Chapter 2: Stomatal conductance

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Stomatal conductance estimates the rate of gas exchange (i.e., carbon dioxide uptake) and transpiration (i.e., water loss) through the leaf stomata as determined by the degree of stomatal aperture (and therefore the physical resistances to the movement of gases between the air and the interior of the leaf). Hence, it is a function of the density, size and degree of opening of the stomata; with more open stomata allowing greater conductance, and consequently indicating that photosynthesis and transpiration rates are potentially higher. The hand-held porometer provides rapid measurement of leaf stomatal conductance in irrigated trials, though it is not a recommended measurement under water stress (unless very mild) as the stomata are generally closed.

A relatively rapid drop in pressure, fast gas flow rate, or a rapidly changing relative humidity (RH) gradient through the instrument indicates that the resistance to gas conductance are relatively small and that the stomatal conductance is high. Results can be used as a proxy for measuring photosynthetic rate. The heritability of stomatal conductance is reasonably high, and gives high correlation with yield; greater leaf conductance under warmer temperatures has been associated with cooler canopy temperatures. Research at CIMMYT has shown that increased yield of CIMMYT wheat lines in favorable environments over a 30 year period reflects proportional increases in leaf conductance.

Types of leaf porometers available:

- **Steady State** (e.g., Decagon: SC-1, Figure 2.1; PP-Systems: PMR-5) – an effectively open chamber is clamped to the leaf surface and water vapor released through the stomata sets up a RH gradient along the chamber. The instrument monitors RH at two points along the flux path and, once the flux gradient reaches a steady state, it calculates and displays the leaf diffusion conductance (the reciprocal of resistance). A leaf with a rapidly changing gradient indicates that the stomata are relatively open.
- **Dynamic Diffusion** (e.g., Delta-T Devices: AP4) – measures the rate of RH increase in a chamber clamped to the leaf surface; as water vapor is released through the stomata, this causes the chamber RH to rise. A relatively rapid rise in RH indicates that the stomata are relatively open.
- **Viscous or Mass Flow** (e.g., Thermoline) – measures the time (in 1/100th of a second) to force a fixed volume of pressurized air through the leaf. This gives a measure of resistance to mass flow, which is inversely proportional and linearly related to conductance. A relatively rapid drop in pressure or a fast flow rate means the resistances are relatively small.
- **Null Balance** (e.g., LICOR: LI-1600) – measures the vapor flux and vapor gradient near the leaf surface by calculating the flow rate needed to keep stable RH inside the chamber (including air and leaf temperature). A leaf with low rate of gas exchange/ transpiration needs a relatively low dry flow rate to maintain a null balance.

Site and environmental conditions

Measurements should be taken when the sky is clear and there is not more than a slight wind. The operating environment for the porometer is 5–40°C and 10–70% RH. It is important that the leaf surfaces are dry and not wet from dew, irrigation or rain.

Only take measurements in reasonably well watered trials, as porosity may be too low in drought trials to give a reliable reading.

Time of day

Take measurements close to solar noon; typically from 11:00h to 14:00h.

Plant developmental stage

Measurements can be taken at any developmental stage and/or at regular intervals from mid tillering to late grain-filling, depending on the experimental objectives/ timing of peak stress. To compare between genotypes, do not take measurements during heading and anthesis where differences in phenology may confound results.

Typically, take one or two measurements between mid tillering and the end of booting, then one or two measurements during grain-filling.

Number of samples per plot

Take three readings on different, randomly chosen leaves from each plot.

Procedure

The following procedure describes taking measurements using the Decagon: SC-1 hand-held porometer (Figure 2.1).

Take the following equipment to the field:

- Hand-held porometer
- Field form and clipboard

Advice on taking measurements

Remember that stomata are sensitive to physical manipulation, so avoid physical stress/contact with the leaf as much as possible. Make measurements as quickly and accurately as possible, as use of the porometer will alter the leaf surface and the boundary layer environment causing a drift in the conductance/ resistance value. Note that stomata are also sensitive to light, RH, carbon dioxide, water stress, pathogens and pollutants, and that agro-chemical products affect stomatal responses.

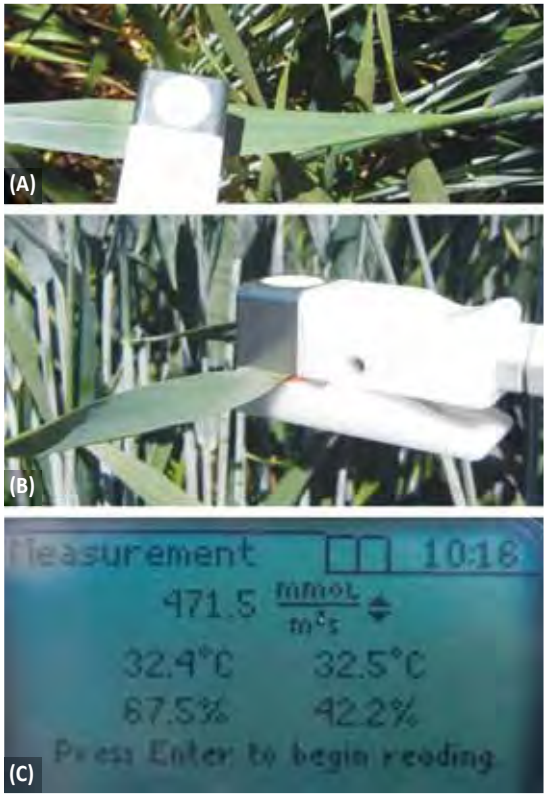

Figure 2.1. Using the Decagon: SC-1: (A) top view showing chamber clamped at the mid-point of the sample leaf; (B) side view with the white Teflon disc clearly visible; and, (C) data output view showing the stomatal conductance reading of 471.5 mmol m^{-2} s^{-1}.

Measurements should be made on the youngest fully emerged leaf receiving sunlight; typically the flag leaf once fully expanded. Be sure to select leaves which are exposed to the sun, and not those in the shadow or shade as these will have very different readings to those leaves in the sun. The leaves must be clean, dry, intact, green, with no sign of disease or damage. Readings should be within 10% or approximately 50 mmol m^{-2} s^{-1} of each other, if not, then a further reading should be taken.

Measurements are typically made on the upper (adaxial) surface of the leaf. In wheat, the ratio of stomatal frequency on the upper and lower leaf surface approaches 1.0, but the stomata on the upper surface show a greater degree of difference between genotypes in mid-day closure (when the temperature and radiation increases). Ensure that the leaf is consistently placed into the clamp in the same way, with the upper surface always facing upwards.

When using the SC-1 porometer, it is of paramount importance that at no point do you touch the white porous Teflon filter disk, as this will cause inaccurate readings and the disk may need to be replaced. Do not breathe near the disk, leaf or chamber as this effects the humidity and carbon dioxide concentration gradient within the sensor head, do not take measurements when there is smoke in the air (e.g., from fires, cigarettes or pollution), and do not bring the sensor into contact with any sort of chemical vapor (e.g., glue, alcohol or gasoline).

Preparations

Check that the batteries are fully charged, and that the chamber seal and gaskets and sensor are free of dust, pollen, etc.

1. After turning on the porometer, allow the instrument to equilibrate with the ambient temperature for around 10 minutes. Press the 'MENU' button, choose the 'CONFIG MENU' screen and use the arrows and 'ENTER' button to make necessary changes.
2. Check that the 'MODE' is set to 'manual' (not 'automatic'), and that the 'UNITS' are set to 'mmol m^{-2} s^{-1}' – this ensures that measurements are made in units of conductance, as the other two units (m^2s mol^{-1} and s m^{-1}) are of resistance. Return to the 'MAIN MENU'.

Trial measurements

3. Choose a flag leaf that is clean, dry, free of disease and receiving sunlight to the adaxial surface.
4. Place the leaf into the chamber at the mid-point of the leaf and ensure that the selected area of the leaf completely covers the aperture of the sensor. During the measurement take care to keep the white filter facing upwards and in full sun (do not allow other plants to shade the filter).
5. To start taking measurements press 'ENTER'. Once the readings have equilibrated press 'ENTER' again to hold the reading. The reading can then either be recorded manually or saved to the instrument. It should take approximately 30–120 seconds to take the measurement. If the reading takes longer than 3 minutes to equilibrate then discard this sample.
6. There are three options on the screen: 'SAVE' to save the data; 'DISCARD' to discard this measurement; or, 'ANNOTATE' and press 'ENTER' to name this data file. After you have annotated and given your data a file name subsequent measurements can just be 'SAVED'.
7. Between measurements, the porometer will request that the chamber is opened to ventilate any residual humidity.

Data and calculations

Depending on the instrument set-up, either take note of the values given during sampling, or save the data to be downloaded with the software supplied with the instrument. Data is typically downloaded as a 'comma delimited' text file and imported into MS Excel.

Typical values for irrigated trials are: 300-700 mmol $m^{-2}s^{-1}$; and for mildly water stressed trials are: 80-300 mmol $m^{-2}s^{-1}$.

Troubleshooting

Problem	Solution
Values are low (<200 mmol $m^{-2}s^{-1}$).	The soil is too dry and stomata have closed. Only take measurements in reasonably well watered trials - irrigate and then repeat measurements. Ensure to minimize physical manipulation of leaves as stomata are sensitive.
Large error variance in data.	Uniform the leaf selection criteria. (e.g., same position, age, orientation etc.).
Erratic values from porometer.	Irregular soil moisture across the field – possibly due to patchy drying of soil – irrigate and then repeat measurements. Clouds passing in front of the sun – measurements are best taken with cloudless skies.
Anomalous values (from steady state, dynamic diffusion or null balance porometers).	Avoid exposing sensor head to solvent fumes (e.g., alcohol, acetone, gasoline). If this occurs, re-calibrate the sensor. Do not use solvents to clean sensor head.

Useful references

Decagon Devices. (2011) Available at: http://www.decagon.com/ (accessed 11 August 2011).

Fischer, RA., Rees, D., Sayre, KD., Lu, Z-M., Condon, AG. and Saavedra, AL. (1998) Wheat yield progress associated with higher stomatal conductance and photosynthetic rate and cooler canopies. *Crop Science* 38, 1467–1475.

Rebetzke, GJ., Read, JJ., Barbour, MM., Condon, AG. and Rawson, HM. (2000) A hand-held porometer for rapid assessment of leaf conductance in wheat. *Crop Science* 40, 277–280.

Rebetzke, GJ., Condon, AG., Richards, RA. and Read, JJ. (2001) Phenotypic variation and sampling for leaf conductance in wheat (*Triticum aestivum* L.) breeding populations. *Euphytica* 121, 335–341.

Rebetzke, GJ., Condon, AG., Richards, RA. and Farquhar, GD. (2003) Gene action for leaf conductance in three wheat crosses. *Australian Journal of Agricultural Research* 54, 381–387.

Reynolds, MP., Balota, M., Delgado, MIB., Amani, I. and Fischer, RA. (1994) Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. *Australian Journal of Plant Physiology* 21, 717–730.

Reynolds, MP., Calderini, DF., Condon, AG. and Rajaram, S. (2001) Physiological basis of yield gains in wheat associated with the LR19 translocation from *A. elongatum*. *Euphytica* 119, 137–141.