Measuring osmotic potential and turgor loss point using a vapor pressure osmometer

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Overview

This protocol explains how to rapidly measure turgor loss point and osmotic potential at full hydration using a vapor-pressure osmometer instead of the standard but more labor- and time-intensive pressure-volume curve method.

Background

This detailed protocol is based on the method presented and validated in Bartlett et al. 2012.

Turgor loss point is an important determinant of ecological and physiological drought tolerance (Cheung et al. 1975; Tyree et al. 1978; Sack et al. 2003; Baltzer et al. 2008; Bartlett, Scoffoni, & Sack 2012). The amount of solute, as assessed by the osmotic potential at full hydration, is the cellular trait that plants vary to adjust turgor loss point in response to drought, and species differences in these traits translate into variation in drought tolerance (Lenz et al. 2006; Bartlett, Scoffoni, & Sack 2012). These traits are typically estimated using the pressure-volume curve method (available from *PrometheusWiki* by Sack et al. 2011), which characterizes the relationship between leaf water potential and leaf relative water content in a drying leaf (Tyree & Hammel 1971; Koide et al. 1989). Because that method takes 1-2 days to complete for 5-6 leaves, it has been impractical to measure osmotic potential at full turgor and turgor loss point for many species in diverse clades and communities. This protocol presents a rapid method for measuring these traits, requiring 1-2 hours for 6 leaves, or about 10-15 minutes per sample, enabling characterization for diverse communities relatively rapidly (Maréchaux et al. 2015).

In brief, sample discs are collected from rehydrated leaves, and frozen in liquid nitrogen to fracture cell membranes and cell walls and to allow symplastic contents to mix upon thawing (Kikuta & Richter 1992; Callister et al. 2006). The leaf disc is then punctured to facilitate equilibration, and then sealed in a vapor-pressure osmometer. The osmometer compares the electrical resistance through the thermocouple in the sample chamber with the resistance under ambient conditions to determine the change in dew point temperature, and calculates the solute concentration and the osmotic potential of the leaf tissue (Vapro Manual, Appendix C). From this osmotic potential at full hydration, one may estimate the pressure-volume curve osmotic potentials at full hydration and turgor loss point using a calibration dataset of 30 species with diverse leaf traits and native habitats (Bartlett et al. 2012).

Materials/Equipment

Vapor pressure osmometer (Wescor, Vapro 5520, Logan, Utah, USA)

2 sizes of Whirl-Pak bags (Whirl-Pak, Nasco, Fort Atkinson, Wisconsin, USA), including one large enough to hold the sample leaves and one somewhat larger for double-bagging
Paper towels
Kimwipes
Aluminum foil
8mm diameter cork borer
Pipette tips (any size)
Sharp-tipped tweezers (Wescor, Logan, Utah, USA)
Liquid nitrogen container
Approximately 1-2L of liquid nitrogen per day of sampling
Tongs
Latex or nitrile gloves
Cold-protective gloves
Calipers (at least 0.1mm resolution)
Envelopes
Drying oven

Units, terms, definitions

Procedure

1. Collect shoots and recut underwater at least 2 nodes distal to the original cut. Place shoots in water and cover with a humidified but not wet opaque plastic bag, with wet paper towel. Allow shoots to rehydrate overnight in a cool, dark location.

2. In the morning, cut at least 2 leaves from each shoot in air. Immediately place the leaves in Whirlpak bags, and breathe into the bags to fill them with high humidity. Double-bag the leaves and put a piece of wet paper towel in each outer bag to prevent evaporation.

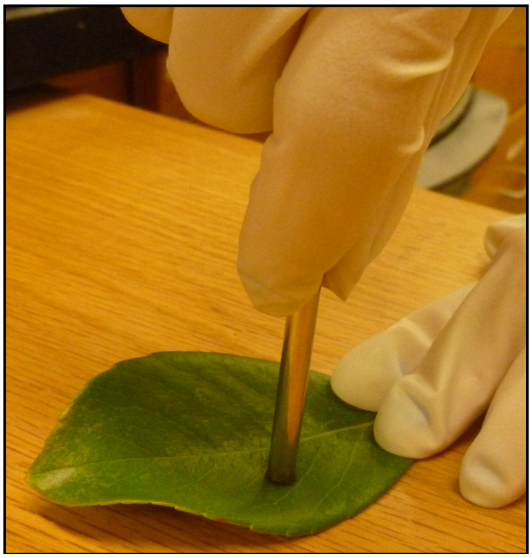
3. Place the double-bagged leaves in an opaque plastic bag and keep refrigerated until sampling (see Notes).

4. Prepare aluminum foil to later make envelopes for the leaf discs by cutting or tearing squares of ≈ 2 × 2 cm. Label the foil squares to tell them apart later.

5. All of the following steps should be done while wearing latex or nitrile gloves to protect from liquid nitrogen splashes and to prevent fingerprints from contaminating the osmometer.

6. Take out a leaf and quickly wipe both sides so it is clean and dry. Rub away trichomes, if possible (see Notes). The leaf will begin drying as soon as it is out of the bag, so complete all of the following steps as quickly as possible. For consistency with the calibration dataset, we recommend 15-20 seconds between taking the leaf out of the bag and putting the disc in the liquid nitrogen.

7. Use the cork-borer to cut a disc out from the leaf, midway between the leaf tip and base, and between the midrib and margin. **It is very important to avoid sampling secondary veins** (see Notes)**.**

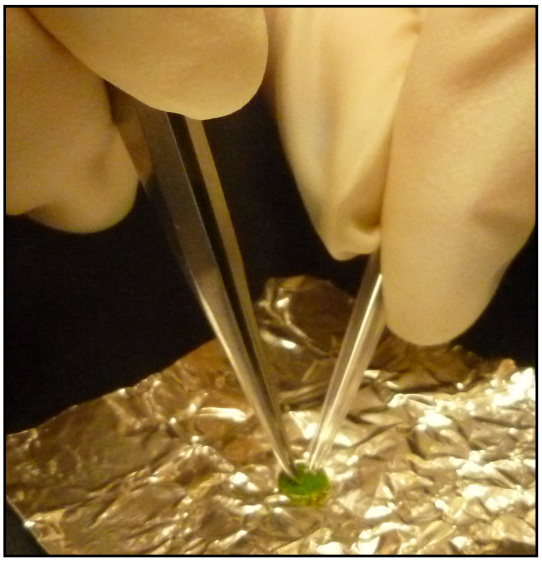


8. Quickly fold the disc inside the foil square, and use tongs to place the foil envelope into liquid nitrogen for at least 2 minutes. Freezing will keep the disc from drying out.

9. Open the osmometer chamber and hit 'Select" to bring up the Mode menu, and select "Auto Repeat" to automatically take up to 10 repeated measurements.

10. Take out the foil envelope and quickly open it next to the chamber. Hold the disc down with a pipette tip and puncture it 5-10 times with the tweezers, and immediately seal it in the chamber. This allows for faster evaporation out of the disc

into the chamber, and reduces your equilibration time. It is extremely important to do this step as quickly as possible- we strongly recommend 15-20 seconds.



11. Keep track of the osmometer measurements. When the osmolality value changes less than 5 mmol/kg then the value has reached equilibrium. This usually takes about 10-15 minutes, or 4-5 measurement cycles.

12. When equilibration has been reached, open the chamber and remove the disc with a Kimwipe, and carefully clean the chamber. The chamber must be perfectly dry, shiny, and lint-free to prevent contamination.

13. Measure leaf fresh area, thickness, and dry mass to calculate leaf density to improve estimations of pressure-volume curve osmotic potential. Measure

thickness using calipers at the bottom, middle, and top of each leaf, avoiding the secondary veins. Leaves should be dried > 48 hours in a 70^{o}C oven before weighing.

14. See attached spreadsheet for conversions to pressure-volume curve parameters.

Other resources

Notes and troubleshooting tips
General osmometer tips:

1. Calibrate the osmometer according to the manufacturer instructions every day that you make measurements, and every 6-8 hours if you're making a lot of measurements in one day.

2. The osmometer requires a fairly stable temperature to maintain its calibration. Close the chamber without a disc inside and allow it to return to its "Temperature Stability" display every 5 or 6 samples, so it can correct its calibration for small shifts in temperature. If you're using the osmometer in a room without air-conditioning, you may need to check the calibration using the calibration standards every 3-4 hours to ensure accurate results.

3. Use the osmometer on the "osmolality" instead of the "water potential" setting, even though the method is intended to measure water potential. The Vapro 5520 has a programming bug that makes calibration less reliable in the water potential setting.

Leaf sampling tips:

1. Depending on how many samples you have, you may have more than you can measure in one day. Collect all of the rehydrated leaves in Whirlpak bags at the same time, so they all have the same rehydration time. They can sit in the dark, humidified, refrigerated bags for at least overnight, and possibly up to a week, although this needs further testing. If you freeze more leaf discs than you can measure in one day, it is possible to double-bag the foil envelopes in humidified Whirlpak bags with moist paper towel, and freeze them overnight. It is important to ensure the foil envelopes are in high humidity but do not get wet. We found no effect of storing the envelopes overnight, but the discs did dry out over 2 nights, so try to only freeze what you can measure in 1-2 days.

2. Trichomes can interfere with the precision of your measurements by absorbing some of the water evaporated into the chamber, or coming loose and contaminating the thermocouple. Rub the trichomes off the leaf, if possible, or at least take note of which samples have trichomes.

3. Secondary veins should be avoided because they contain dilute apoplastic water, which can mix with the more concentrated symplastic water and make the osmotic potential appear closer to 0 than it actually is.

Links to resources and suppliers

The Vapro 5520 has since been discontinued and replaced with the 5600, which can be used in the same way for leaf discs.
http://www.wescor.com/biomedical/osmometer/vapro5600.html

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Health, safety, and hazardous waste disposal concerns

Always wear close-toed shoes, long trousers, and long sleeves when handling the liquid nitrogen. Use latex or nitrile gloves to handle the tongs, and cold-protective gloves to move the liquid nitrogen container. Always use liquid nitrogen in a well-ventilated room, and do not transport a dewar of LN2 in an elevator. Two or three breaths of nitrogen gas can empty your lungs of oxygen and unconsciousness can occur in less than one minute. Accidental nitrogen asphyxiation is thought to kill 8 per year in the United States alone (http://en.wikipedia.org/wiki/Inert_gas_asphyxiation). Stay safe kids!