Supplementary Data

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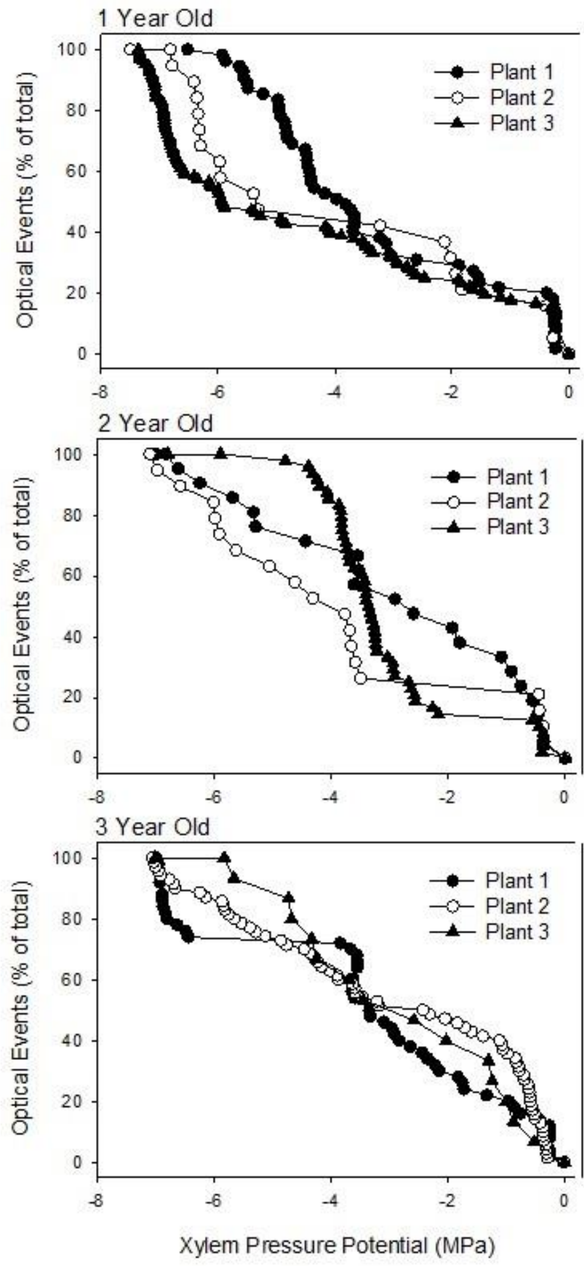

Figure S1. Optical vulnerability to embolism curves for stems of three different ages measured on three different trees (n=3). These curves represent the raw data and no modifications have been made to them.

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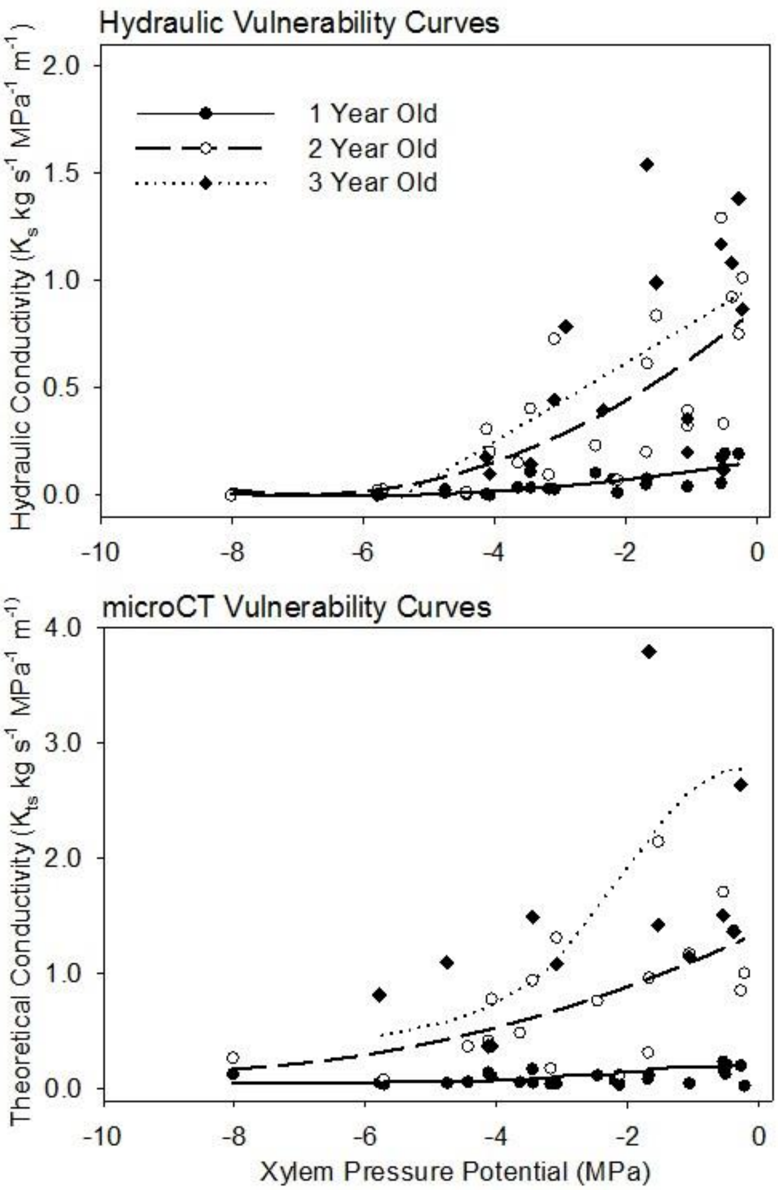

Figure S2. Benchtop hydraulic and microCT vulnerability to embolism curves for stems of three different ages measured on three different trees (n=3). These curves represent embolism as specific hydraulic conductivity (K_s) and theoretical conductivity (K_{ts}). Each data point represents a unique stem. Best fit lines are shown as a visual guide using power or sigmoid models. Note that the y-axes are on different scales.

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Testing for artificial air in cut stems: Validation of hydraulic-based methods

Cutting stems underwater can lead to artificial embolism being introduced during sample preparation (Wheeler *et al*. 2013). This could lead to artificially elevated embolism (PLC) levels. We removed very large branches in the field far enough away from the target stems to avoid embolism (maximum measured vessel length was 1.29 m and our target stems were at least 1.5 m away). When excising stem segments from the large branches in the lab, we cut samples off under water and allowed the xylem pressure to relax, but we did not typically cut the stems longer than the longest vessel length.

To test the possibility that our protocol artificially introduced air, we used the best practices recommended by Torres-Ruiz *et al*. (2015). In this experiment, we cut large branches in air that were much longer than the longest vessel length, consistent with our sampling methods generally. Plants were then dehydrated in the lab so that they had significant negative xylem pressures. After this, the cut end of the large branch was cut under water to remove embolized xylem while the plant was still bagged. The plant rehydrated for 2 hours and the water potential was measured to verify this. This step allowed for the relaxation of the xylem that should prevent or minimize any air introduction due to cutting as recommended by Torres-Ruiz *et al*. (2015). After the xylem pressure was relaxed, 3-year-old stems were cut off underwater, about 20 cm away from the target stem, cut to length underwater as previously described, and their hydraulic conductivity measured. We chose to use only 3-year-old stems for this experiment because they had the longest vessels, which could make them more vulnerable to such artifacts. We sampled two plants from this experiment using a paired comparison, to minimize error variation, where each large branch from a plant had 3 stems under tension and 3 relaxed for a total of n = 6 for stems under tension and for relaxed stems. See a more extensive comparison in Venturas *et al*. (2016).

We tested for a cutting artifact using an ANOVA that included PLC as the response and the model included plant (two levels), treatment (cut under tension and cut relaxed), a repeated measures term nested within treatment for the different stems sampled within a branch, and an interaction between plant and treatment (JMP 13.2.1, SAS Institute, North Carolina, USA) (**Figure S3**).

In a separate experiment, we compared embolism levels at the cut ends and in the middle of excised segments. All of our microCT measures were done some distance (>4 cm) away from cut ends, thus it was possible that air bubbles at the cut ends could lead to reduced hydraulic conductivity and higher estimates of embolism in the hydraulic data compared to the theoretical conductivity and embolism estimates from microCT. Such air-bubbles could be a form of cutting artifact. To test for this, we used our previously described sampling protocol for branches, and we sampled hydraulic conductivity of three 1-year old stems from one large branch removed from one plant after the large branch had been dehydrated in the lab. After measuring native-state hydraulic conductivity, each stem was scanned at their cut ends and at their middle and we estimated PLC at each point. The cut ends, during scanning, were submerged under water in plastic tubes and x-rays penetrated tubes, water, and stems for this protocol. After scanning, we again measured conductivity. The stems were then flushed and maximum conductivity was measured. In addition to analyzing the PLC at the ends and at the middle of the stems, we calculated stem PLC by summing the resistance of the ends and middle in series (resistance = 1/conductance) and calculating PLC.

These data were analyzed in a mixed 2-way ANOVA that included theoretical PLC and K_s as the response variables, and treatment (cut ends, apical and basal, and stem center) and stem as a random factor as the predictor variables. A contrast was conducted between cut ends (both basal and apical) compared to the middle of the stem to test for a cutting effect (JMP 13.2.1, SAS Institute, North Carolina, USA) (**Figure S4**).

As part of this same experiment, hydraulic conductivity was measured before and after scanning. The hydraulic estimates of PLC were compared to microCT using a t-test. This dataset also allowed us to test the possibility of a scanning effect on conductivity (**Figure S5**). To examine this, we compared hydraulic PLC and K_s estimates before scanning to after scanning using paired t-tests.

Finally, as an additional test of the potential influence of x-ray scanning on samples, we examined vulnerability to embolism curves for 1-year old stems generated using a benchtop dehydration method. Some of the stems were scanned with x-rays in a microCT system prior to measurement (open symbols) and the other subset were not scanned (closed symbols) (**Figure S6**). Curves were analyzed in the same way described in the Methods section of the manuscript.

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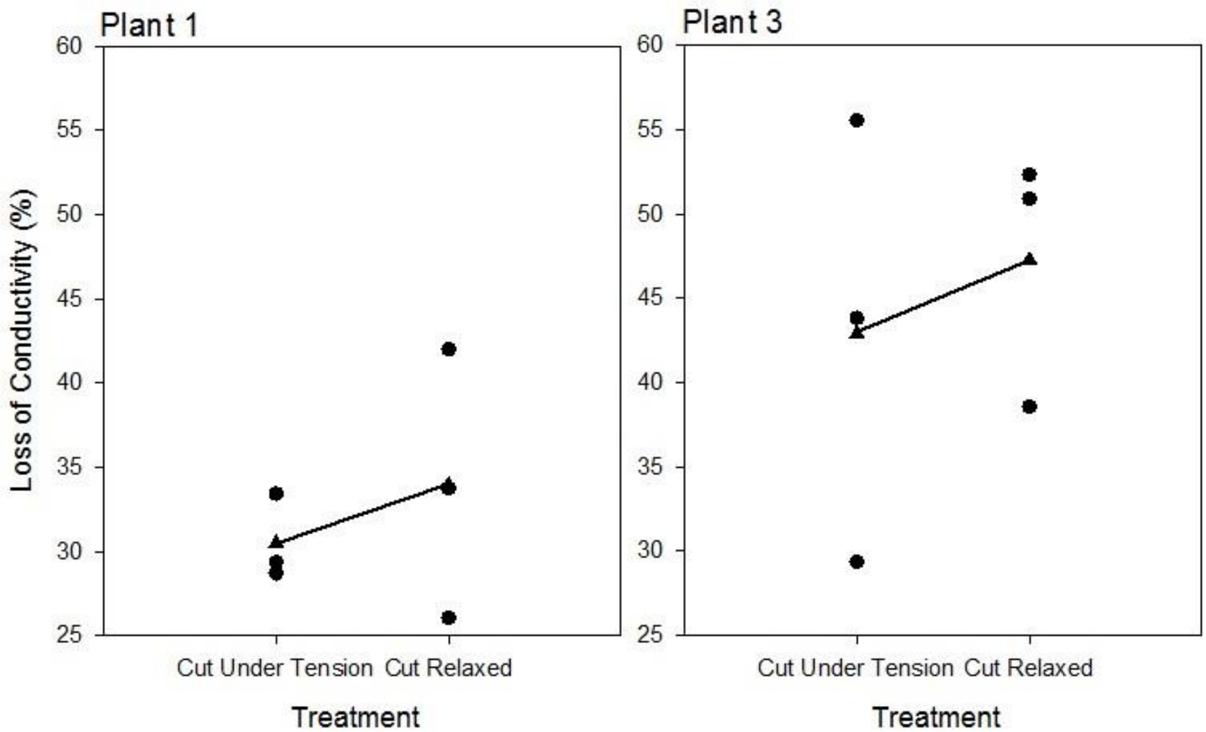

Figure S3. Cutting stems to sample conductivity may introduce embolism and this experiment tested for this using two large branches from two different plants (1 and 3). Stems were cut under tension (-2.32 for plant 1 and -2.91 for plant 3) and in a relaxed state (-0.20 for plant 1 and -0.23 for plant 3). Three 3-year-old stems were cut under tension from each large branch and a different three were cut from each branch in their relaxed state (n = 6 for tension and relaxed). Circles represent each replicate and triangles represent the mean with lines connecting the two means. The existence of a cutting artifact would show significantly higher embolism in the cut under tension group than the relaxed group. No significant difference was found between the two groups suggesting that there was not a cutting artifact ($F_{1,4}$ = 0.453, P = 0.5378). The level of embolism (PLC) was greater for plant 3, consistent with its more negative xylem pressure ($F_{1,4}$ = 10.176, P = 0.033).

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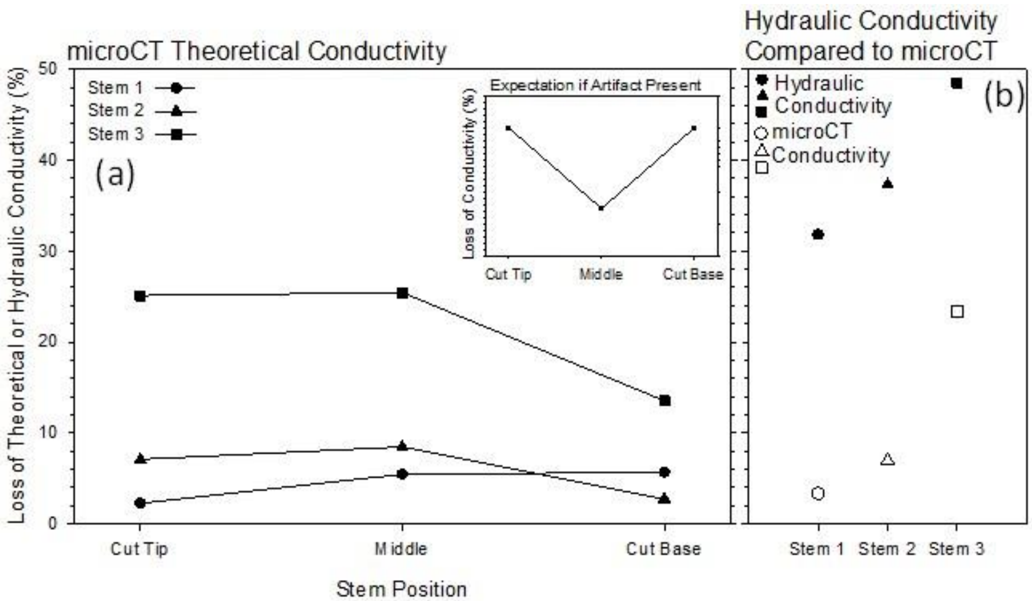

Figure S4. Cutting stems could lead to gas bubbles being introduced into the cut ends that could lead to losses in hydraulic conductivity. If this were the case, then we would expect to see higher levels of embolism (loss of conductivity) at the cut ends when compared to the middle of the stems (Inset in panel a shows this expected pattern). Stems were scanned using microCT at the cut ends (while underwater) and in the middle to test for this possibility. Theoretical percentage loss of conductivity (PLC_t) was compared and found to not differ between cut ends and the stem middle ($F_{1,4}$ = 1.743, P = 0.257 for PLC; $F_{1,4}$ = 3.677, P = 0.127 for K_{ts}, which is not shown). The expected pattern of greater embolism at the cut ends (inset in panel a) was not supported, indicating that there was no artifact caused by embolism at the cut ends. Hydraulic conductivity was measured before and after scanning the stems and used to calculate hydraulic PLC (see Figure S6) and compared to PLC theoretical from the microCT method. The hydraulic PLC was significantly greater than the theoretical PLC (panel b; t_4 = 3.545, P = 0.023). We conclude that the significant discrepancy between hydraulic PLC measurements and those estimated with microCT are not due to an artifact associated with cutting of stems.

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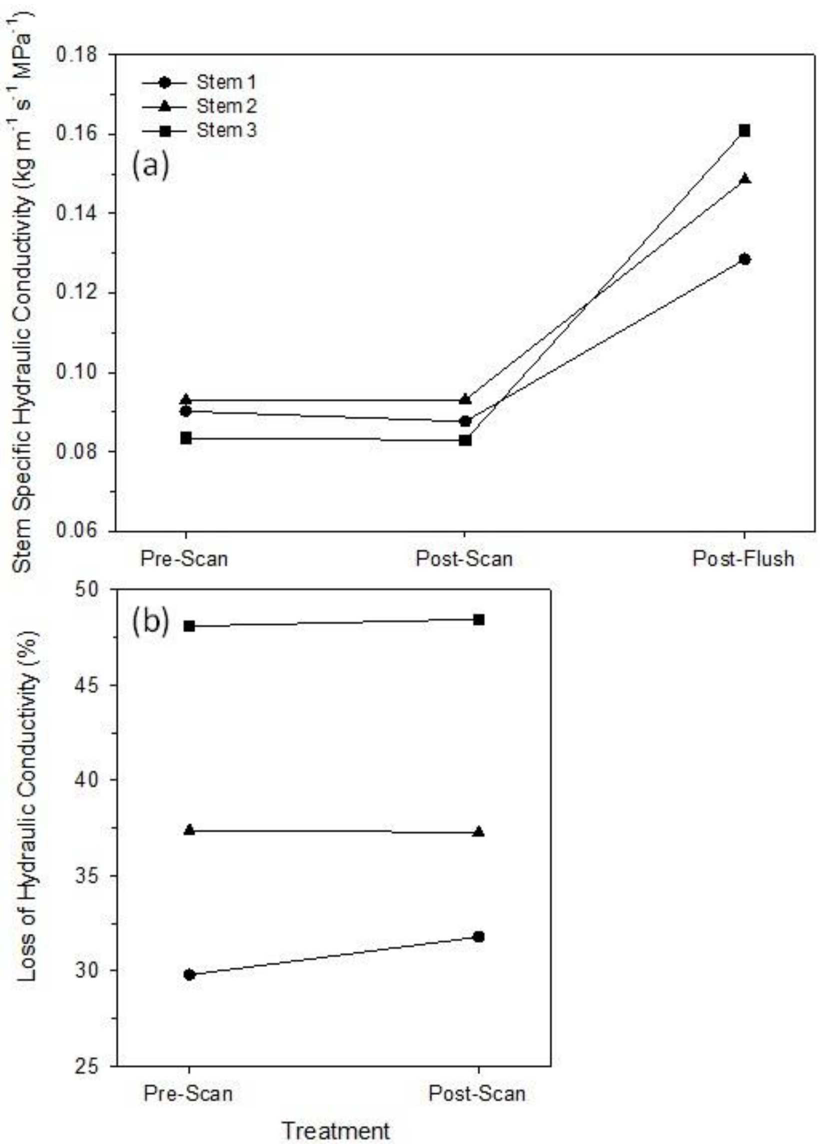

Figure S5. Scanning of stems with x-rays in the microCT system could lead to artificially induced embolism that would manifest as lower stem specific conductivity (K_s) or elevated level of percentage loss of conductivity (PLC). Alternatively, because stems are kept in contact with free water at their cut end, refilling of embolized conduits could occur, which would manifest as higher K_s and lower PLC after scanning. To examine this, we measured hydraulic K_s and PLC before and after scanning stems three times. The pre and post-scan K_s (panel A) and PLC (panel B) were not significantly different (t_2 = 1.177, P

= 0.360 for PLC; t_2 = 1.226, P = 0.345 for K_s). This suggests three things: 1. that x-rays were not leading to artificial embolism; 2. that stems were not refilling when in contact with free water to keep hydrated; and 3. that clogging of stems due to wounding was not occurring. Another observation is that the significant increase in K_s after flushing (panel a) suggests that any refilling was very incomplete, at best, and that any clogging was also a minor factor if present at all. We did not measure any direct response of living cells to the x-ray treatments and cannot rule out any damage that could have occurred to them (Petruzzellis et al. 2018). With respect to artificially-inducing embolism, this experiment represents the worst-case scenario for our experiments, because stems were scanned three times instead of only one time as was done in all other experiments. The same argument holds for refilling, because scanning three times meant that stems were being held with two cut ends in contact with free water under a slight pressure head for an extended period (about 25 minutes). In other experiments, stems only had one cut end in free water and there was no pressure head because the water was basal and leaves were left intact on the shoot.

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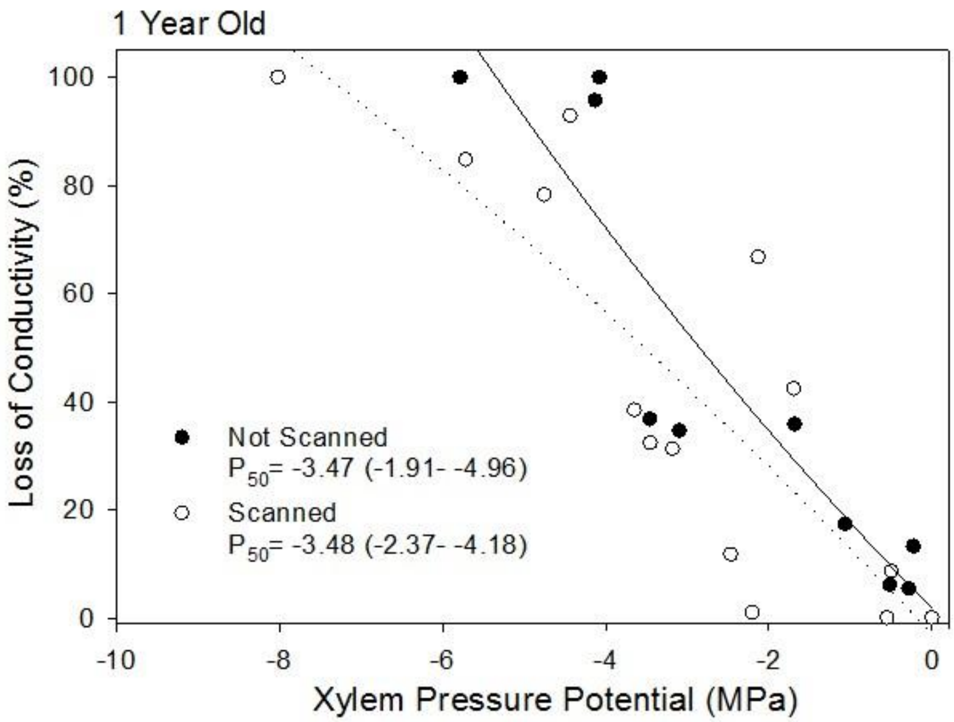

Figure S6. Vulnerability to embolism curves for 1-year old stems generated using a benchtop dehydration method. Some of the stems were scanned with x-rays in a microCT system prior to measurement (open symbols) and the other subset were not scanned (closed symbols). The water potential at 50% loss of conductivity (P50) was not significantly different between the scanned and non-scanned stems (P50 and lower and upper 95% CL shown in parentheses). This suggests that scanning stems did not affect vulnerability to embolism measurements.

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