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Silicone Injection of Vessels
(for Vessel Length Determination)

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Important!

See the following references for where this technique was first described:

Plant, Cell and Environment (2005) **28**, 456–465

Comparative analysis of end wall resistivity in xylem conduits

JOHN S. SPERRY, UWE G. HACKE & JAMES K. WHEELER

Department of Biology, University of Utah, 257S 1400E, Salt Lake City, Utah, 84112, USA

Plant, Cell and Environment (2005) **28**, 800–812

Inter-vessel pitting and cavitation in woody Rosaceae and other vesselled plants: a basis for a safety versus efficiency trade-off in xylem transport

JAMES K. WHEELER, JOHN S. SPERRY, UWE G. HACKE & NGUYEN HOANG

Department of Biology, University of Utah, Salt Lake City UT 84112, USA

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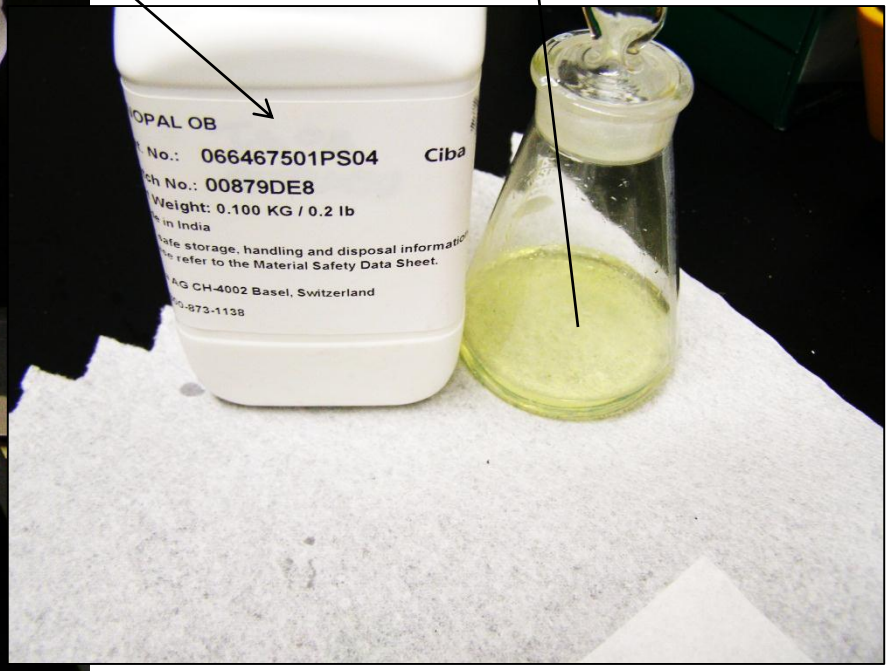
Mixing the Silicone:

10 parts A to 1 part B
(1 gram of mixture per stem)

UV-stain dissolved in chloroform (1% by weight)
1 drop added per gram of the A/B silicone mixture

Mix very well!



Allow the silicone mixture to sit for 2 hours after mixing. This allows the small bubbles that are incorporated into it during mixing to come out of the silicone.

The solution should appear clear when it is ready. A solution that appears milky or white has too much chloroform-UV stain added to it.

Just after mixing

After sitting for ~2 hr

(Photos: Martin Venturas)

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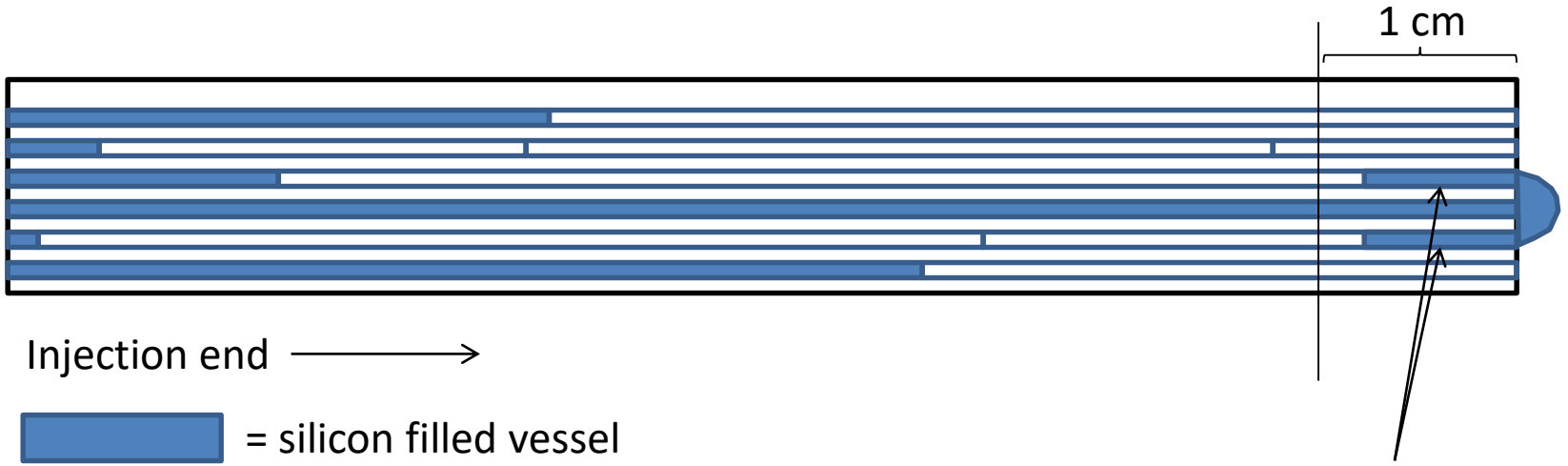
The ends of samples are trimmed under water and the samples are cut to the appropriate length. The ends are then shaved with fresh razor blades.

Samples that are going to be injected are flushed for 1 hour using degassed ultra-filtered KCl solution (using the same flushing method as for conductivity measures).

Samples should be *at least* 1 cm longer than the last distance from the injection site that you are going to sample. This is because if there are vessels that are longer than your sample, the silicone tends to form a drop at the end of the stem and sometimes pushes back up into other vessels at that end of the sample (this would cause you to over estimate how many vessels are actually filled at that distance).



Back filling of vessels because of silicone build up at the far end of the stem. (Usually this does not extend very far into the sample, but sampling at the very far end of the sample should be avoided).

I usually sample segments that are at least 25 cm in length and serially section up to 24 cm. For very long vesselled species, we inject samples that are >50 cm in length and section up to 48 cm.

The basal section of a stem is fit with a grommet and a piece of tubing. These will be thrown away at the end of the experiment (the silicone will not come off of them).

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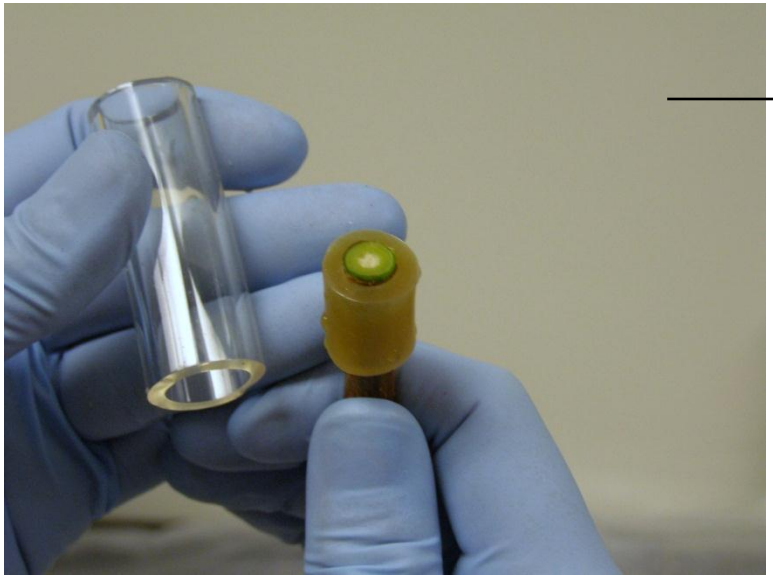
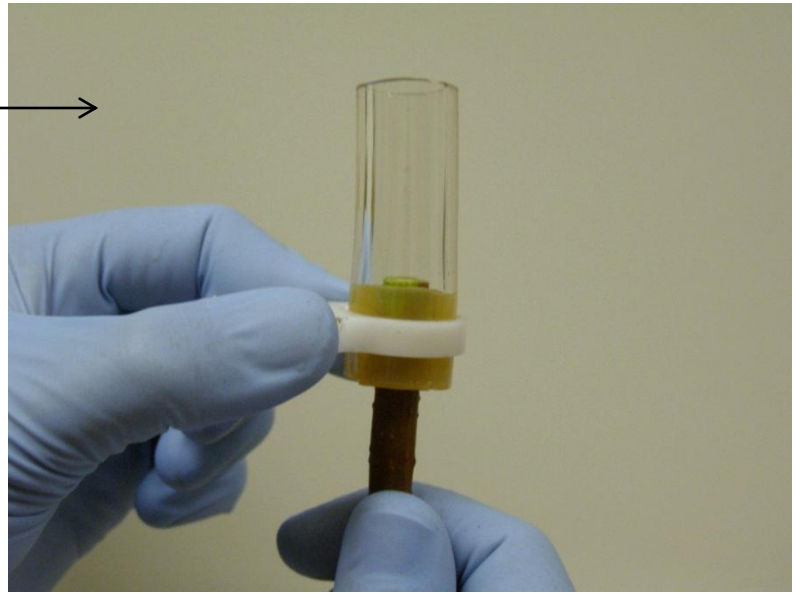
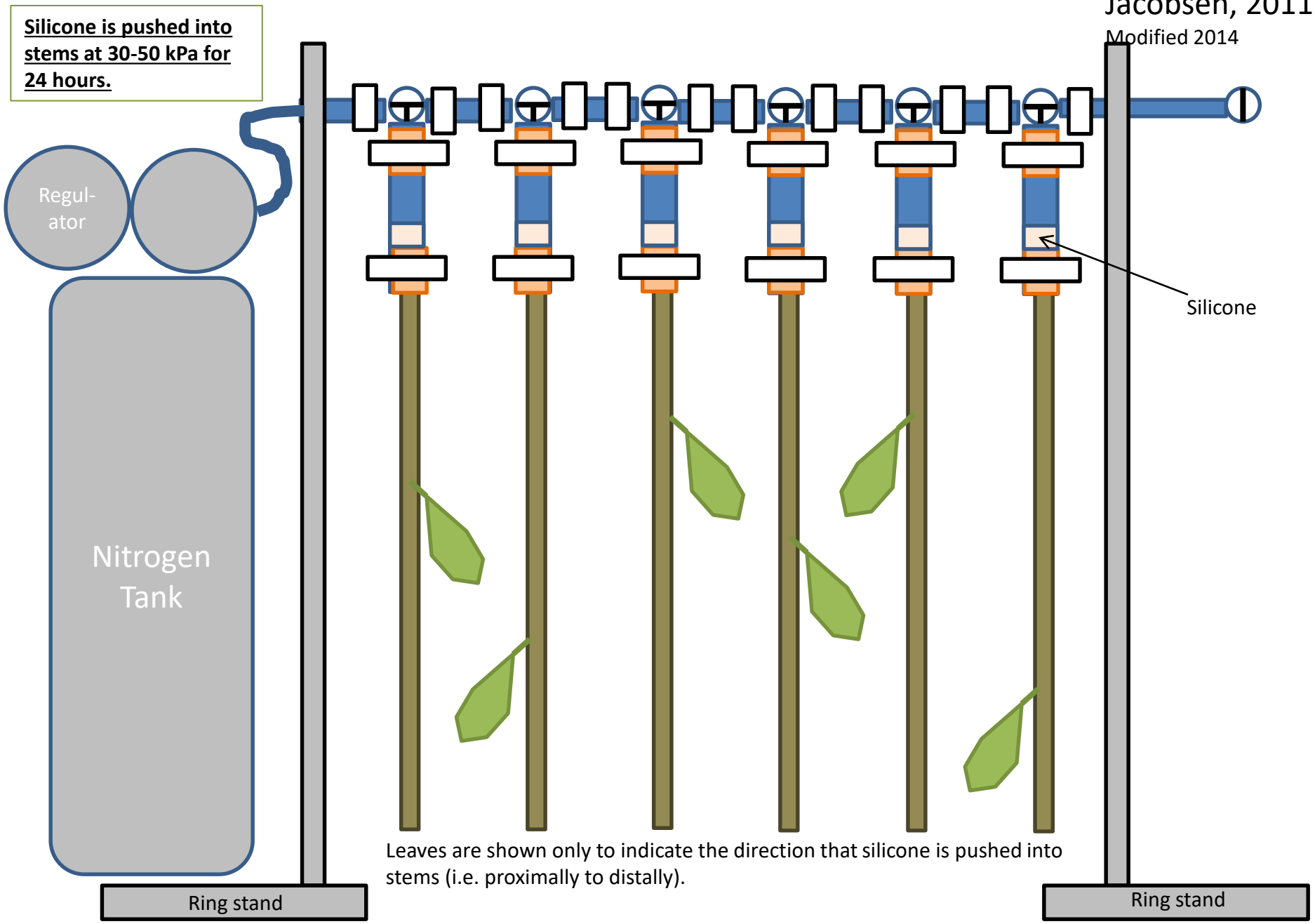




Silicone is carefully poured into the tubing. If silicone gets on the sides of the tubing, then the tubing will need to be replaced and the silicone re-poured because the silicone is quite slippery and the seal of the tubing will not hold under pressure if there is silicon in the seal.

Photos on this page were taken by Mike Tobin


Silicone is pushed into stems at 30-50 kPa for 24 hours.
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Regul-
ator
Nitrogen
Tank
Silicone
Leaves are shown only to indicate the direction that silicone is pushed into stems (i.e. proximally to distally).
Ring stand
Ring stand

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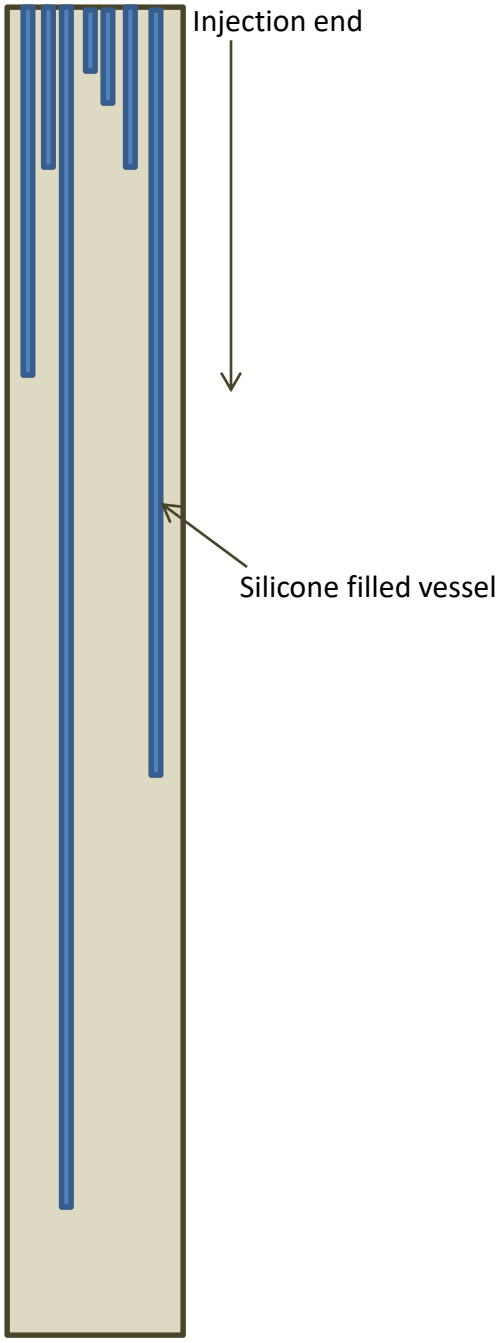

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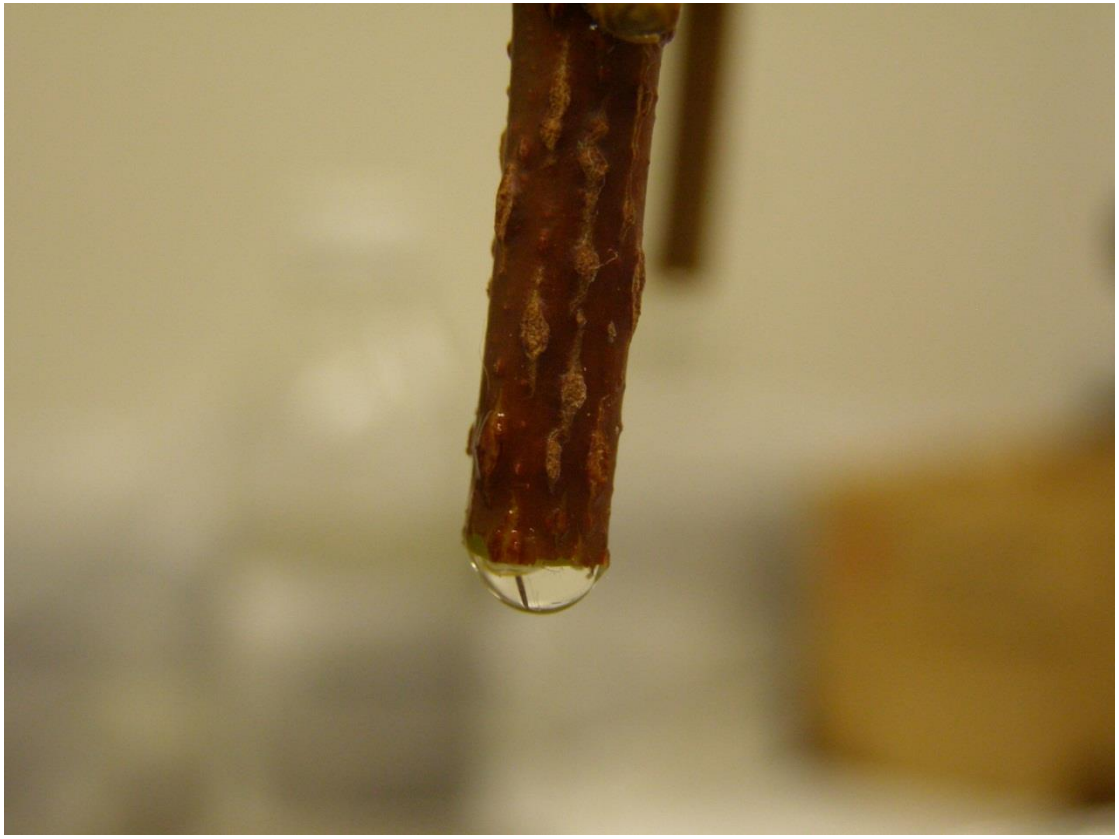
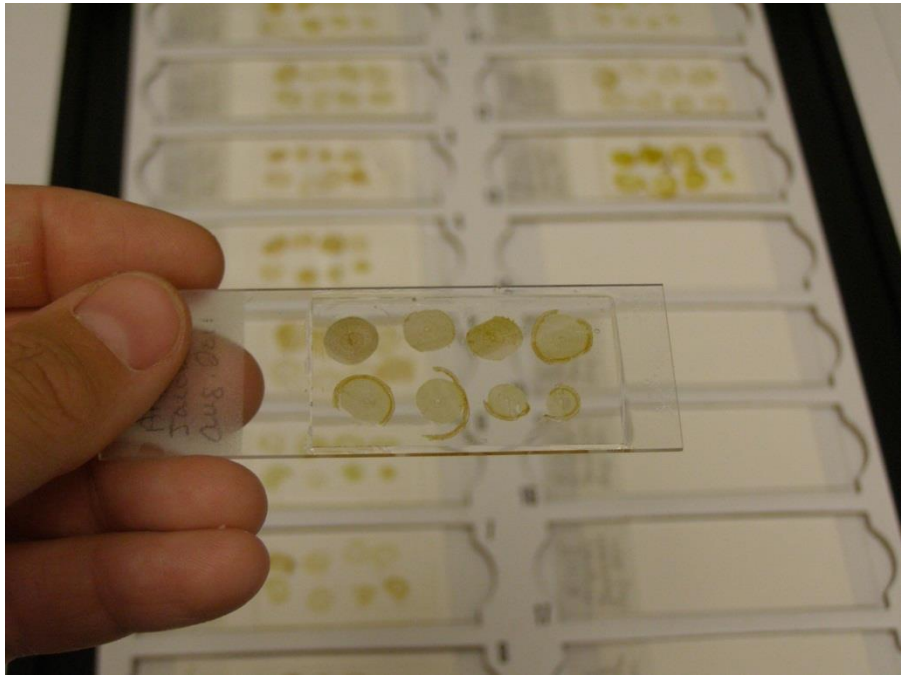
Not very much silicone is actually used (only enough to fill vessels that are cut open to the first terminal vessel element) so the silicone amount in the tubing will not change noticeably over the 24 hour injection period. Typically, the only visible sign that the silicone is moving into the stem is a small drop of water that forms at the far end of the stem immediately after the pressure is turned on. There is typically only a small amount of water that comes out (equal in volume to the silicone being pushed in) and this drop dries as soon at the silicone reaches the terminal ends of most vessels and is no longer displacing water. This happens relatively rapidly. The injection is conducted for 24 hours to make sure that the longest and narrowest conduits fill with silicone and so that the silicone has time to begin to cure before the stems are moved.



After the 24 hour injection period, stems are allowed to cure at room temperature for at least 48 hours before being sectioned.

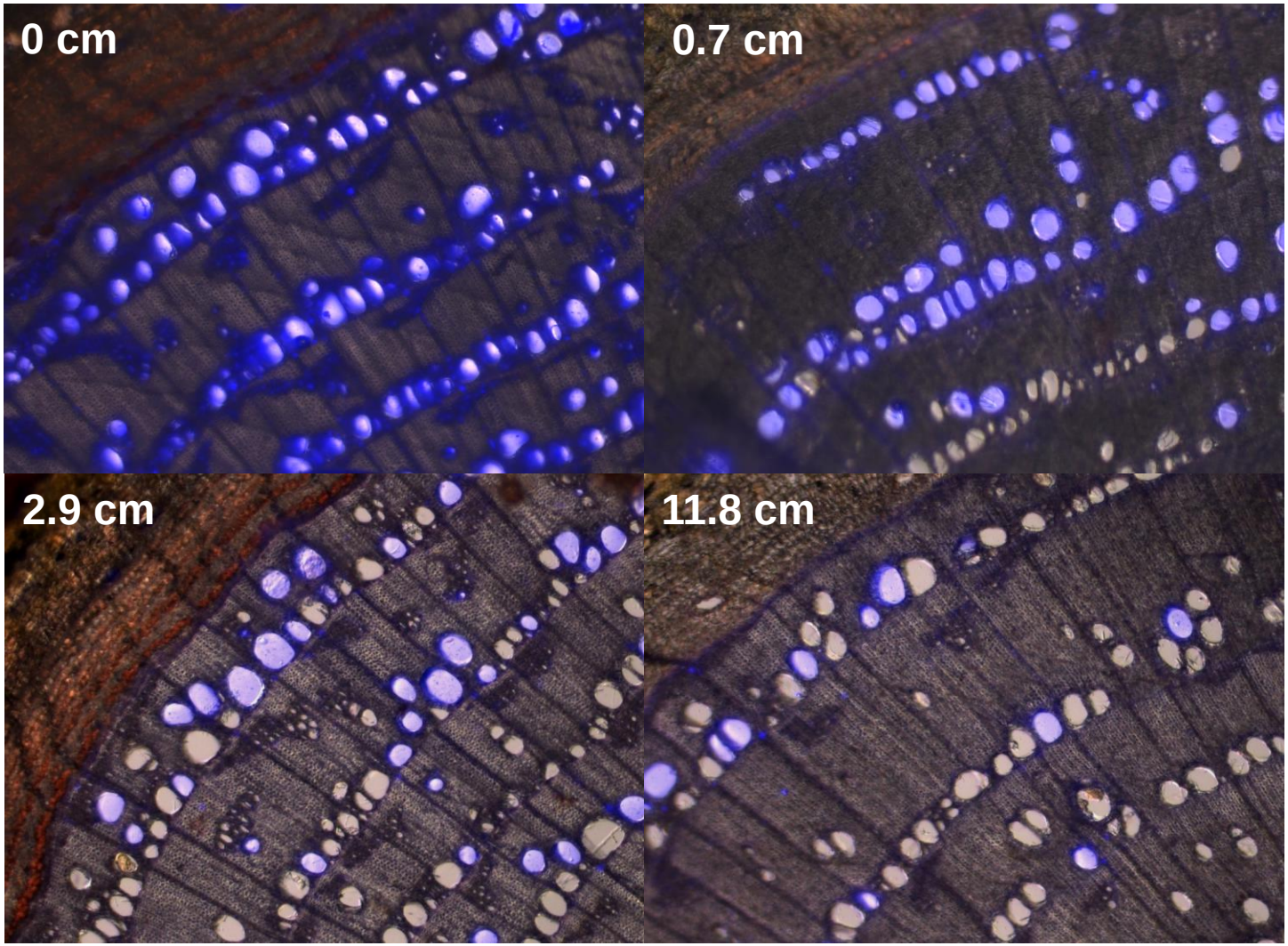
If samples have been allowed to dry for significantly longer than that, we usually soak them in water overnight (or longer) to rehydrate the samples, which makes it easier to section.

Samples are sectioned serially (at a log distributed number of sampling points from the injection end). Because most vessels are very short, many sections are taken from the first few centimeters of the sample and then sections can be taken further apart as the distance from the injection point increases and the density of filled vessels rapidly declines.



Sections are examined using UV fluorescence and the % of filled vessels is determined for each sampled distance from the injection point.

These data can then be used to calculate the vessel length distribution and the mean vessel length of the measured samples. There are equations for these calculations in the publications listed at the beginning of this document. Alternatively, Dr. John Sperry has recently posted wonderful excel files on his methods webpage that can calculate these parameters for you (http://biologylabs.utah.edu/sperry/methods.html).



Finally, there are some other neat ways that you can use silicone injected stems to examine xylem vessel structure. Silicone fills vessels completely, including completely filling terminal vessel elements and excised vessels can be interesting to examine for traits such as vessel element length and the areas of vessels that contain inter-vessel pits.

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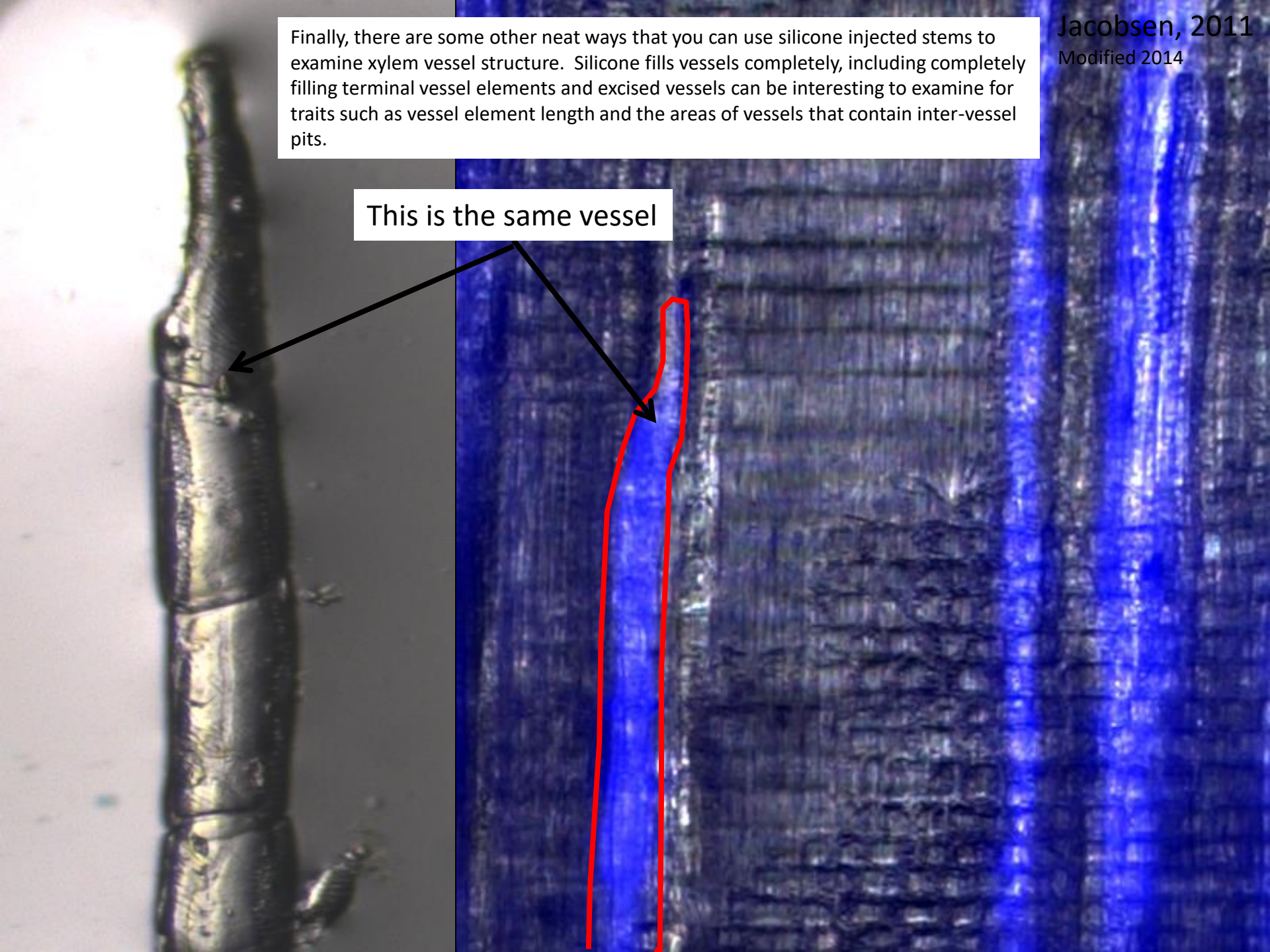

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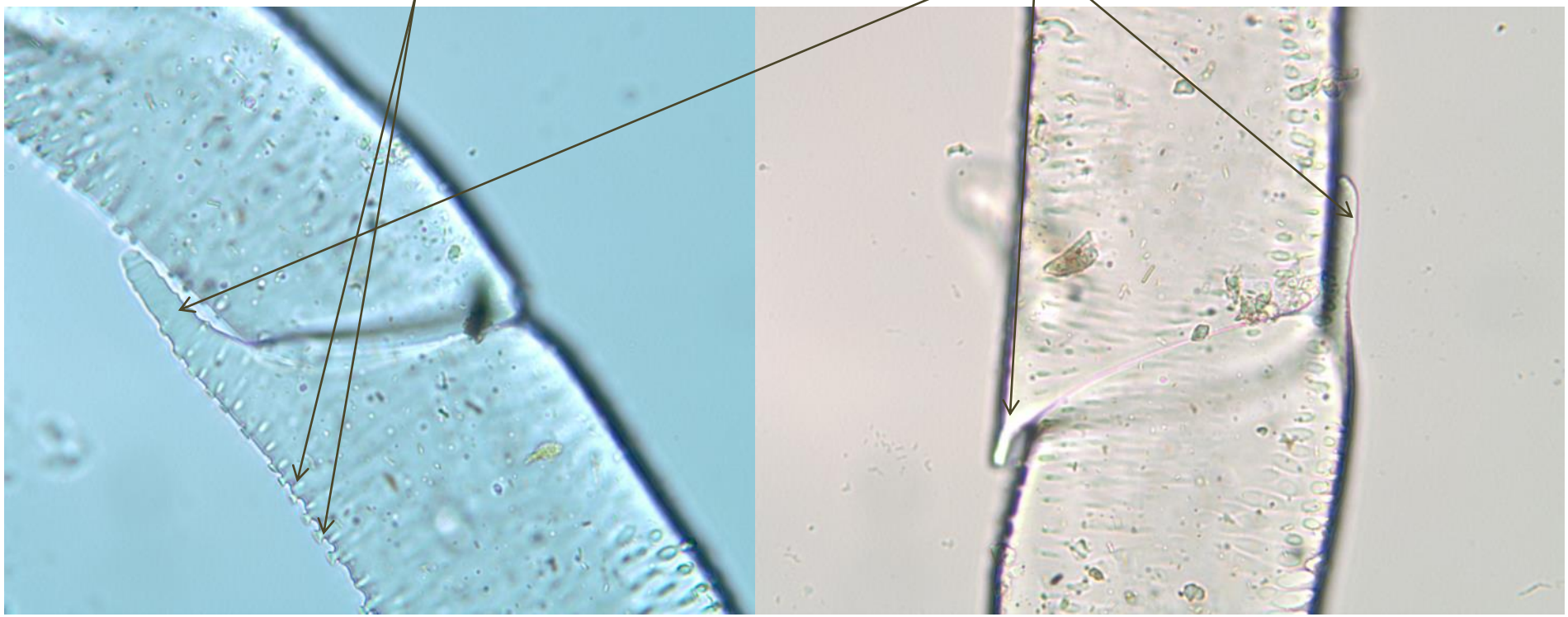
Excised vessel elements and vessels also show neat xylem structures such as vessel element tails and half filled (because the silicone does not go through pit membranes) pit chambers.



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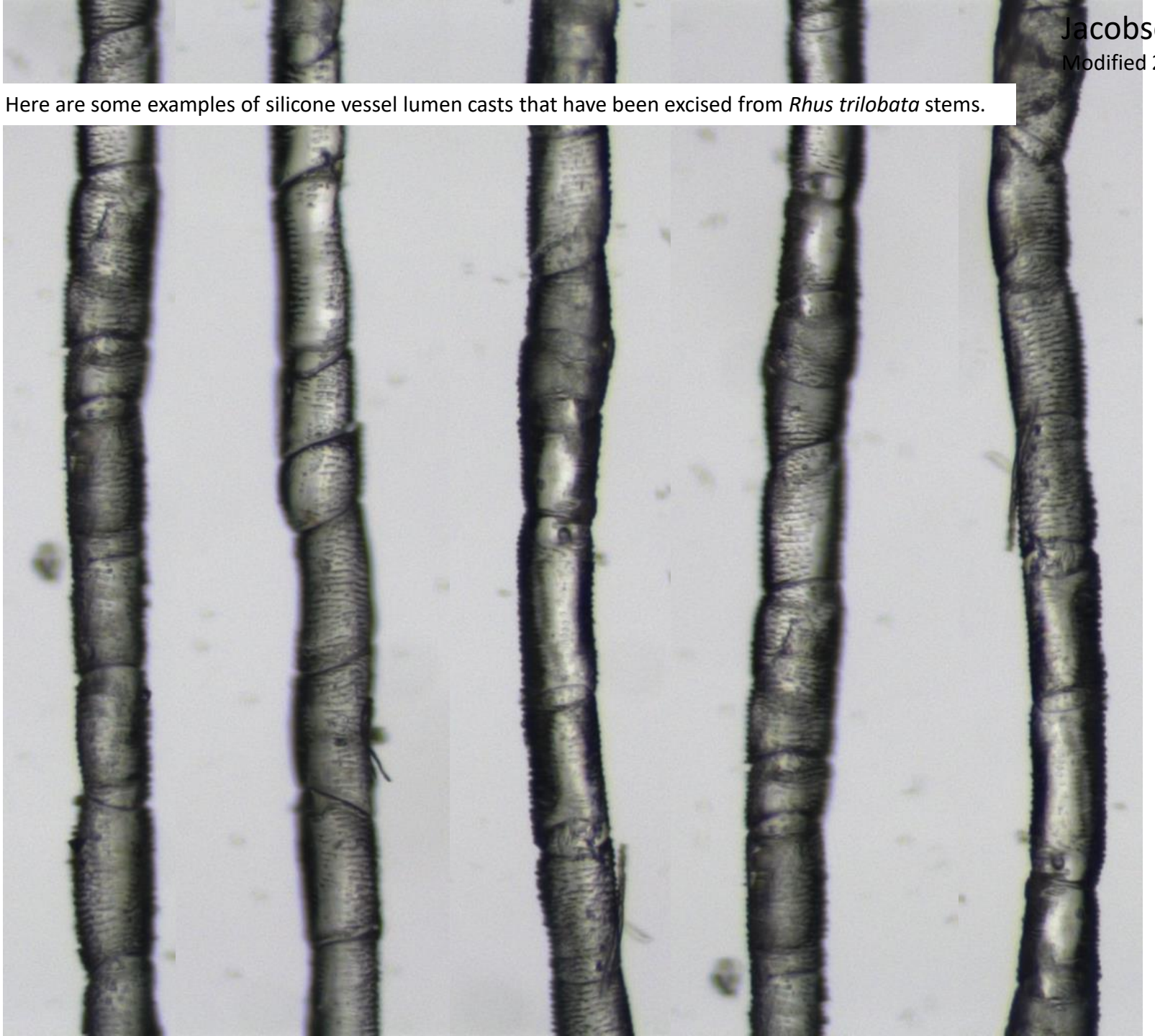
Here are some examples of silicone vessel lumen casts that have been excised from *Rhus trilobata* stems.



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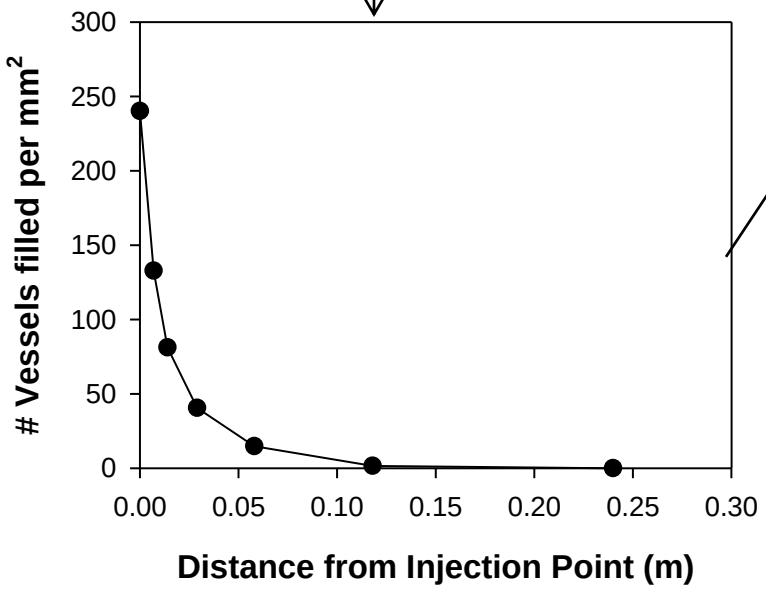
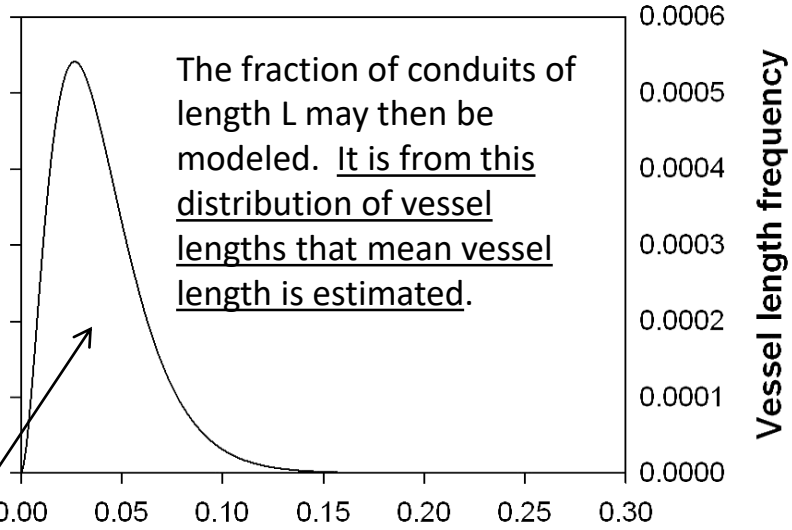
Data from sections are used to determine the number of vessels filled at increasing distances from the injection point. I usually count all of the vessels that are filled at each distance from the injection point, because these complete counts tell you exactly the number of conduits that remain open to the injection point. These whole-cross-section counts are then sometimes scaled by vessel density or percentage of filled vessels.

These data are then fit with a model, such as the exponential decay function reported in Wheeler et al. 2005.





Dr. John Sperry has written two excel macros to do these calculations based on your measured number of vessels filled with section distance. They are freely available on his methods webpage: http://biologylabs.utah.edu/sperry/methods.html#vessel_lengths