

PROTOCOL: Measurement of Specific Leaf Area and Water Content

(i) Equipment

- Epson 4990 high-resolution scanner with transparency table
- Portable Balance (0.001 g)

(ii) Consumable materials

- Paper bags (~ 8"x10")
- Coin envelopes (5"x7")
- Light heavy duty tissue.
- Wash cloths.
- Paper towels

(iii) Sample preparation

- Working with one sample at a time, pick all the leaves from the branches.
- Select as many mature, healthy leaves that are representative of sample as possible.
- Snip off all petioles and thick mid veins.
- If necessary, rub leaves vigorously with a damp rag to remove dirt and epiphylls from the surface.

(iv) Measurement procedure

- Select enough leaves to fill the scanner bed, cut large leaves if necessary and increase the number of scans for these leaves.
- Pat leaves dry with a paper towel to remove excess water.
- Place leaves on scanner, filling the entire scan area, and being careful to not let them touch or overlap.
- Scan each set of leaves, saving each file as a black and white TIF image.
- Clean scanner with a light duty tissue between each measurement to ensure there are no particles or smudges.
- Weigh all scanned leaves from one sample together on a portable balance (0.001 g). Be sure the balance is calibrated properly and located away from any drafts that may hinder its stability.
- Record weight as "leaf wet weight" for the sample.
- Transfer all leaves to a labeled coin envelope.
- Dry samples at 65 °C for 72 hours.
- Also dry the remainder of the leaves from the branches in separate paper bags.
- Re-weigh each sample from the coin envelopes and record weight as "leaf dry weight" for the sample.

(v) Data preparation and finalization

- Calculate the leaf area of each scan from the black portion (= leaves) relative to the entire scan area using IDL or Scion software. Sum the areas from multiple scans together to get the 'Total leaf area'.
- Calculate Specific Leaf Area (SLA)

$$\text{SLA (cm}^2\text{/g)} = \text{Total leaf area (cm}^2\text{)}/\text{leaf dry weight (g)}$$

- Calculate Leaf Water Content (%H₂O)

$$\%H_2O = 100 * \frac{\text{leaf wet weight(g)} - \text{leaf dry weight (g)}}{\text{leaf wet weight (g)}}$$