

Measuring Mesophyll Conductance During Photosynthesis in C₃ Plants

Mesophyll conductance in C₃ plants refers to the movement of CO₂ from the intercellular air spaces within leaves to the site of carboxylation by Rubisco inside the chloroplast. This is partly movement of CO₂ by diffusion, but probably also involves metabolic steps, based on its sometimes high apparent temperature sensitivity (Warren 2006). Just based on the diffusion pathway, it is anticipated that mesophyll conductance (g_m) often substantially limits photosynthesis by lowering the CO₂ concentration at the site of fixation (C_c). In other words, C_c is often significantly less than C_i during photosynthesis.

Current experimental methods of estimating mesophyll conductance are based on determining simultaneously the rate of CO₂ assimilation and the difference between C_i and C_c ($g_m = A/(C_i - C_c)$). Many researchers consider that the most reliable method of estimating mesophyll conductance currently involves simultaneous measurements of net CO₂ flux and carbon isotope discrimination (Evans et al. 1986). Unfortunately, on-line carbon isotope measurements are available to few researchers. However, four different methods of estimating mesophyll conductance use more standard leaf gas exchange apparatus, some combined with chlorophyll fluorescence measurements, and are available to users of the CIRAS-3 system. These are described below. While measurement PFD seems to have only minor impact on mesophyll conductance, it strongly affects the activation state of Rubisco, and both mesophyll conductance and Rubisco can also be strongly temperature dependent.

Method 1

(without chlorophyll fluorescence measurements)

The initial slope of A vs. C_i curves is, in theory, controlled both by the carboxylation capacity of Rubisco, and by mesophyll conductance. Ethier and Livingston (2004) proposed that mesophyll conductance could be estimated from the degree of curvature of the initial slope of A vs. C_i curves (see also Harley et al. 1992). Publicly available software for analyzing photosynthetic response curves, such as that by Sharkey (2016) provides estimates of mesophyll

conductance based on the Ethier and Livingston method. The A vs. C_i curves generally require several data points in the low C_i region for reliable estimates of g_m , and those estimates usually must assume that g_m does not change with C_i (Sharkey 2016) which is currently a subject of debate. However, using the linear CO₂ ramping capability of the CIRAS-3, enough data points in the low C_i range may be available to provide estimates of mesophyll conductance over different ranges of low C_i and allow testing of the assumption that mesophyll conductance is independent of C_i , or even to permit estimates of the response of mesophyll conductance to C_i . [See CIRAS-3 Application Note on linear ramping of CO₂] For sunflower and soybean, we have found no evidence of changes in mesophyll conductance with C_i using CO₂ ramping to develop A vs. C_i curves. Scripts for collecting both steady-state A vs. C_i curves and rapid A vs. C_i curves are available from PP Systems.

Method 2 & 3

Harley et al. (1992) developed two different methods of estimating mesophyll conductance by combining measurements of leaf gas exchange with chlorophyll fluorescence. The two methods are referred to as the “constant J” and “variable J” methods, where J refers to photosynthetic electron transport, in this case estimated using fluorescence. Subsequent modifications of these methods alter the values only slightly (c.f. Singh and Reddy 2016).

Both methods depend on generating steady-state A vs. C_i responses coupled with fluorescence measurements of the quantum efficiency of photosystem II at each step in the response curve, and ideally also a measurement of leaf respiration in the light. A script which collects “multi-pulse” measurements of ψ_{II} , with parameters suitable for plants such as sunflower and soybean is available from PP Systems. With fluorescence measurements, we prefer to use manual recording of the data on the A vs. C_i curve, based on assimilation rates being constant at each step for about a minute, because the pulses of high light used for fluorescence temporarily disrupt gas exchange analysis.

Method 2

(variable J method)

This method additionally requires a measurement of photosynthesis, C_i , and ψ PSII at very high CO₂ (i.e. $C_i > 1000 \mu\text{mol mol}^{-1}$) or at low O₂, to determine the proportionality between J and ψ PSII (Harley et al. 1992).

Method 3

(constant J method)

For the constant J method of estimating mesophyll conductance, more measurements at intermediate CO₂ levels (where J tends to be constant) than are normally obtained are useful. Since J is displayed after each measurement of fluorescence, it is easy to tell if J is unchanged with CO₂.

Method 4

(oxygen sensitivity method)

The response of photosynthesis to oxygen concentration depends on the CO₂ concentration at Rubisco (C_c), and thus

provides an estimate of mesophyll conductance from corresponding measurements of A and C_i (Bunce 2009). A vs. C_i curves at normal (21%) and 2% oxygen can also be used to estimate the dependence of mesophyll conductance on C_i (Bunce 2010). Singh and Reddy (2016) provided an Excel-based calculator for estimating mesophyll conductance from oxygen responses of photosynthesis in the V_{Cmax} -limited part of A vs. C_i response curves. With the CIRAS-3 no corrections of CO₂ and H₂O readings between 2 and 21% O₂ are needed, as they are with some other instruments. Air with altered O₂ concentration is provided to the air inlet of the CIRAS-3 using a "T" connector to avoid over-pressures. A 2% O₂ concentration is convenient to use, so that dark respiration is unaffected, and "respiration" needed in the Singh calculator can be obtained from the extrapolated value of A at zero C_i at 2% O₂.

References

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