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Australian University Provider Number PRV12105
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PROTOCOL
Residual conductance - mass method

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Background

Leaf residual conductance (gres) is the conductance to vapor diffusion across the leaf once stomata are closed, i.e., through the cuticle and any leaky stomata. Gres varies many-fold across species, and can be a potentially strong determinant of drought tolerance, since a lower gres better enables maintenance of hydration based on stored water.

Relationship between gres and other traits
Climate →In general, there were no meaningful relationships with gres and climate of origin. Therefore, gres cannot be easily explained by variation in leaf, or chemical and structural components of the cuticle, suggesting a significant role of incomplete stomatal closure
Stomatal density ➔ strong positive correlation between stomatal density and gres;
Drought ➔ there is a general tendency for a decreased gres in plants acclimated to drought stress. Gres is also reduced in leaves that are subjected to conditions that increase evaporative demand (vapour pressure deficit - VPD);
Wind ➔ Further evidence that exposure to strong winds or even just gentle rubbing may cause a strong increase in gres, leading to the suggestion that with rising altitude,gres may reach values that may cause unsustainably high water loss during the winter;
Leaf age ➔ a possible explanation for the increase in gres for older leaves is the continued exposure to wind, rain and abrasives, which have been shown to damage the cuticle and increase its conductance. Another possibility is that the contribution of stomata to gres increases with leaf age;
Cuticle thickness ➔ water does not diffuse as a gas through the cuticle. Instead, it dissolves into the medium of the cuticle, diffuses through the solid matrix and is desorbed at the outer edge of the cuticle. The main barrier to diffusion is actually a very thin layer of wax at the leaf surface. Because most of the resistance is located in such a thin layer, gres does not correlate with the thickness of the cuticle. This is good evidence that it is primarily the molecular architecture of cuticular waxes in the limiting skin which determines cuticular water permeability, and not the membrane thickness, the wax load or the presence of pores.

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Materials

- Two lab stands (to hang the leaves)
- Paper towels
- Lab tape or little clothes pegs
- Analytical balance (to 0.001 g)
- Leaf area meter (or flatbed scanner for determination of leaf area)
- Candle and lighter (or glue - to seal petioles)
- Razor blades
- Fan
- Thermometer and humidity sensors (for determination of VPD)

OBS.: Measurements should be made in a closed room with a stable temperature.

Procedure

1. Cut 1 meter long branches (or whole for herbaceous species) of at least 5 individuals per species.
2. Recut samples under water and rehydrate in buckets with water, covered with dark plastic bags and stored in a cooler room for a minimum of 3 h and a maximum of 24 h.
3. Set up lab stands up to 1 m apart, and run lab tape around them, sticking to itself between them, such that a “clothesline” runs between the stands, about 0.5 m above the bench.
4. In the morning, remove leaves from shoot by cutting at base of petiole with a razor blade
5. Seal the cut petiole ends with melted candlewax (optional) or glue.
6. Number the leaves with a Sharpie and measure leaf areas using a desktop scanner. (Then use the leafarea macro in Image J for determination of leaf area https://benjaminblonder.org/leafarea/)
7. Stick individual leaves to the lab tape “clothesline” with pieces of tape (or use little pegs and a rope).
8. Place a fan under the clothesline, such that the leaves sway gently in the breeze.
9. Allow the leaves to dehydrate for at least 30min to 1 hour so that stomata are closed. This may take up to 4 h, depending on the species. You will be able to check this when you have your data plotted.
10. Set up temperature and humidity sensors next to the leaves.
11. Start timer
12. Take the leaves off the clothesline, place in a plastic bag previously exhaled in (to minimize water loss). Stop the timer and record the time (hour, minutes, seconds). This will be your time 0.
13. Weigh leaves, removing from the bag one at a time and replacing in the bag after weighing.
14. Tape leaves back onto the “clothesline”.
15. Repeat steps 11-14 at every 15-20 min intervals.

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16. Aim for at least 8 points per leaf.
17. At the end of the experiment, take leaves off the fan, and measure leaf area again.
18. Input your data (i.e. the leaf mass values, the initial and final leaf area values, temperature and relative humidity values) in the gres spreadsheet tool.
19. Input the VPsat for the given temperature values, using a Table (e.g., Table 7 in Pearcy et al., 1989), or computed from the Goff-Gratch(https://en.wikipedia.org/wiki/Goff%E2%80%93Gratch_equation) or Arden Buck Equations(https://en.wikipedia.org/wiki/Arden_Buck_equation).

For measurements of gres in detached leaves, the relationship between the change in mass (due to water loss) and time is typically curvilinear and characterised by three phases: 1) the rate of water loss initially increases as stomata temporally open in response to being placed under light; 2) the rate of water loss declines as stomata begin to close in response to increasing leaf water deficit; and 3) once stomata have closed (as much as possible) the rate of water loss is at minimum and is constant over time until leaf relative water content nears zero. In this case, gres is calculated from the linear portion of the curve during phase 3.

20. Determine the points to use for gres calculation (the linear part of the leaf mass vs time plot, at least 3-4 points).
21. Calculate gres based on the mean values (cell K16) or based on the slope (cell K17); they should be similar to each other.
22. Oven dry the leaves for three days at 70o C and weigh them for dry mass.

Notes

To achieve a good curve for gres calculation, it is important to ensure that the environmental conditions under which gres is measured are conducive to stomatal closure. From experience, leaves placed under high light and/or high VPD conditions are often unable to effectively close stomata in response to desiccation, possibly as a result of rapid epidermal drying and hydro-passive stomatal opening. These conditions lead to a rapid decline in leaf water status and an overestimation of gres, as compared to control leaves from the same species. Additionally, sometimes the stomatal response of replicate leaves from the same species can be highly variable, even under the same environmental conditions. In light of these potential issues, we recommend measurements of gres on detached leaves to be conducted under mild conditions (low light and low VPD), or at least that the measurement conditions are tested for different species and adjusted if necessary. We also note that previous studies have shown that gres decreases substantially with very low relative water content; again it is beneficial to dry the leaf slowly (especially rapid drying conditions may lead to excessive drying of the epidermis, which can physically pull apart the stomata).

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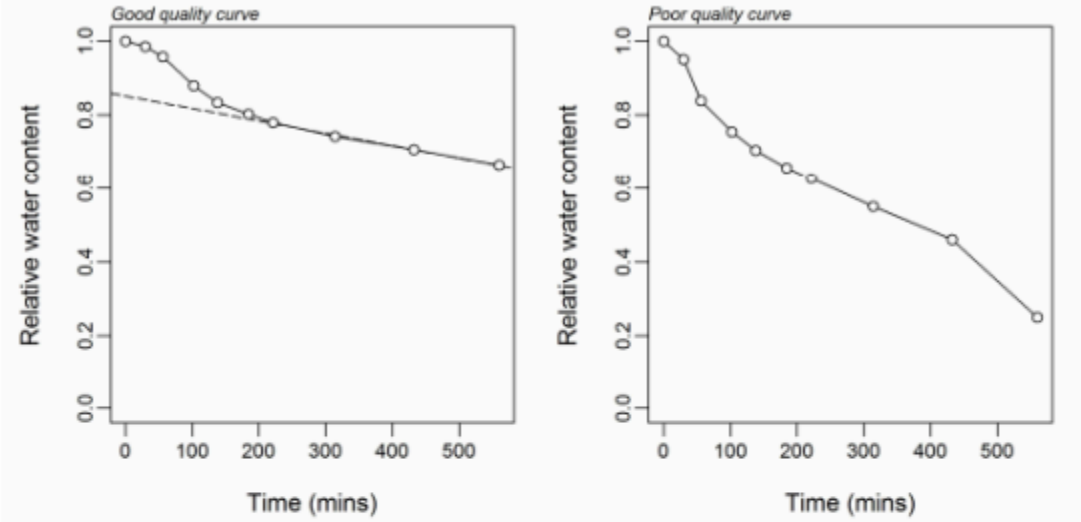

Figure 1. Two example curves for determination of minimum conductance (gmin) via mass loss of detached leaves (MLD). Leaf weights are expressed as relative water content (RWC). The good quality curve shows a distinct linear portion at the end of the drying curve, from which minimum transpiration can be calculated (using actual leaf weight, and leaf area). The poor quality curve shows a quicker decrease towards the end of the drying curve (> 400min), indicating an increased transpiration rate despite the low water content. We speculate that this increase - which we have observed in a number of curves - is due to the epidermis drying so quickly that the stomata are 'pulled open', increasing water loss.

Tips

• Be sure to choose leaves that do not have cracks in the cuticle (unless this is what you wish to measure).
• Note that gres can show plasticity within species, and within canopies, and care should be taken to sample the right leaves for one's questions.
• Ensure that your lab provides a realistic temperature and relative humidity. The gres can be sensitive to these conditions (especially when these are extreme), and so the average conditions during measurement should be reported with the gres. Some applications might require measurement in a chamber of given temperature and humidity.
• Typically, one person with a setup as described above can measure gres for 10-20 leaves in the course of a day of measurement.
• This method aims to simulate field conditions during severe drought, when water supply to the leaf has practically ceased.
• All conductance values were converted to per unit projected surface area, allowing direct comparison with stomatal conductance data which are typically presented in these units.

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Resources

https://prometheusprotocols.net/function/gas-exchange-and-chlorophyll-fluorescence/stomatal-and-non-stomatal-conductance-and-transpiration/minimum-epidermal-conductance-gmin-a-k-a-cuticular-conductance/

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