Air Injection of Vessels
(for Maximum Vessel Length Determination)

Based on the methods originally introduced in this paper:

AN APPROACH TO THE STUDY OF VESSEL LENGTH IN HARDWOOD SPECIES
K. N. H. Greenidge

And described in this paper:

A GLOBAL ANALYSIS OF XYLEM VESSEL LENGTH IN WOODY PLANTS
Anna L. Jacobsen, R. Brandon Pratt, Michael F. Tobin, Uwe G. Hacke, and Frank W. Ewers
Air is pushed into stems at 100 kPa.

Initial sample length is quite long (>1m) so that when pressure is first applied to the stem there are no open vessels.

A latex grommet and tubing are fit onto the proximal end of the stem and filled with water. This allows for the stem to be inverted and for you to use a magnifying glass to carefully look for signs of bubbles.

Leaves are shown only to indicate the direction that air is pushed into stems (i.e. distally to proximally—the opposite direction of water flow).

Tubing is attached to the distal end of the stem after it is trimmed under water and shaved with a fresh razor blade. **This end of the stem should be your target sample diameter.** This is important because vessel length changes with sample diameter and so you want to make sure that you are measuring the vessel length that is most relevant to the samples that you are measuring for other parameters, such as vulnerability to cavitation.
This grommet is removed each time the sample needs to be shortened. The stem is trimmed incrementally from this end (cut underwater and reshaved with a fresh razor blade each time). Then the grommet and tubing are reapplied and filled with water and the sample is checked to see if any air bubbles can be seen emerging. (We usually cut in 5 cm increments initially, followed by 1 cm increments as the sample gets shorter).

Air tank

Regulator

Air is pushed into stems at 100 kPa

Jacobsen, 2011 (modified 2014)
The length of the “longest” vessel is reached with air can be seen emerging from a single cut open vessel.

Air is pushed into stems at 100 kPa.

Jacobsen, 2011 (modified 2014)
A note on this technique:

We have found that this technique produces different maximum vessel lengths when different pressures are used for the air-injection (i.e. using 30 kPa instead of 100 kPa results in shorter vessel length estimates and 500 kPa results in much longer estimates).

This suggests that this technique is not measuring the length of the longest vessel *per se*, but is rather measuring the longest (multiple-)vessel pathway through which air can travel at a given pressure. **Thus, the length of the “longest” vessel measured with this technique is most likely the length of more than a single vessel.** Air is probably able to move through a very large pit pore or a damaged pit membrane at these relatively modest pressures (these conduits would probably not be functional in the intact plant because they would not be able to maintain flow under negative pressure).

This technique still gives you an estimate of how far air could move through sample, even if it is not telling you the exact longest vessel length and maximum vessel length measured with this technique correlates well to other measures of mean vessel length (see Jacobsen et al. 2012 AJB). In my lab, we have found that maximum vessel lengths measured using this pressure are predictive of (but much longer than) mean vessel lengths as measured using the silicon injection technique.

Even with this pressure sensitivity, the most commonly used (and therefore, the “standard”) pressure is 100 kPa for stems and some researchers report using 50 kPa for roots.