

Turgor Loss Point (TLP)

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Introduction

We assessed the leaf turgor loss point, TLP in MPa, from a previously established relationship with the osmotic potential at full hydration, π_{osm} in MPa. π_{osm} is linked to the equilibrium solute concentration value C_0 (in $mmol\ kg^{-1}$) directly measured with a vapor pressure osmometer (Vapro 5600, Wescor, Logan, UT). This is referred as the osmometer method ([Maréchaux et al. 2020](#); [Bartlett et al. 2012](#)).

Protocol

Installing Vapro

1. Turn on Vapro the day before for the thermocouple's stability
2. Test Water Quality
3. Clean
4. Calibration
5. Control tests
6. Verify temperature
7. Always have the black diamond at the center of the display
8. Use daily: * clean beforehand * select automatic mode (10 runs)

First day

1. Carefully remove the leaf from the zip lock bag
2. Cut off the petiole under water
3. Place the leaf in a plastic upright glass with the petiole in the water
4. Put 12h in fridge to hydrate overnight

Second day

- Vapro:
 1. check distilled water in vapro reservoir
 2. clean
 3. select automatic mode (10 runs)
 4. make sure vapro software is on
- Sample measurement:
 1. Sample from a leaf a 5 mm disc with a cork borer: avoid 1st and 2nd order veins to avoid apoplastic dilution that would lead to less negative osmometer values
 2. Wrap disc in tin foil
 3. Immerse in liquid nitrogen for at least 2 min using metal tea ball
 4. Puncture 10-15 times with needle

5. Place in vapro chamber In total, discs are exposed to air for less than 40 seconds for all the steps.
 6. Record value C0 when the difference between consecutive 2-min measurements fell below strictly 5 mmol.kg⁻¹ after at least three runs.
 7. If error! or Nr_Run > 10 : + try a 2nd cycle with same leaf + try a 3rd cycle with another leaf + otherwise record NA
- Maréchaux, Isabelle, Laurent Saint-André, Megan K Bartlett, Lawren Sack, and Jérôme Chave. 2020. "Leaf Drought Tolerance Cannot Be Inferred from Classic Leaf Traits in a Tropical Rainforest." *Journal of Ecology* 108 (3): 1030–45.

Material

- Vapor pressure osmometer (Vapro 5520, Wescor, Logan, UT)
- Vapro software (Vapro Lab Report)
- Fridge
- Plastic glass
- Liquid Nitrogen
- Ziplock bag
- Paper towel
- Distilled water
- Metal tea ball
- Tin foil
- Needle
- Liquid nitrogen gloves
- Liquid nitrogen goggles
- Liquid nitrogen container
- 2 Tweezers
- Cork borer

References

Bartlett, Megan K, Christine Scoffoni, Rico Ardy, Ya Zhang, Shanwen Sun, Kunfang Cao, and Lawren Sack. 2012. "Rapid Determination of Comparative Drought Tolerance Traits: Using an Osmometer to Predict Turgor Loss Point." *Methods in Ecology and Evolution* 3 (5): 880–88.