**How to Use a DroughtBox to Measure Petal Minimum Conductance (gmin)**

This guide will help you use a DroughtBox to measure minimum conductance (gmin) of flower petals under different temperature conditions.

**Reference**

This guide is adapted from: Billon, L. M., Blackman, C. J., Cochard, H., et al. (2020). The DroughtBox: A new tool for phenotyping residual branch conductance and its temperature dependence during drought. Plant Cell and Environment, 43, 1584-1594. https://doi.org/10.1111/pce.13750

**What You'll Need**

* A DroughtBox setup (your teacher or lab supervisor will provide this)
* Fresh flower petals
* Cyanoacrylate glue or petroleum jelly (for sealing cut surfaces)
* Analytical balance (resolution = 0.0001g)
* Scanner or camera (for measuring petal surface area)
* Drying oven (set to 70°C)
* Lab notebook for recording data

**Understanding the DroughtBox**

The DroughtBox is a special climate-controlled chamber that lets you:

* Maintain stable temperature and humidity
* Automatically record weight changes as petals lose water
* Test how petals respond to different temperature conditions

**Step-by-Step Guide**

**1. Preparing Your Petal Samples**

1. Carefully excise flower petals at their base. For fused petals, keep them intact as a unit to minimize cut surfaces.
2. Immediately seal all cut surfaces with cyanoacrylate glue or petroleum jelly.
3. Weigh each petal sample on the analytical balance and record the initial weight.
4. Attach each petal sample to a piece of wire or thread for hanging in the DroughtBox.

**2. Setting Up the DroughtBox**

1. Open the front door of the DroughtBox.
2. Attach each sample to one of the strain gauges in the upper compartment.
3. Make sure the petals hang freely and don't touch anything.
4. Close the door carefully.
5. Ensure the fan is on to maximize boundary layer conductance.

**3. Programming the DroughtBox**

1. Use the connected Raspberry Pi computer to set your desired conditions:
   * For standard measurements: Set temperature to 30°C and relative humidity to 40%
   * For temperature response test: Program a series of temperatures (e.g., 30°C, 35°C, 40°C, 45°C, 50°C)
2. Set the measurement interval to record weights every 5-20 minutes.
3. Start the program and let the DroughtBox begin recording data automatically.

**4. Collecting Your Data**

1. The DroughtBox will automatically record:
   * Changes in petal weight over time
   * Temperature and humidity conditions inside the chamber
2. Continue measurements until you have approximately 10 data points for each sample.

**5. Processing Samples After Measurement**

1. After collecting sufficient data points, remove each sample from the DroughtBox.
2. Scan or photograph each petal sample for surface area measurement.
3. Place samples in a drying oven at 70°C for at least 72 hours.
4. Weigh the dried samples to determine dry mass.

**6. Analyzing Your Results**

1. For samples showing non-linear change in mass over time, discard the initial 1-2 measurements (which may reflect incomplete stomatal closure).
2. Calculate minimum conductance (gmin) by fitting a linear regression to the relationship between mass change and atmospheric vapor pressure deficit.
3. Calculate additional parameters:
   * Petal mass per area (PMA) = dry mass ÷ surface area
   * Water content per area (Warea) = (initial mass - dry mass) ÷ surface area
   * Water content per dry mass (Wmass) = (initial mass - dry mass) ÷ dry mass
   * Water residence time (τ) = W ÷ E where E is the transpiration rate, calculated from gmin assuming a vapor pressure deficit of 1 kPa

**Troubleshooting**

* If weight readings fluctuate unexpectedly, check that petals aren't touching the sides.
* If temperature/humidity isn't stable, check that the door is properly sealed.
* If the computer isn't recording data, restart the Raspberry Pi program.

Good luck with your experiment! The DroughtBox will help you understand how temperature affects water loss in flower petals, providing insights into their drought responses and water-use strategies.