Protocol for minimum conductance (G_{min}) measurements

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Background

Light capturing is the specialization of the upper side of the leaf (adaxial) and the lower (abaxial) surface for gas exchange. Adaxial epidermis has thin cuticle and Abaxial epidermis has thin layer of cuticle. In general, stomata are present on the lower side of the leaf, but they might also be present in lower numbers in the upper part of leaf.

The g_{min} is the conductance to vapor diffusion across the epidermis once stomata are closed, i.e., through the cuticle and any leaky stomata. The g_{min} varies many-fold across species, and can be a potentially strong determinant of drought tolerance, since a lower g_{min} better enables maintenance of hydration based on stored water (Kerstiens, 1996, Sack *et al.*, 2003).

Why G_{min} measurements?

Materials:

- Analytical balance (to 0.001 g better 0.0001 g if it is needles)
- Candle and lighter
- Petri dish: to place the leaf/needle sample in the petri dish
- Temperature and humidity sensors (to maintain with stable temperature, or log temperature and humidity).
- spreadsheets,
- pen,
- permanent marker,
- tissue paper,
- tweezers/tongs
- petri dish for the wax,
- cutting board, razor blade,
- gloves (optional), samples
- acetone??,

Units, terms, definitions

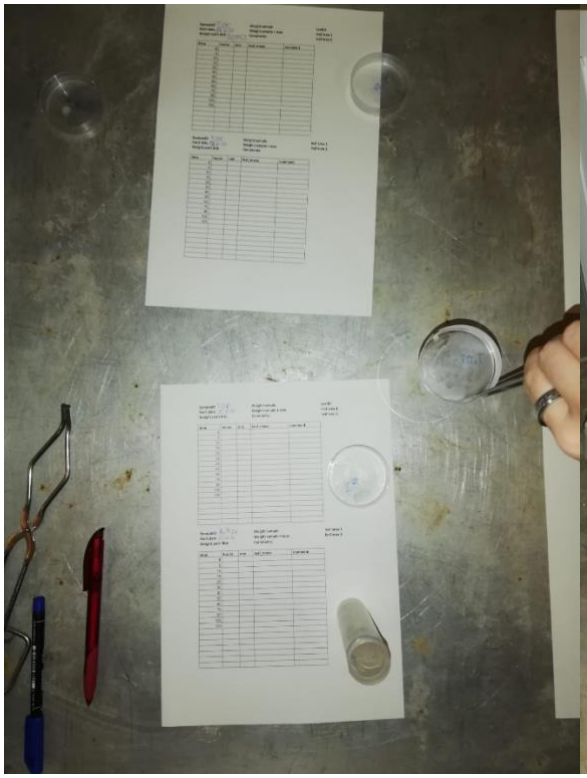
- G_{min} - minimum epidermal conductance
- VPD - mole fraction vapor pressure deficit
- VPsat – saturated VP
- RH = relative humidity (%)
- T = air temperature (°C)

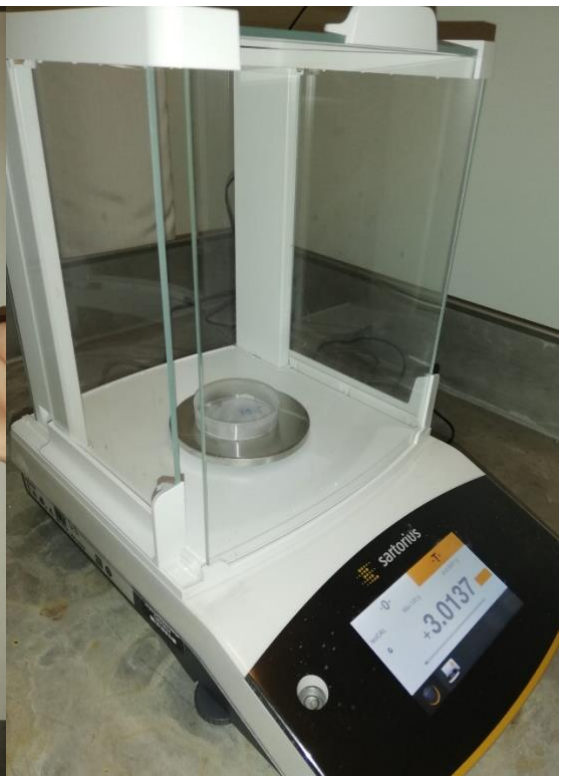
Sample Collection and preparation

1. The plant material harvested a day before analysis and recut under water and saturated – to avoid open vessel artefact (Wheeler et al. 2013),
2. Samples are brought to lad, scanned and stored overnight in fridge to enable saturation,
3. Let shoots rehydrate overnight (optional).
4. Confirm rehydration by measuring xylem water potential before starting preparation for measurements
5. In the morning, remove leaves from shoot by cutting at base of petiole with a razor blade. In case of needles, carefully pluck out 30 (roughly) needles
6. Seal the cut petiole ends with melted candlewax
7. number the leaves/needles and measure leaf area

Before getting the samples out of the fridge:

- clean table and sampling containers (petri dish etc.)
- turn on scale + make sure it is balanced (water bubble in the middle)
- label sampling containers and spreadsheets
- weigh all sampling containers (empty) and write down weight on the spreadsheet





➡ **Get samples out of the fridge**

General information:

- Do not touch the samples or the sampling containers (even if you are wearing gloves). Better work with tweezers / tongs !
- Always put the samples back in their designated spot (on the paper sheet so they do not catch any mositure).
- Make sure the leaves stick out of the sampling container as much as possible, you can try to get them into the upper part with your tweezers. This is important so the sample can dry uniformly throughout the measurement period).
- Make sure the leaves aren't touching the walls of the scale while you weigh them.
- Make sure you do not squeeze or damage the leaf, for moving leaf to measure the weight use tweezers at petiole.
- Decide for a sampling container depending on the species and leaf size.

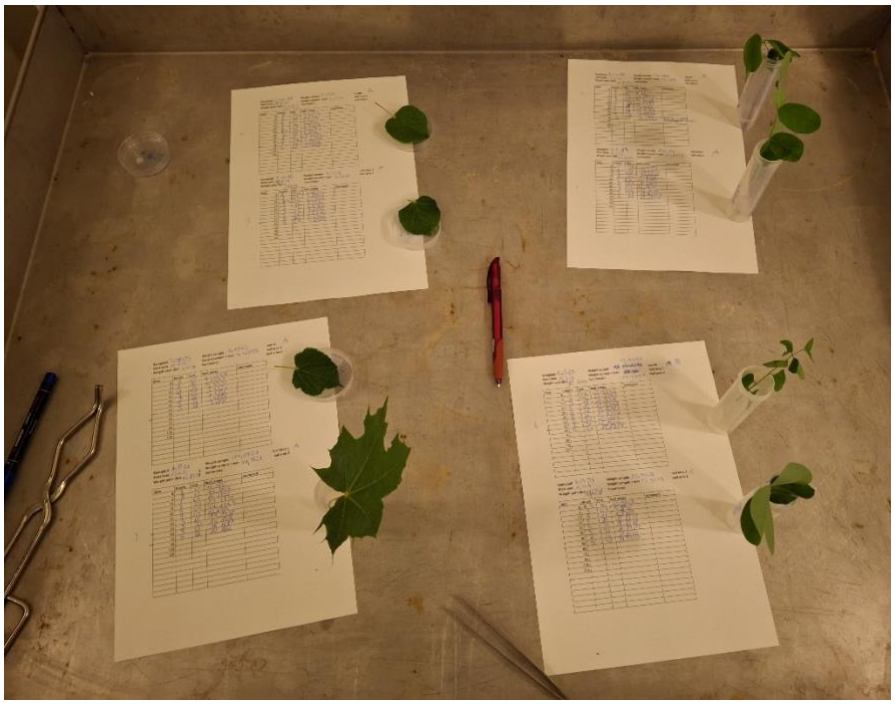
Weighing process:

- Light the candle.
- Put on gloves (optional).
- Get sample and cut the marked leaf with razor blade. Make sure the cut is clean and straight.


- Put your leaf in the sample container and weigh it.


- Get the leaf out, drop some wax into a petri dish and dip the tip of the leaf petiole into the liquid wax.
- Make sure the samples are not burnt due to heat from the candle. This could bias the measurements.

- Put the sample back in sample container and weigh it again.
- Write weight down on the spreadsheet; this weight is also the starting weight (time = 0).
- You don’t have to get started with all your samples at once; you can do 2 or 3, by the time you finish with the third one, 5 mins will have passed since you prepared the first one; weigh that one again, then continue.
- The time you have to wait between the measurements depends on the species.
- Start with weigh them every 5min (has to be adjusted depending on the species), then extent the time between weighing, depending on how the weight develops; if it declines a lot in a short time, keep the time between weighing short; if the weight doesn’t decline much, extend time e.g., (in 5/10/20/30/40min... steps).
- All samples should be positioned in the sampling holder in the same manner, e.g. always upper leaf-side facing upwards.
- Cover branches with plastic bags and put them back in the fridge after taking all the samples for G_{min} (in case if some of the measurements go wrong, it would be good to have backup, however after successful measurement discard them).



- Comment column: write down any errors (e.g. dropped leaf), changes in leaf morphology (e.g. wilting, rolled in leaves) or anything else worth documenting.

Possible errors:

- Leaf weighs less after being dipped in wax:
  try again with leaf b, check if leaf is still moist, remove water carefully with a tissue, if it happens again, the loss of water is probably heavier than the added weight of the wax; continue
- Leaf dropped: if not damaged or dust particles on it, document and continue

Collecting & preparing samples

- Cut a 60 cm long branch off of your desired tree species
- Store it in a bucket with water, covered by e.g. a bag
- Makes sure leaves are not getting wet!
- Find two leaves (one "back-up leaf") on the branch that are representative for the tree and healthy
- The leaves should be complete with no damage
- Back at the institute: Mark the leaf stem next to the petiole with a pen (do not mark leave parts that we want to measure!) and write A / B on the leaves next to it (this will help you to find the leaves later)
- Scan the leaf to acquire leaf area
- Recut the branch after scanning under water and store it in a double bagged box over night at 8°C. Make sure that leaves do not touch moist paper or get wet during rehydration!

NOTE:

- Keep the lights on so that we have constant light intensity
- Comment if anything unusual (e.g.: if the needles fall out of the petri dish)
- Comment when the leaf/needle starts to desiccate could be interesting if the visual observation match results from the relative leaf mass & relative time curve
- Comment color of the needles/leaf when they start turning yellow/brown

Calculations

1. Minimum leaf conductance (g_{min}) will be calculated from the slope of the latter linear region of the relationship between decreasing leaf mass (g) and increasing time (mins).
2. The results will be converted from g^{-1} min^{-1} to mmol m^{-2} s^{-1} by dividing by projected leaf area (m^{-2}) and the mean chamber VPD, (approx. 0.5 kPa), and converting the mass loss from g to mmol H_2O.

References:

1. **Sack L, Cowan PD, Jaikumar N, Holbrook NM**. **2003**. The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. *Plant, Cell & Environment* **26**: 1343–1356.
2. **Kerstiens G**. **1996**. Cuticular water permeability and its physiological significance. *Journal of Experimental Botany* **47**: 1813–1832.
3. **Wheeler JK, Huggett BA, Tofte AN, Rockwell FE, Holbrook NM**. **2013**. Cutting xylem under tension or supersaturated with gas can generate PLC and the appearance of rapid recovery from embolism. *Plant, Cell & Environment* **36**: 1938–1949.