Protocole Ptlp

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18/02/2021

Introduction

We assessed the leaf turgor loss point, π_{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* (Bartlett et al. 2012a; Maréchaux et al. 2016).

Materials

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

Methods

Installing Vapro for measurements

- Turn on Vapro the day before for the thermocouple's stability
- Test Water Quality *cf Vapro_cheatsheet*
- Clean
- Calibration *cf Vapro_cheatsheet*
- Control tests *cf Vapro_cheatsheet*
- Verify temperature
- Always have the black diamond at the center of the display

Used daily: * clean beforehand * select automatic mode (10 runs)

Sampling on the field

- Collect at least 3 healthy mature leaves on branch
- Place them in sample ziplock bag with:
  - wet paper towel
  - Exhale in bag to saturate in CO_2
  - Annotate bag with sample information
- Zip bag and stock in cooler

Lab measurements

Field day

- Recut branch under water
- Replace in ziplock bag with wet paper towel
- Put 24h in fridge to hydrate overnight

N+1 Field day

Vapro:

- check distilled water in vapro reservoir
- clean
- select automatic mode (10 runs)
- make sure vapro software is on

Sample measurement:

- 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*
- Wrap disc in tin foil
- Immerse in liquid nitrogen for at least 2 min using metal tea ball
- Puncture 10-15 times with needle
- Place in vapro chamber

In total, disc are exposed to air for less than 40 seconds for all the steps.

- Record value C_0 when the difference between consecutive 2-min measurements fell below strictly 5 mmol.kg^{-1} after at least three runs.

If error! or Nr_Run > 10 : + try a 2nd cycle with same leaf + try a 3rd cycle with another leaf + otherwise record *NA*

- Beware of the stuck leaf inside the vapro! If so *cf Vapro_cheatsheet*

For METRADICA PROJECT:

- Place measured leaf in envelop for VIENNA

End measurements

Clean Vapro