is unable to repair embolized xylem vessels under negative pressure, but its hydraulic vulnerability segmentation provides a significant protection of the perennial stem.

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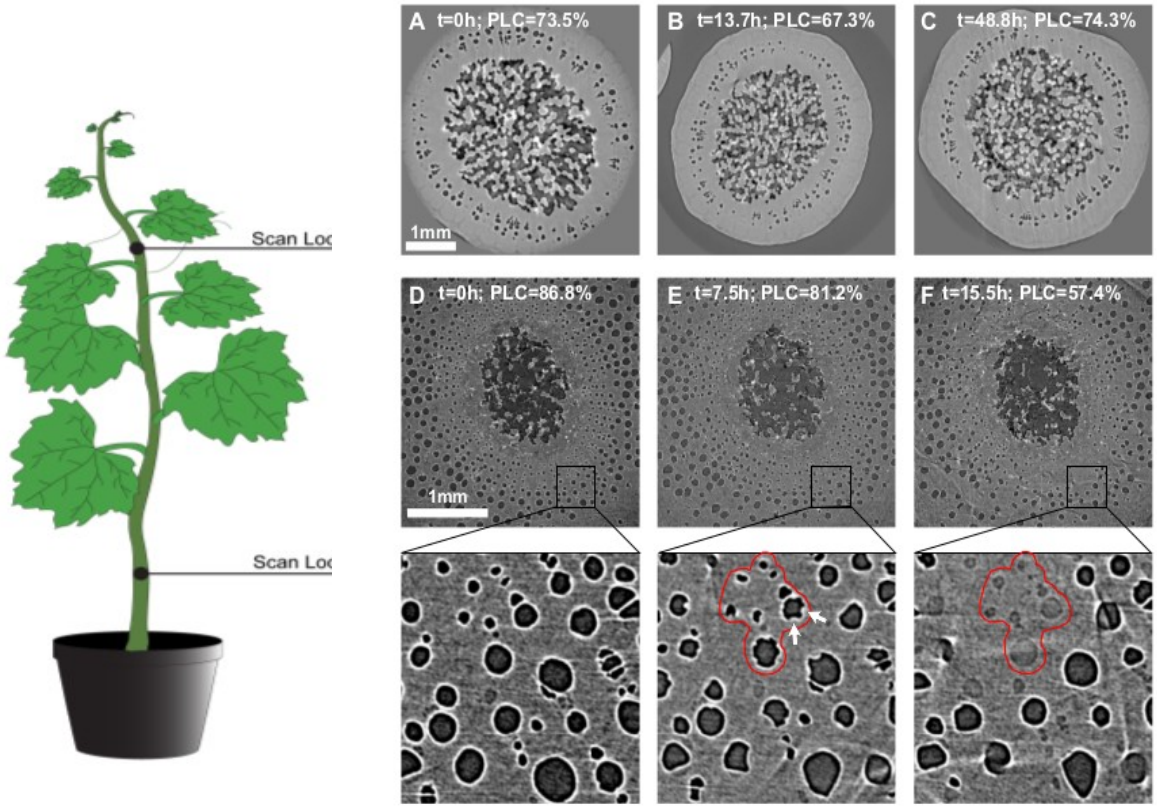

Figure 1: Figure 1. Cross section of Vitis vinifera stems at two different height levels i.e. the upper, distal part (A-C) and bottom, proximal part above the graft (D-F), after re-watering drought-stressed plants. Time relative to rewatering (t = 0 h, i.e. the rewatering time) and the theoretical losses of hydraulic conductance (PLC, %) are indicated. White bar = 1 mm scale.