

***Supplemental Online Material for:***  
**Large volume vessels are vulnerable to water-stress-induced embolism in stems of poplar**

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**Content: Figures S1–S4**

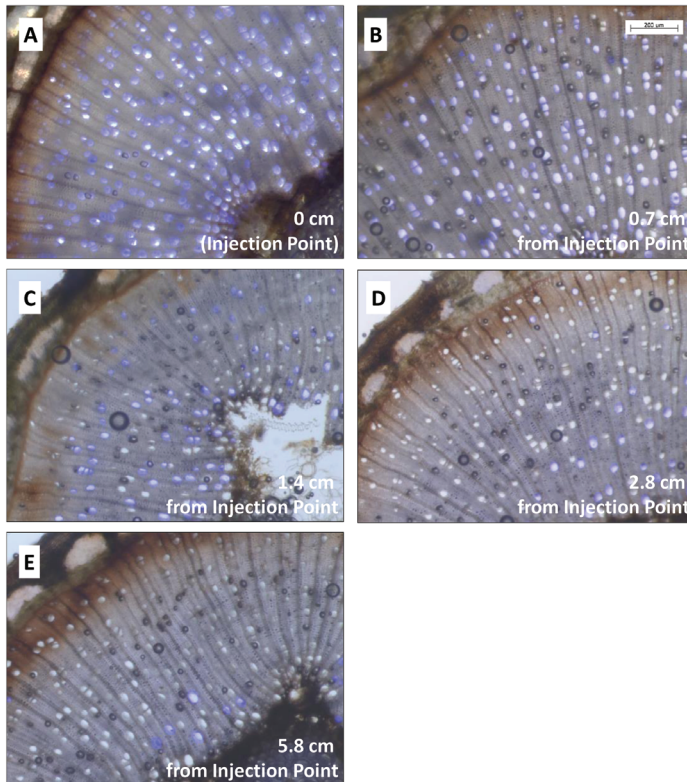


Figure S1. Representative micrographs showing the number and size distribution of silicone-filled (light blue) vessels with increasing distance from the point of silicone injection (A) into stems. The injection point was 35 cm from the stem apical meristem and samples were injected from the proximal stem end in the direction of water flow (toward the stem apex). The micrographs shown above were all taken at the same magnification (100×) and a scale bar is included in the upper right panel (B).

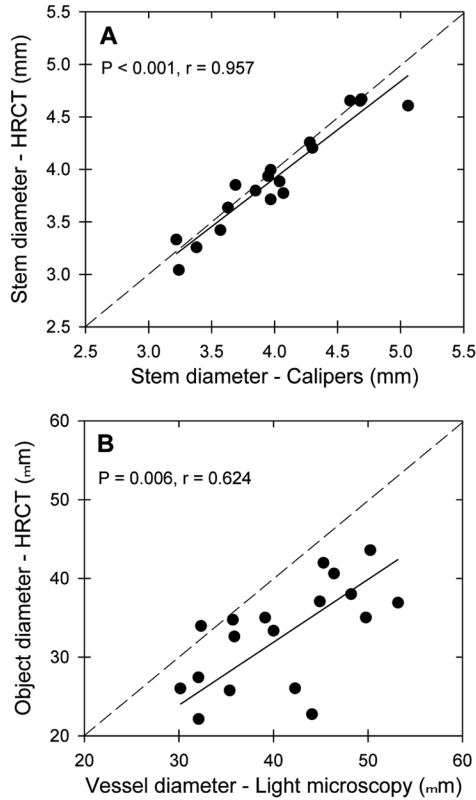


Figure S2. Measurement derived from different analyses were compared to ensure that analyses were giving comparable results and object dimensions. Stem diameter was measured from microCT scans and digital calipers (model 500-196-20, Mitutoyo America, Aurora, IL) were used to measure the stem diameter at the scanned point ( $n = 18$ ) (A). The correlation between these parameters and their location on the 1:1 line indicates that measures of stem dimensions from HRCT scans were consistent with the size of sampled objects (*i.e.*, there was no scaling error within measures from scans). With samples, individual vessels were selected for a similar analysis. Only individual vessels that could be matched between 3D analysis and thin cross sections of the scanned region examined via light microscopy were selected ( $n = 18$ ) (B). Mean object diameter was strongly correlated with cross-sectional vessel diameter estimated from thin sections, but vessel diameters from light microscopy were larger than object diameters. This is consistent with differences in the way that these parameters are calculated: The calculation of object diameter was based on the largest diameter sphere that could fit within the object. This excludes the “corners” of a vessel that departs from circular and results in smaller diameter estimates than our 2D light microscopy cross-section analysis, which was calculated the diameter of a circle of equivalent area to the measured vessel lumen area. Each panel includes the p value and Pearson correlation of the shown parameters, the dashed line indicates the 1:1 line, and the solid line represents the regression between the shown parameters.

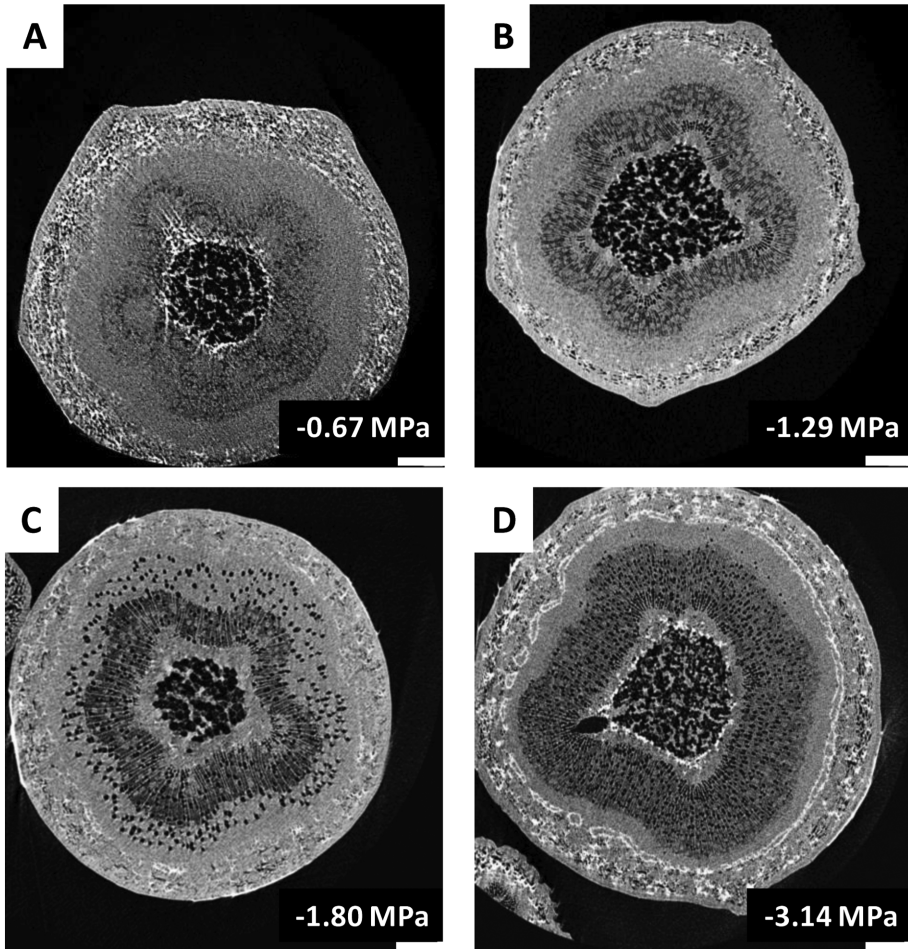


Figure S3. Representative cross sections from branches that were scanned using HRCT at differing water potentials. The scans were all taking at 3  $\mu\text{m}$  resolution. Water-filled vessels, cell walls, and other cell types appear as grey, gas-filled spaces are black, and dense particles (typically crystals) are visible as bright white spots. The light grey band located within the xylem near the cambium is due to fluid-filled vessel lumens of living fibers. A scale bar is included in the lower right corner of each panel (white band = 400  $\mu\text{m}$ ).

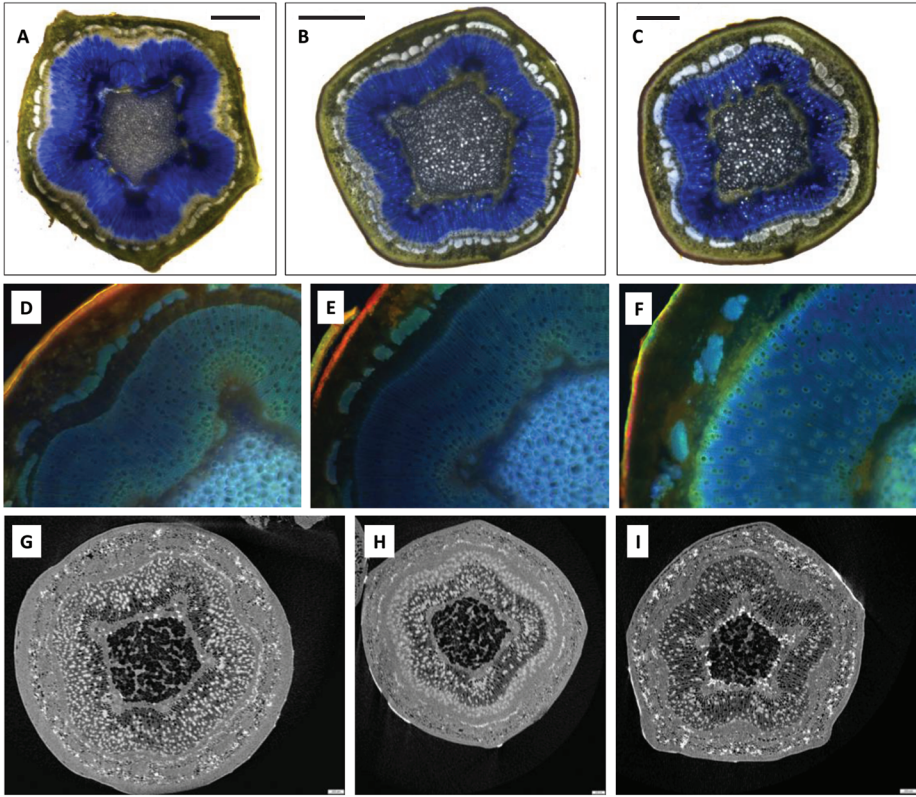


Figure S4. Representative cross sections from branches that were either stained for active xylem (A, B, C), examined using fluorescence microscopy (D, E, F), or fed an iodine-based solution and scanned using HRCT (G, H, I). Active stained xylem sections were all hydrated and show that vessels were conductive throughout the cross section, both near the pith and near the vascular cambium. Sections examined using fluorescence were the same sections that were used for native stem measures within the current study. In the fluorescence images, differences in cell wall chemistry and lignin content within the xylem appear as differences in color, and immature xylem would be visible as a band of different colored tissue near the cambium; however, this band was not present in these images, indicating that most of the xylem within the sections was fully developed. HRCT scanned segments were native stems collected at the same time and from the same plot as other stems examined within this study. Within these scans, fluid-filled vessels appeared white, indicating that they contained iodine and that they had been conductive. There were no grey fluid-filled and non-conductive vessels observed. The HRCT scans were all taken at 3  $\mu\text{m}$  resolution. – Scale bar in A & B = 1 mm, in C = 500  $\mu\text{m}$ .