

NGEE Tropics: BNL - STRI Leaf Water Potential Protocol

This protocol is for the measurement of leaf water potential in support of NGEE-Tropics measurements. This protocol is adapted with minor changes from the 2016 NGEE Tropics ENSO Leaf Water Potential Protocol..

Instruments & Equipment

- PMS pressure chamber
- Instrument hose
- Compression gland or stoppers of varying sizes
- Compressed gas (220 cf N) nurse tank
- Regulator
- Magnifying glass

Abbreviated Sampling Plan

We will measure and sample upper canopy full sun leaves on the trees that have been selected for sap flux. This requires sap flux trees to be located in the footprint of canopy access (tower, crane). Leaves will be sampled before dawn and around noon on two days. For each sampling round, 2 - 3 leaves of each age category (young, mature, old) should be sampled. After LWP is determined, leaf spectra is measured, then discs will be taken for measurement of LMA (g m^{-2}), elemental CHN content and to provide an archive sample.

Leaf Water Potential Instrument preparation

www.pmsinstrument.com/file_download/6/Model+1000+Operating+Instructions.pdf

1. Attach regulator to nitrogen cylinder
2. Connect hose to regulator
3. Insert other hose end in pressure chamber gas inlet by retracting quick-connect fitting
4. Tug on hose lightly to ensure secure connection
5. Turn Control Valve on the instrument to "OFF" position.
6. Slowly open gas cylinder valve
7. Adjust Rate Valve
 - a. Place solid rubber stopper inside recessed area under lid.
 - b. Seal lid on chamber by pushing down and turning clockwise to the stop. Lid must be turned completely to the stop to close the brass Safety Valve. If stainless steel piston is not depressed, chamber will not pressurize.
 - c. Turn control valve to CHAMBER position and pressurize chamber.
 - d. Adjust Rate Valve until pressure in chamber increases at desired rate ($\frac{1}{2}$ Bar/second recommended).
 - e. Turn control valve to EXHAUST position to release pressure from chamber.
8. Test chamber safety valve

- a. Turn the lid back so the cam is not blocking the piston.
- b. Slowly pressurize chamber by turning control valve to CHAMBER position
- c. Ensure safety valve piston releases before pressure reaches 2 bars (0.2 MPa).--see manual (link above) if not
9. Turn the control valve to EXHAUST and remove the lid from chamber by turning counter-clockwise and lifting.
10. Proceed to measurement (procedure below)
11. When measurements are complete, disassemble system
 - a. Close valve on nitrogen tank
 - b. Release pressure from hose using hose purge valve.
 - c. Release excess pressure in chamber by turning control valve to EXHAUST position.
 - d. Check regulator and instrument gauge to ensure all pressure has been released.
 - e. Disconnect hose from nitrogen tank and then from instrument.
 - f. Store lid completely locked on chamber, or completely removed

Sampling and measurement

1. Harvest leaf with pruning shears.
 - a. Sample length should fit within chamber and diameter within gasket or stopper depending on chamber model (check instrument in advance).
2. Measurements should be taken as soon as possible after the sample is cut.
 - a. If immediate measurement not possible, place sample in plastic bag with barcoded envelope, blow into bag, remove air, seal, place in ice bucket for measurement within the hour
3. Re-cut leaf petiole with razor blade to reveal clean edge for viewing (cut minimal material).
4. Seal sample into lid using compression gland or stopper
 - a. Compression gland: insert the cut end of the petiole (stem) through the hole from the bottom side of the chamber lid. Twist the Compression Screw clockwise.
 - b. Stopper: Push insertion tool through hole in narrow end of stopper. Insert cut end of petiole (stem) into insertion tool through stopper. Insert stopper and sample into lid.
 - i. Note: for short petioles, samples can be wrapped with teflon tape for use with tapered stopper
5. Attach lid to chamber as above
 - a. Cut end should extend out of chamber far enough to get your face close to it for a good visual of the cut end.
 - b. Use a magnifying glass and get close enough to cut end to verify you have a very clear view of the xylem prior to increasing pressure.
6. Turn control valve to CHAMBER to pressurize chamber
 - a. Ramp up pressure slowly by adjusting rate valve as necessary
7. Watch for the first sign of moisture on the cut end.
 - a. Do not mistake sap or latex for water - water usually follows sap or latex and will be visible as a dilution of latex.

- b. Most often phloem (outer layer of twig) begins to bubble first. Wait for the water in the xylem to emerge, which causes a color change (often it darkens the xylem and latex appears diluted).
 - i. For problem species, phloem can be cut back (CAREFULLY!) if necessary (e.g. TOCOPI)
8. Record pressure at the moment of xylem surface wetness. This takes some trial and error to identify what is truly water, and what might be sap or latex.
 - a. Datasheet:
https://docs.google.com/spreadsheets/d/1CBCfSw4KCjE1D0WsnToWOJd-IGhn6vQiomS_2XWvPw/edit#gid=0
9. Turn the control valve to OFF.
10. Release pressure after recording data by turning control valve to EXHAUST.
11. Lid and sample can be removed for once chamber pressure gauge reaches zero