Jacobsen, 2011
(updated 2014)

The standard centrifuge method for the determination of xylem vulnerability to cavitation

Materials:

The rotor that we are currently using was constructed using custom rotor schematics supplied by Dr. John Sperry (Thank you, John!)

Our rotor was constructed by the Department of Physics and Astronomy Machine Shop at Michigan State University, East Lansing, MI, USA.

Changes to the rotor design relative to those described in Alder et al. 1997 (Journal of Experimental Botany 48: 665-674) include the modification of the rotor to hold three stems at once and the construction of the water reservoirs out of Lexan instead of Plexiglas (the old Plexiglas reservoirs sometimes shattered but the new Lexan reservoirs are very tough). Rotors can be constructed with differing diameters to accommodate different stem lengths—in this file rotors are show than can accommodate 14 and 27 cm long samples.

As of 2012, we are now also using "hydration reservoirs" to prevent sample ends from drying before and after spinning. This addition was first described and tested in Tobin et al. 2013 (Plant Biology 15: 496-504).

We are using a Sorvall Instruments RC5C refrigerated centrifuge.

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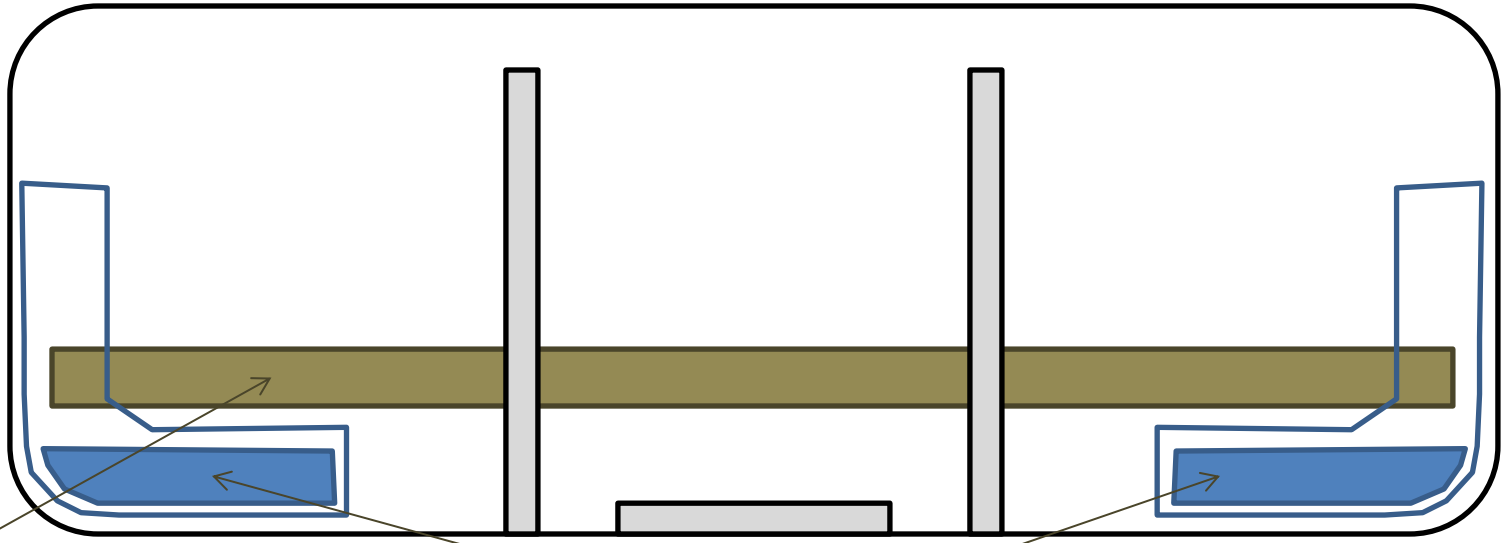

Stem or root sample is placed in the centrifuge between two reservoirs. The sample should be centered and should pass directly over the center of the rotor.

NOTE: Grommets should be removed from sample ends for spinning. We have found that the latex grommets interfere with samples if they are left on during spins.

Reservoirs are filled with equal amounts of fresh, degassed solution. We fill them to a height of 6 mm.

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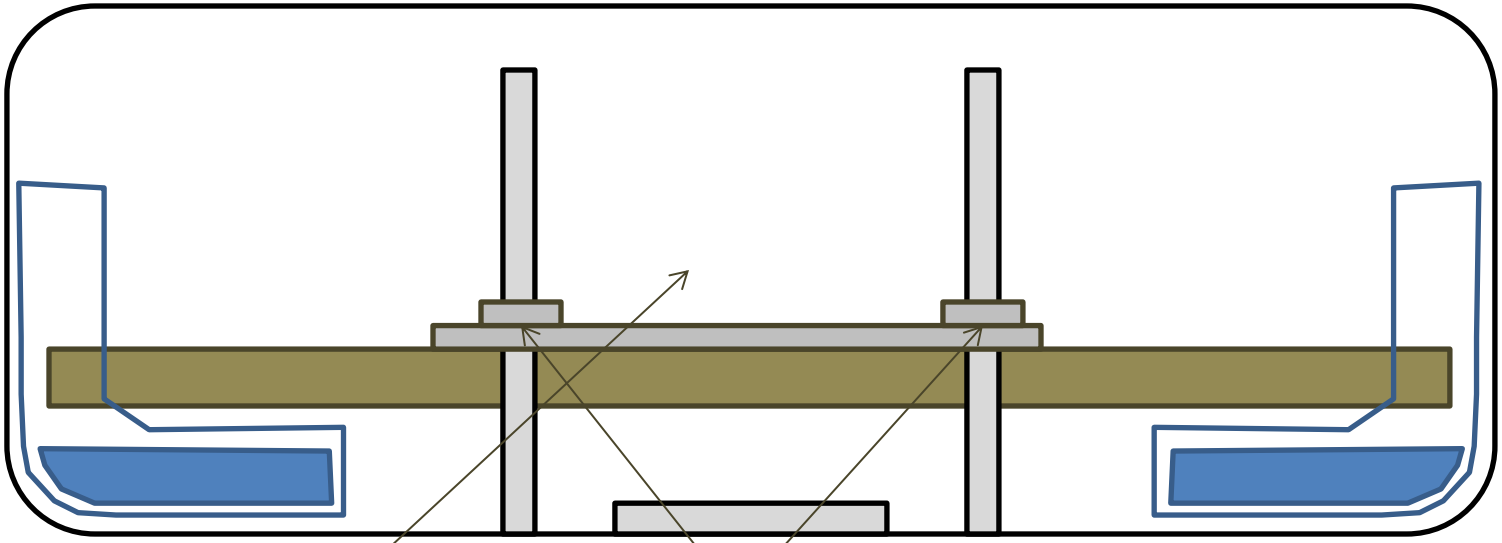

Sample is held in place by a thin metal plate and nuts that are screwed onto the upright metal bolts of the rotor.

The next sample is then loaded above this sample (but traverses two different reservoirs) and the process is repeated.

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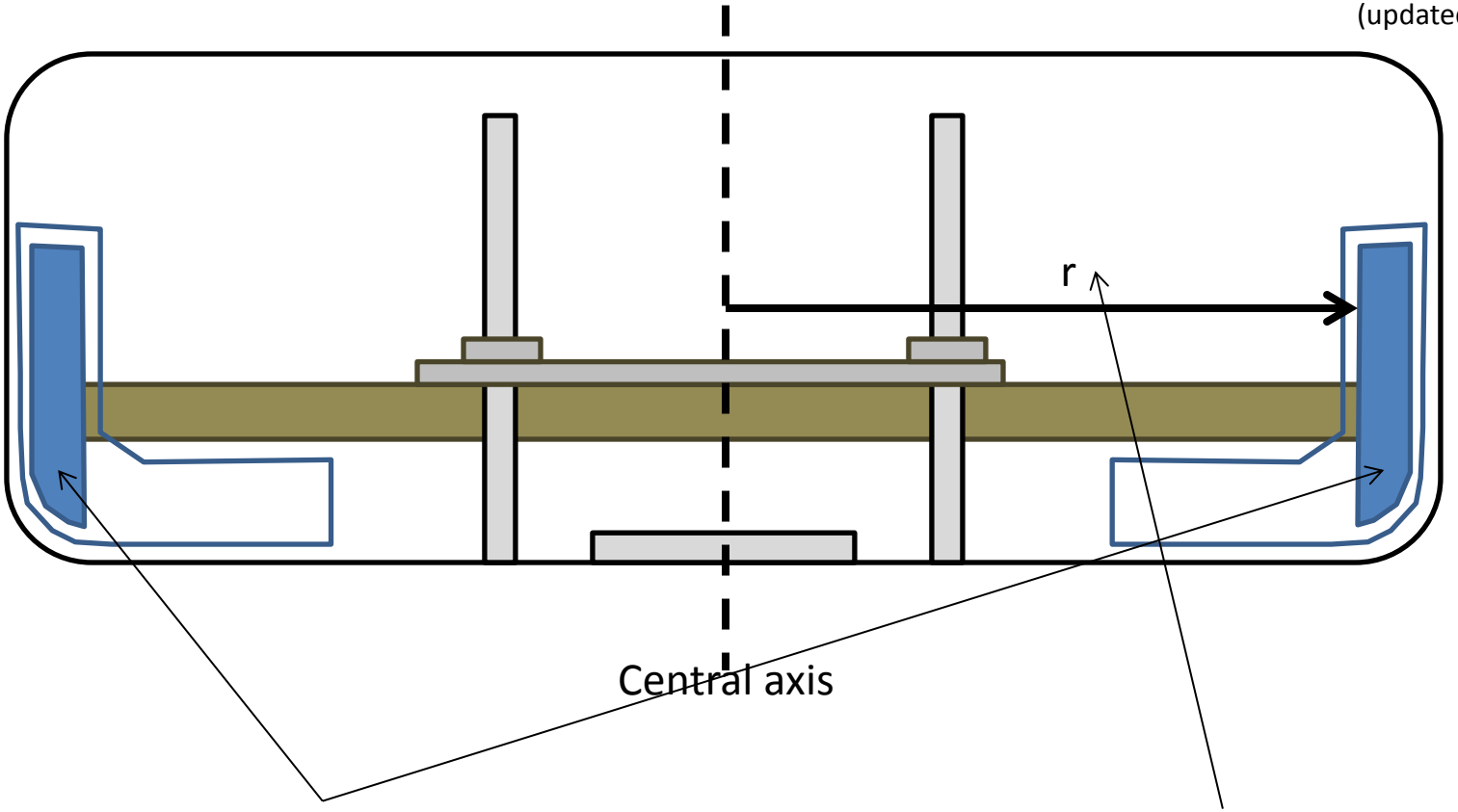
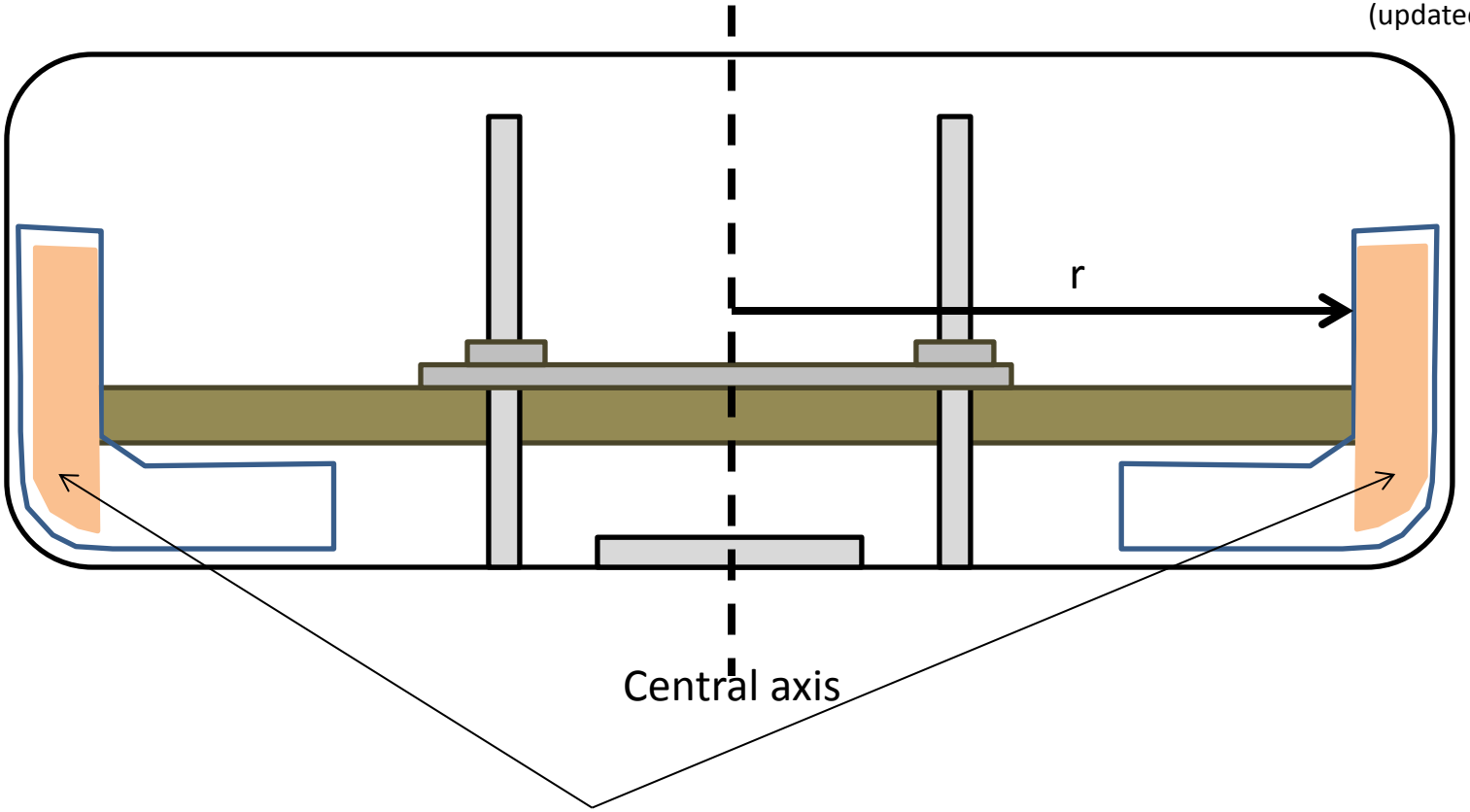


When the rotor is spinning, the solution is held against the vertical walls of the reservoirs and so the ends of the samples under water. Because the same amount of solution is loaded into each reservoir, they balance each other and there is no net flow through the stem while it is spinning.

The radius for calculation of the angular velocity is the distance from the center of the sample to the surface of the solution of the reservoirs when the sample is spinning. Because of the design of the reservoirs, the depth of the solution when spinning is the same as the height of the solution when the rotor is not spinning (i.e. 6 mm in this example)



With the hydration reservoirs, the solution is held entirely within sponges both before and after spinning. We fully saturate the sponges with degassed ultrafiltered solution and we have trimmed them so that they reach to the surface of the reservoirs. When using the hydration reservoirs, we keep enough solution in the reservoirs to fill them to their rim when the sponge and solution are present. This makes the calculation of r easier (i.e. from the sample center to the resevior; see above).

The solution in the sponges is replaced between every centrifuge run.

We tested a variety of sponge types and we've found that CoverGirl Make-up Master cosmetic sponges work quite well because they are hydrophilic and have a small pore size. Other types, including generic brands, did not work as well. There are likely other suitable brands that we did not test and this is something that one could easily evaluate if a different type of sponge were going to be used.

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The RPM needed to created a specific pressure can be calculated using the following equation:

$$\mathbf{RPM} = \left(\left((2 * (-\mathbf{P} * 10^6)) / (\mathbf{r}^2 * \boldsymbol{\rho}) \right) \text{^ } (1/2) \right) * 9.5493$$

Where **r** is the distance from the center of the rotor to the surface of the solution in the reservoirs (units: **m**),

ρ is the density of water (units: **kg m^{-3}**) [Note that this changes with temperature],

EXAMPLES: Density of water at:
18 C = 998.60 kg m^{-3}
20 C = 998.21 kg m^{-3}
22 C = 997.77 kg m^{-3}

and **P** is the desired Pressure in **MPa** (a negative number because the centrifuge generates tension).

The 9.5493 is in the equation to convert angular velocity (ω) in rad s^{-1} into rotations per minute.

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Sample numbers:

	radius	20 C	
P (MPa)	r	p	RPM
0.25	0.0645	998.21	3313
0.5	0.0645	998.21	4686
0.75	0.0645	998.21	5739
1	0.0645	998.21	6627
1.25	0.0645	998.21	7409
1.5	0.0645	998.21	8116
1.75	0.0645	998.21	8767
2	0.0645	998.21	9372
2.5	0.0645	998.21	10478
3	0.0645	998.21	11478
3.5	0.0645	998.21	12398
4	0.0645	998.21	13254
4.5	0.0645	998.21	14058
5	0.0645	998.21	14818
5.5	0.0645	998.21	15542
6	0.0645	998.21	16233
7	0.0645	998.21	17533
8	0.0645	998.21	18744
9	0.0645	998.21	19881
10	0.0645	998.21	20956
11	0.0645	998.21	21979
12	0.0645	998.21	22957

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For construction of a vulnerability curve:

1) Samples are cut to length underwater, the ends are trimmed with a razor blade (often the bark is trimmed away from the ends as well to prevent bark and phloem exudates from blocking the xylem), and samples are fit with appropriate sized latex grommets.
2) Samples are flushed with filtered degassed solution for 1 hr.
3) Maximum Kh is measured.
4) Samples are loaded into the centrifuge and spun at an initial high pressure (usually -0.25 or -0.5 MPa).
5) Samples are removed from the centrifuge and Kh is remeasured so that the loss in Kh can be determined.
6) Samples are reloaded into the centrifuge and spun at a more negative pressure (for instance, -1 MPa).
7) Samples are removed from the centrifuge and Kh is remeasured.

ETC…

Samples are usually run until they have greater than 95% PLC or the flow is so low that it is no longer measureable.


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RC5C


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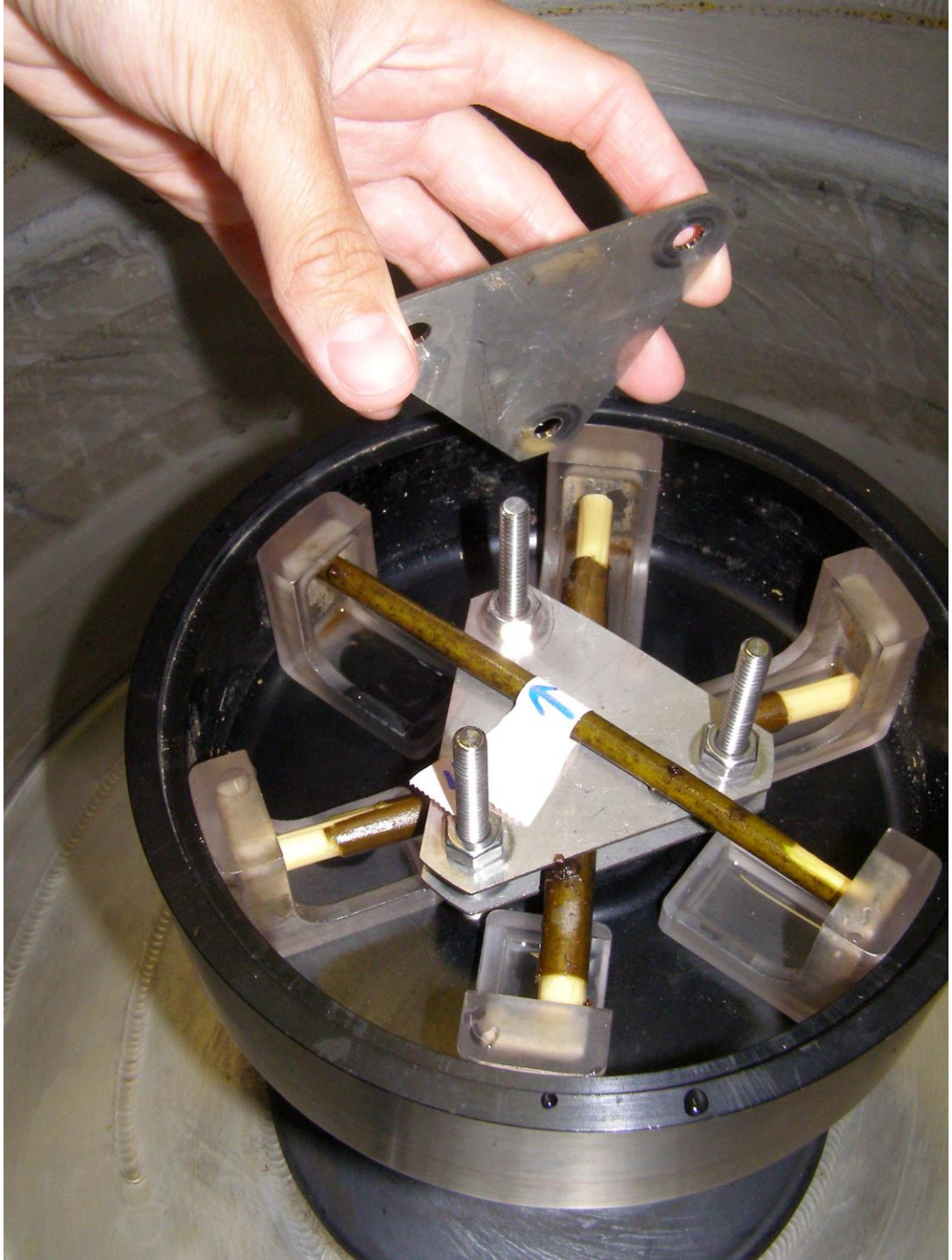

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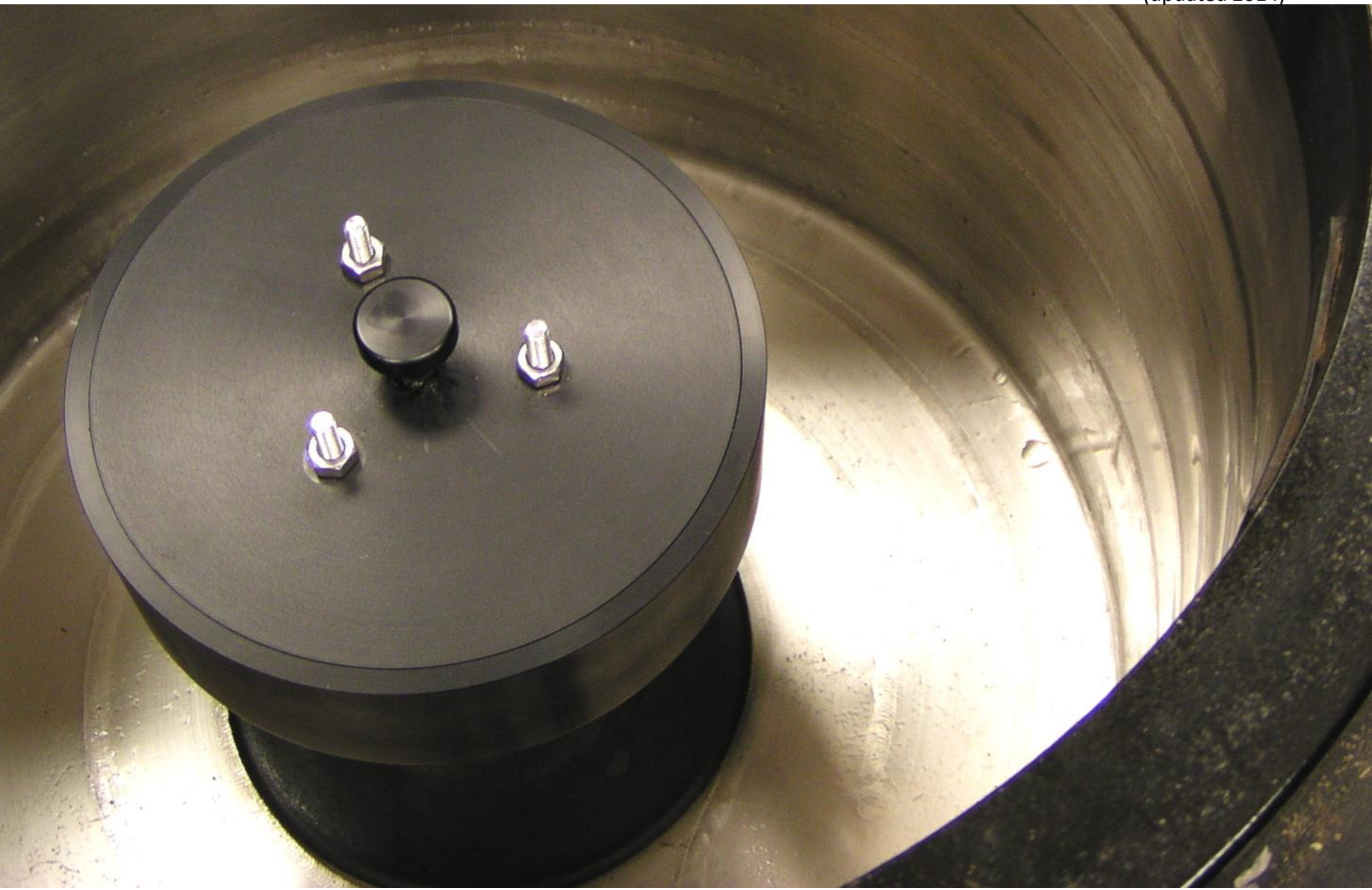

The Big Rotor – for 27.1 cm long samples

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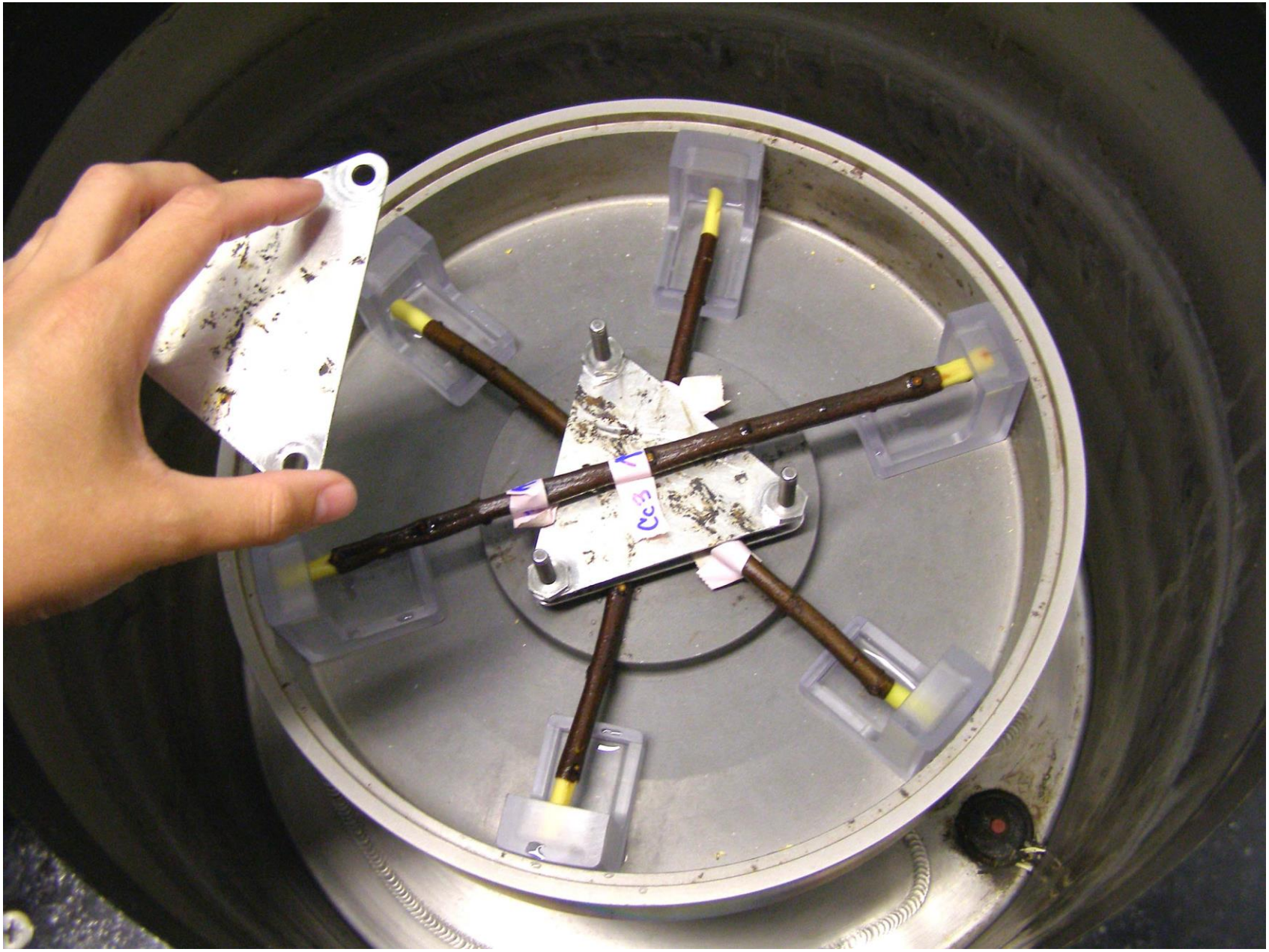

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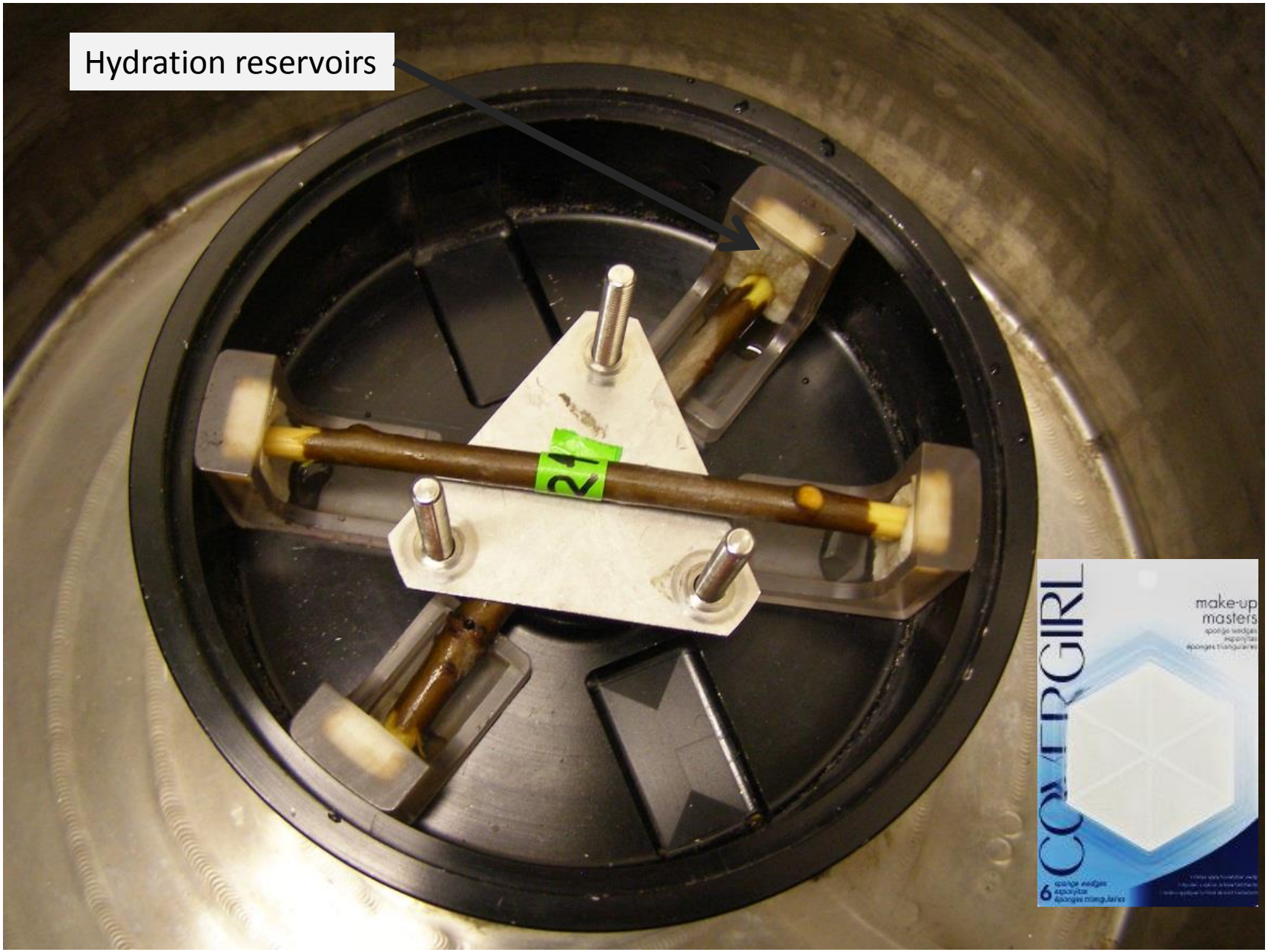

Stems are typically spun in the centrifuge for 5-6 minutes at the desired RPM. (The time varies because for faster spins it takes some time for the centrifuge to get up to speed).

Stems are removed from the rotor as soon as it has stopped spinning so that cut ends are not in air for any length of time.

Some species are particularly sensitive having cut ends in air, even for a very brief period of time (causing the "repeat-spin effect"; Tobin et al. 2013). We are currently including small artificial sponges in the reservoirs to hold water against the cut ends of stems even when the rotor is not spinning (CoverGirl makeup sponge wedges because they hold up well to being vacuum infiltrated with fresh degassed solution in between each spin). Other types of sponges would probably work provided that they can handle being degassed and sterilized. The wedges are cut down to fit in the back wall of the reservoirs.

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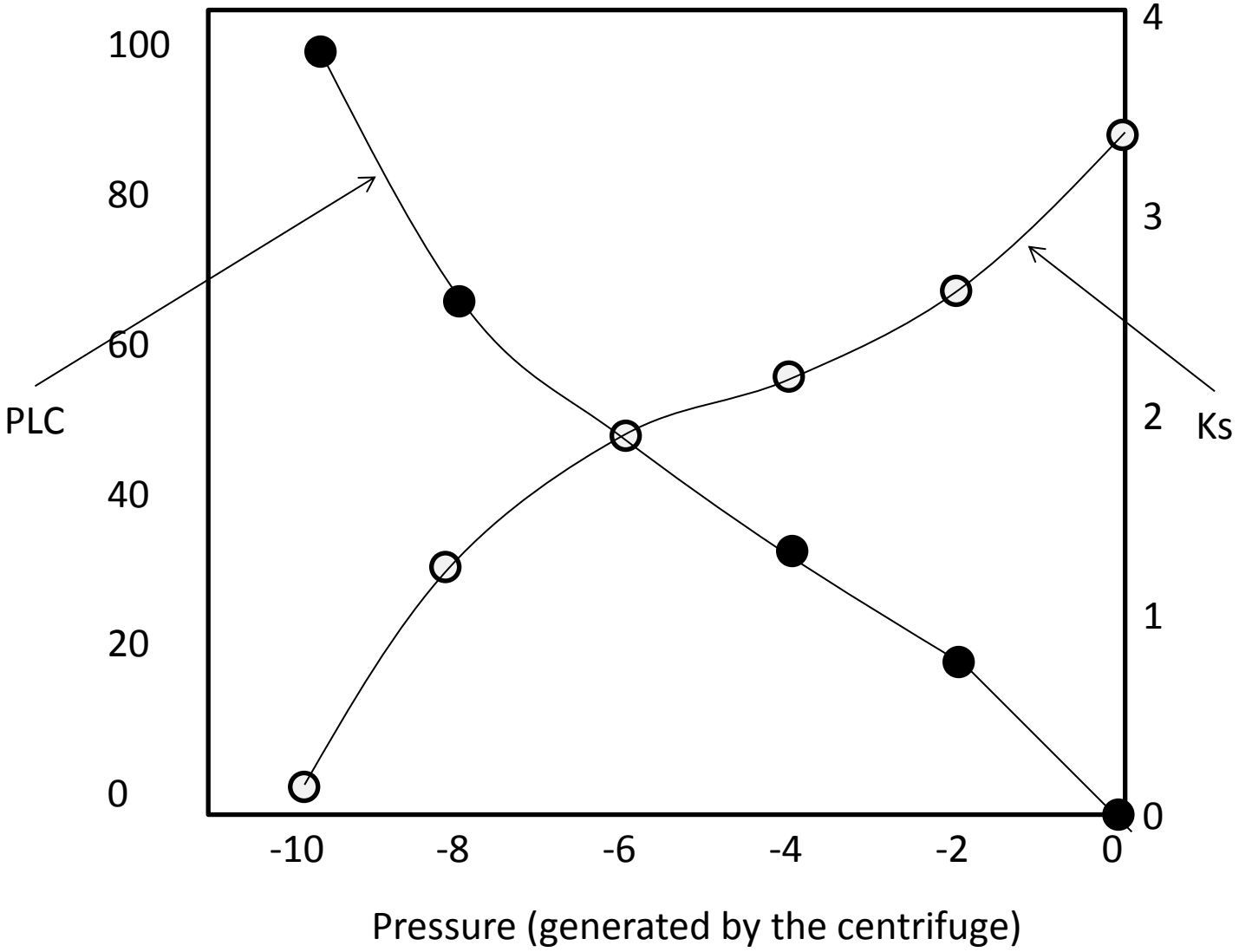
A vulnerability to cavitation curve is constructed by plotting the percentage loss in hydraulic conductivity (PLC) (relative to a flushed Kmax) with declining pressure. It is recommended that maximum Ks (Kh divided by the cross sectional xylem area) be reported or that Ks be plotted as well as PLC (Sperry et al. 2012, Hack et al. 2014).



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Fatigue Correction

Sometimes “fatigue-corrected” vulnerability curves are constructed (see Hacke et al. 2001 for a description of cavitation fatigue). These curves use the Kh at a high pressure (usually -0.25 or -0.5 Mpa, but also potential the maximum predawn water potential measured seasonally on plants in the field) as the Kmax instead of the flushed value. This is to remove vessels that may be artificially more susceptible (due to damage) or which may never have been functional in the intact plant from the curves.