ForestGEO - sampling protocol for preparing plant material for osmometry, to measure rehydrated leaf structure, and to preserve leaves for leaf venation and anatomy

Norbert Kunert, June 2018

1. General information

Per species, at least three tree individuals should be sampled. To conduct all leaf trait measurements about 12 well-sun exposed leaves will be needed, hence enough branches should be sampled from each sample tree. The leaves should be mature, as complete/undamaged and "pretty" as possible.

2.1. List of material needed for field sampling

- Pruner, or other tools to cut branches
- Wet paper towel
- Flagging tape to mark branches
- Waterproof permanent marker
- Large plastic bags, preferably black plastic bags to reduce any photosynthesis or transpiration of the sampled plant material

2.2. Field sampling

- Collect one branch or if necessary various branches from each tree.
- Branches should be collected with at least three nodes of stem proximal to the leaf.
- Wrap the cut end of the branch with wet tissue paper immediately after sampling.
- Mark branch after sampling with flagging tape and write tree individual and species on flagging tape.

- Place branch in black plastic bags with some wet paper tissue.

- Seal bag well!
- Do not expose bags to direct sunlight or hot environment.
- Bring sampled material to lab as soon as possible!
- Samples should be processed in lab within a couple of hours.

3.1. List of material needed for processing samples in the lab

- Hand pruner
- Plastic buckets
- Large black plastic bags
- Water

3.2. Preparation of samples in lab (same day as sampling!)

- Cut back 2 nodes of the branches under water.
- Label the branches with trail tape, with species and individual number in sharpie.

- Put the branches in a bucket so the water covers the stem, but the leaves to be used are not in contact with water.
- Place a trash bag over the branches in the bucket.
- Allow to sit (re-hydrate) at least 6h or overnight.

4. *Lab analysis on the next day*

4.1. *Osmometry*

Save one leave per individuals per species and put into whirl pak bag with moist paper towel for osmometry (separate protocol document).

4.2. *Preserve leaves for major vein and cross sectional anatomy measurements*

Material needed:

- formalin-acetic-acid solution (FAA) 1l solution:
  - 500ml 95% Ethanol
  - 50 ml Glacial acetic acid,
  - 100ml Fromalin (37% fomaldehyde)
  - 350ml DI water

- Glas jars or whirl pak bags if material will be shipped.
- Paper
- Permanent marker and pencil

Store 6 to 8 leaves of each tree individual in glass jars with about 20-30 mL of FAA. Prepare paper labels written in PENCIL! Place paper label with information on tree individual and species with the leaf samples in the jar. In addition. Label the jar on the outside with a permeant marker. Seal the the jars well with the lid and store in a ventilated area for further analysis.

To reduce weight for shipping, samples can be stored in whirl pak bags.

4.3. *Leaf structure*

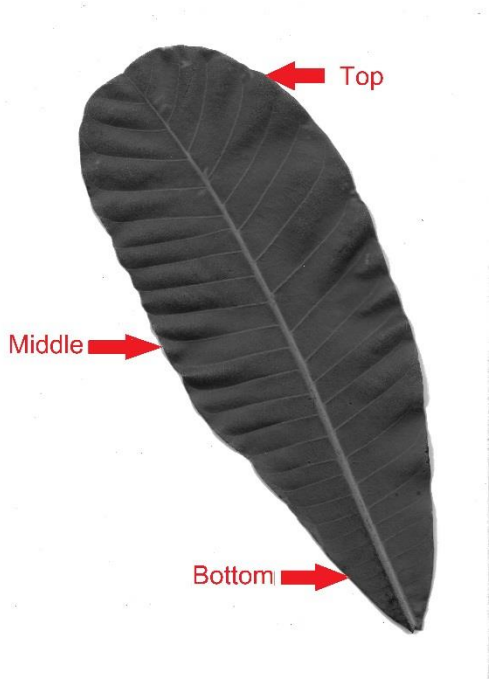
Material needed:

- Racer blades
- High precision scale
- Caliber
- atLEAF chlorophyll meter
- Flatbed scanner
- Whirl pak bags
- Wet paper towel
- Envelopes
- Dry oven

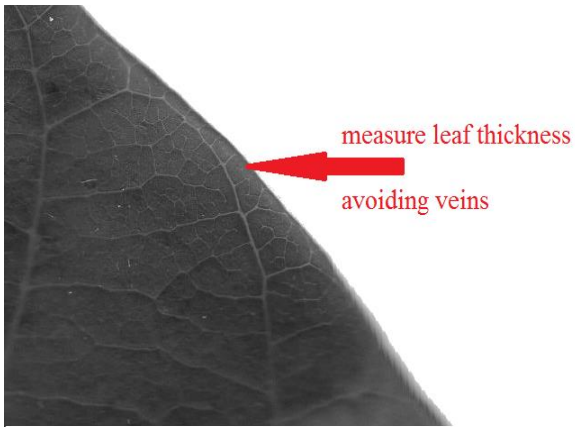
- Three leaves per tree individual (one large leaf, one medium leaf, and one small leaf) should be cut off from their petioles with a razor blade and put into whrilpack bags with moist paper towel.

- When ready to conduct the measurements, with minimal time spent outside of the whirl pak bags place leaf on balance and record mass.

- Measure relative chlorophyll content using atLEAF chlorophyll meter on the top, middle, and bottom thirds of the leaf.



- Using calipers measure leaf thickness, avoiding veins at top, middle, and bottom of the leaf.



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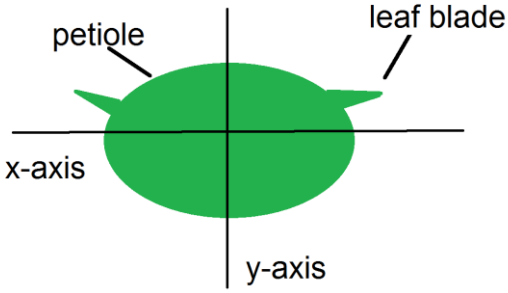
- Scan leaf on flatbed scanner at 600 dpi and at a standardized size for all leaves, ensuring the leaf is completely flat. The leaf may need to be torn in order to fit on the scanner and lie completely flat.

- Using calipers measure petiole thickness on the x- and y-axis at the base, in the center, and at the point of attachment to the leaf.



- Measure petiole length in centimeters.
- Store petiole and leaf in a labelled coin envelope
- Place labeled coin envelope with samples to dry in oven for >48h at 60°C or higher.
- After oven drying, weigh each leaf and scan each leaf for dry leaf area.

Note: Values will be averaged, so the leaves from a given individual can be scanned together and weighed together.