Commentary

What gas exchange data can tell us about photosynthesis

This title is a modification of the title of Long and Bernacchi (2003), who described the then current state of analysis of A/C_i curves, plots of photosynthetic CO_2 assimilation versus CO_2 inside the leaf (to remove any influence of stomata). These curves were made possible by the concept of a calculated CO_2 partial pressure inside a leaf (Moss & Rawlins 1963) and have been studied since the 1970s when Graham Farquhar was a post-doc and I was a graduate student in Klaus Raschke's lab. In 1980, Farquhar, von Caemmerer and Berry (1980) published a seminal paper describing photosynthesis as either 'Rubisco limited' or 'RuBP limited', and soon, a third limitation was added, 'TPU limited' (Sharkey 1985). A/C_i curves are ideally suited to assess these three mechanisms that can set the upper limit to the rate of photosynthesis. Analyses of A/C_i curves and the tripartite model of photosynthesis limitations have been very useful for testing mechanistic models of photosynthetic metabolism and for predicting photosynthetic responses to global change (Wullschleger 1993). This issue of *Plant, Cell & Environment*, includes several papers related to the analysis of gas exchange data (Bellasio *et al.* 2015b; Bellasio *et al.* 2015a; Walker & Ort 2015). Here, I highlight the importance of these contributions and also announce an update to the PCE Calculator (version 2.0) that can be used for a simple analysis of gas exchange parameters from A/C_i curves. A separate sheet is provided to fit light response curves following the recommendations of Buckley and Diaz-Espejo (2015).

The success of the Farquhar, von Caemmerer and Berry (1980) model derives at least in part because it allows gas exchange measurements to be interpreted in terms of biochemical and biophysical processes. Soon after publication of the model, programs and algorithms for estimating the underlying parameters became available. One early program was provided by Dundee Scientific (Dundee, Scotland) and called 'Photosyn Assistant' (http://www.ddsci.com/). Several other methods were shared informally among researchers, including one by Carl Bernacchi that used linear versions of some of the equations so that critical parameters could be estimated from linear regressions.

In 2007, *PC&E* made available an Excel spreadsheet to help estimate key parameters from A/C_i curves (Sharkey *et al.* 2007). The intent was to strike a balance between detailed information and ease of use. Theoretically, five parameters could be estimated: V_{cmax}, J, TPU, R_d and g_m (the maximum carboxylation rate of Rubisco, maximum rate of electron transport for the given light intensity, maximum rate of triose phosphate use, day respiration, and mesophyll conductance to CO_2 transfer, respectively). With five parameters that can be adjusted, some very good fits are possible, even if they are not always believable. Instead, each parameter should

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be considered carefully, and when possible, parameters should be fixed using other data to improve estimates of the remaining parameters. Since the publication of this tool in 2007, a number of issues have become more clear about each of the parameters, as outlined in the following.

V_{cmax} A particular problem with estimating V_{cmax} is that V_{cmax} and g_m are almost complimentary, so that very good fits can be had by varying these two parameters inversely over a wide range of values. It is therefore important to have data that constrains g_m, either independent measures of g_m from another method or many data points in the RuBP-regeneration-limited region of the A/C_i curve.

J There has been discussion of the meaning of J. J_{max}, as used in global models of photosynthesis, is a theoretical number requiring extrapolation to infinite light intensity. In global models, J is calculated using J_{max} and two other parameters. J_{max} should not be used for the maximum rate of electron transport at high light intensity, a parameter that has sometimes been called J_{high} (Buckley & Diaz-Espejo 2015). In the PCE Calculator version 1.0, the maximum J obtained over all C_i was just called J, not J_{max}, the maximum rate of electron transport over the A/C_i curve at that light intensity, despite the fact that the irradiance may or may not have been saturating, and of course was not infinite. In version 2.0 of the calculator, the maximum J is still reported as J, and a new sheet is provided to allow calculation of J_{max} from a light response curve. A true J_{max} would require using only RuBP-regeneration-limited points, but in most cases, measurements of A at high light will be limited by Rubisco or *TPU*.

TPU The maximum rate of end product formation that can be observed sometimes reflects the maximum capacity and sometimes not. Wullschleger (1993) found this behaviour in 23 of 109 species he analysed. When the highest data points of the A/C_i curve do not increase with CO_2, *TPU* limitation is assumed. However, sometimes A actually declines at high CO_2. For some time, this was attributed to changes in photorespiration and direct use of glycine and serine by export from leaves and use in protein synthesis (Harley & Sharkey 1991); the best equation for describing this effect was published by von Caemmerer (2000). However, in recent attempts to incorporate this equation into the PCE Calculator, it became clear that even if all of the glycine or serine produced in photorespiration were used directly, it would not give the degree of reverse sensitivity sometimes observed. An earlier explanation for reverse sensitivity now seems a more likely explanation. Phosphoglyceric acid can inhibit stromal phosphoglucoisomerase and, in so doing, limit the capacity for starch synthesis (Sharkey & Vassey 1989). Unfortunately, this effect is not easily described by an equation. It is proposed that the von Caemmerer (2000) equation still be used to model this phenomenon, but that the fitting parameter,

α, be considered an arbitrary parameter useful for comparison of the degree of reverse sensitivity, but without a mechanistic basis (see calculator version 2.0 (R)).

R_d This parameter has been perhaps the most problematic in many fittings of A/C_i curve data. The calculator often estimates unrealistically high values of R_d, a phenomenon whose origins are not clear. This parameter is the best candidate for finding some other method of determining a reliable estimate for R_d and then fixing the value in the calculator so that better estimates of the other parameters are found. The best available method is the method used to determine Γ^*. This is relatively involved and requires very accurate measurements of A at low CO_2. A simpler way to constrain R_d is to measure respiration in the dark and use that as an upper limit or set R_d to be some proportion of dark respiration, perhaps 50%.

g_m There has been a tremendous interest in g_m in recent years (Sharkey 2012), and it is possible to obtain an estimate of g_m from the calculator. However, using the calculator is not as good as other methods specifically designed to measure this parameter. The calculator assumes that g_m is constant with CO_2 even though many studies find it varies with CO_2. Even so, it gives an assimilation-weighted value that works reasonably well in predicting how photosynthesis will respond to changes in CO_2. However, if other estimates of g_m are available, it is best to use those instead of letting the calculator estimate it. Regardless, it should be noted that it has been argued that any reasonable estimate of g_m is preferable to assuming infinite mesophyll conductance (Sun *et al.* 2014a; Sun *et al.* 2014b).

The simplicity of the calculator is both its strength and weakness. A number of parameters need to be specified, for example the affinity (K_m) of Rubisco for O_2 and CO_2. The parameter values in the calculator are mostly from tobacco (Bernacchi *et al.* 2002), but in this issue of *PC&E*, several papers show how more precise values can be obtained. One critical parameter is Γ^*, the Rubisco CO_2 compensation point. This varies among species, and it is among the easier parameters to measure. One paper in this issue directly addresses this. The commonly used technique for assessing Γ^* was first suggested by Agu Laisk and made popular by Brooks and Farquhar (1985). This method requires measurement of A/C_i curves over a range of limiting light intensities. Theoretically, these should intersect at C_i^*, which is related to Γ^* by the equation

$$\Gamma^* = C_i^* + \frac{A}{g_m}$$

This technique has been used by many people, but what is not clear in the publications is that the lines often do not intersect that well, creating uncertainty in the intersection point. In this issue of *PC&E*, Walker and Ort (2015) provide a method for analysing Γ^* data so that the effects of small errors and uncertainties are minimized, giving a more robust estimate of Γ^*.

A new estimate of Γ^* over a range of temperature has been determined for *Arabidopsis thaliana* (Weise *et al.* 2015), and a new spreadsheet in the PCE Calculator with an *Arabidopsis thaliana* Γ^* value is now available (version 2.0 (A)). When reverse sensitivity is observed at high CO_2 in an A/C_i curve, a second new sheet (2.0 (R)) is provided that estimates α, an arbitrary parameter useful for describing the degree of decline of photosynthesis with increasing CO_2 at high CO_2.

As before, the PCE Calculator requires the user to assign which points are controlled by Rubisco, which by RuBP regeneration and which by TPU. The most informative data points are the RuBP-regeneration-limited data points, and so, investigators should be sure to include many points in this region. The fitting program of Gu *et al.* (2010) estimates these transitions within the program and allows users to share data at the website leafweb.ornl.gov.

Now an even more detailed approach is available in the supplemental material of Bellasio *et al.* (2015b), who provide a series of worksheets that allows estimation of many more parameters. This is a comprehensive analysis of photosynthesis making use of A/C_i curves at normal and low oxygen and light response curves. Carrying out such a comprehensive analysis will likely be challenging, but also very rewarding because of the rich dataset that will be obtained. Finally, Bellasio *et al.* (2015a) extend this type of A/C_i curve analysis to C_4 plants. Because the C_4 pump obscures many of the C_3 processes, the C_4 analysis is less mechanistic, but very interesting data can still come from this analysis.

It was not obvious in 1980 that the mechanistic model of photosynthesis would have such far-reaching implications. The original paper has been cited over 4900 times according to Google Scholar, and the peak in the number of citations came in 2013, 33 years after its publication. One reason for its popularity is that underlying biochemical mechanisms can be estimated from leaf gas exchange characteristics. The papers in this issue (Bellasio *et al.* 2015b; Bellasio *et al.* 2015a; Walker & Ort 2015) and the updated version of the PCE Calculator should continue the usefulness of analysis of A/C_i curves and allow ever greater information to be obtained by gas analysis of photosynthesis.

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