The Effect of Leaf-Level Spatial Variability in Photosynthetic Capacity on Biochemical Parameter Estimates Using the Farquhar Model: A Theoretical Analysis\[W\]

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Application of the widely used Farquhar model of photosynthesis in interpretation of gas exchange data assumes that photosynthetic properties are homogeneous throughout the leaf. Previous studies showed that heterogeneity in stomatal conductance ($g_s$) across a leaf could affect the shape of the measured leaf photosynthetic CO$_2$ uptake rate ($A$) versus intercellular CO$_2$ concentration ($C_i$) response curve and, in turn, estimation of the critical biochemical parameters of this model. These are the maximum rates of carboxylation ($V_{c,max}$), whole-chain electron transport ($J_{max}$), and triose-P utilization ($V_{TPU}$). The effects of spatial variation in $V_{c,max}$, $J_{max}$ and $V_{TPU}$ on estimation of leaf averages of these parameters from A-C$_i$ curves measured on a whole leaf have not been investigated. A mathematical model incorporating defined degrees of spatial variability in $V_{c,max}$ and $J_{max}$ was constructed. One hundred and ten theoretical leaves were simulated, each with the same average $V_{c,max}$ and $J_{max}$ but different coefficients of variation of the mean (CV$_V$) and varying correlation between $V_{c,max}$ and $J_{max}$ ($\Omega$). Additionally, the interaction of variation in $V_{c,max}$ and $J_{max}$ with heterogeneity in $V_{TPU}$, $g_s$, and light gradients within the leaf was also investigated. Transition from $V_{c,max}$- to $J_{max}$-limited photosynthesis in the A-$C_i$ curve was smooth in the most heterogeneous leaves, in contrast to a distinct inflection in the absence of heterogeneity. Spatial variability had little effect on the accuracy of steady-state CO$_2$ assimilation in leaves (Farquhar et al., 2001; Gielen et al., 2005). The model also provides a widely used practical method of quantifying the key biochemical limitations to steady-state C$_3$ photosynthesis in vivo from the response of leaf photosynthetic CO$_2$ uptake per unit leaf area ($A$) to intercellular CO$_2$ concentration ($C_i$) as measured in gas exchange systems (Wullschleger, 1993; Long and Bernacchi, 2003).

The Farquhar model of photosynthesis is a mechanistic, biochemical model that is widely used to describe steady-state CO$_2$ assimilation in leaves (Farquhar et al., 2001). Applications of this model range from analysis of transgenic plants to projection of the gross primary production of the terrestrial biosphere under global change (Cramer et al., 1999; Farquhar et al., 2001; Gielen et al., 2005). The model also provides a widely used practical method of quantifying the key biochemical limitations to steady-state C$_3$ photosynthesis in vivo from the response of leaf photosynthetic CO$_2$ uptake per unit leaf area ($A$) to intercellular CO$_2$ concentration ($C_i$) as measured in gas exchange systems (Wullschleger, 1993; Long and Bernacchi, 2003).

One of the basic premises of the Farquhar model, as modified by Sharkey (1985), is that steady-state photosynthesis is limited by either (1) the maximum rate of carboxylation governed by Rubisco, termed $V_{c,max}$; (2) the rate of ribulose-1,5-bisP (RuBP) regeneration, which is assumed to be limited by the maximum rate of electron transport, known as $J_{max}$; or (3) capacity for triose-P utilization, $V_{TPU}$. Once these three are known, the leaf photosynthetic rate can be calculated given light flux, CO$_2$ and O$_2$ concentrations, and temperature. An implication of this is that the A-C$_i$ response of a leaf will show an abrupt decrease in d$A$/d$C_i$ with increasing $C_i$ when $A$ passes from Rubisco- to RuBP-limited photosynthesis and, in turn, to TPU limitation. Represented graphically, this will be evident as inflections in the A-C$_i$ response. Photosynthetic CO$_2$ uptake is routinely measured by enclosing a whole leaf or a few square centimeters of a leaf in a gas exchange cuvette. The A-C$_i$ response measured this way rarely shows the abrupt transitions predicted by the Farquhar model (e.g. Riddoch et al., 1991; Wullschleger, 1993). Further, metabolic control analysis using leaves with transgenically decreased quantities of specific photosynthetic proteins suggest that, for a given CO$_2$ concentration, control is shared between Rubisco and proteins limiting the rate of RuBP regeneration (Quick

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et al., 1991; Price et al., 1998; Raines, 2003). Understanding the basis for these inconsistencies between assumptions of the model and observations may be critical to the application of the model both as an in vivo method of determining biochemical limitations and in projecting photosynthesis at scales from crop canopies to the globe.

Application of the Farquhar model assumes that the photosynthetic properties of the leaf are spatially homogeneous (von Caemmerer, 2000). Based on this assumption, \( V_{c,max} \), \( I_{max} \), and \( V_{TPU} \) are derived by fitting the Farquhar model to measured leaf responses of \( A \) to \( C_i \) (Long and Bernacchi, 2003; Sharkey et al., 2007). Given complex gradients of leaf development, impact of heterogeneous environments, disease, and pest attack, it seems unlikely that the assumption of homogeneity is often met in nature (Terashima, 1992; von Caemmerer, 2000; Aldea et al., 2006), but does this matter for fitting \( V_{c,max} \), \( I_{max} \), and \( V_{TPU} \) and for accurate modeling of photosynthetic carbon assimilation?

The effect of leaf-level heterogeneity in stomatal conductance (\( g_s \)) on the \( A-C_i \) response curve has previously been studied (Cheeseman, 1991; Buckley et al., 1997). Increased stomatal heterogeneity was found to decrease the initial slope of the \( A-C_i \) curve with the result that \( V_{c,max} \) determined from such curves underestimated the true value. However, simulations showed that stomatal heterogeneity could not fully explain the observed leaf-level variability in photosynthetic activity (Cheeseman, 1991; Buckley et al., 1997). Variation in biochemical parameters (i.e. \( V_{c,max} \), \( I_{max} \), and \( V_{TPU} \)) has been implicated as a possible explanation; however, the effects of spatial heterogeneity in these parameters have not yet been investigated.

In addition, there is an exponential decline in light from the upper to lower surface when the leaf is illuminated from above. Photosynthetic capacity (i.e. \( V_{c,max} \) and \( I_{max} \)) may decline with this vertical gradient, a phenomenon known as light acclimation (Terashima and Hikosaka, 1995). This acclimation maximizes nitrogen-use efficiency with respect to \( CO_2 \) uptake (Delucia et al., 1991; Hikosaka and Terashima, 1995, 1996). The effect of leaf cross-sectional gradients in electron transport rate on the light response curve has previously been modeled (Terashima and Saeki, 1985); however, its effect on the \( A-C_i \) response, with regard to fitting the Farquhar et al. (1980) model, has not been studied.

It is difficult to measure variability in these various photosynthetic parameters at high resolution across a leaf. But, it is relatively easy to determine the effects of biochemical variability via a mathematical model incorporating defined degrees of spatial variability in \( V_{c,max} \), \( I_{max} \), and \( V_{TPU} \), and light, while knowing the exact average of these parameters. This study constructs and applies such a model to determine the effect of simulated spatial variance in \( V_{c,max} \), \( I_{max} \), and \( V_{TPU} \) and light on the measured \( A-C_i \) response curve, and estimates of \( V_{c,max} \) and \( I_{max} \) derived from that curve. It addresses the question: Is the Farquhar model able to accurately predict leaf photosynthetic performance from leaf-level measurements of the \( A-C_i \) response in the presence of within-leaf biochemical heterogeneity?

This study shows that biochemical variability within a leaf has little effect on the accuracy of the Farquhar model in predicting photosynthetic capacity (i.e. the average \( V_{c,max} \) and \( I_{max} \) of a leaf) so long as \( V_{c,max} \) and \( I_{max} \) remain closely coupled and \( V_{TPU} \) is nonlimiting or uniform throughout the leaf. When \( V_{c,max} \) and \( I_{max} \) become uncoupled or when significant within-leaf heterogeneity in \( V_{TPU} \) is present, the \( A-C_i \) response assumes a shape close to that commonly observed in practice. For such \( A-C_i \) responses, \( I_{max} \) and, to a lesser extent, \( V_{c,max} \) are underestimated. When these parameters are, in turn, used in models to project whole-leaf photosynthetic \( CO_2 \) uptake, \( A \) is underestimated, the largest error occurring at the transition between \( V_{c,max} \) and \( I_{max} \)-limited photosynthesis.

**RESULTS**

**Effect of Heterogeneity in Leaf Biochemistry on the \( A-C_i \) Response Curve**

A total of 110 theoretical leaves, each with different levels of univariate variability in \( V_{c,max} \) and \( I_{max} \) (defined by the coefficient of variation (CV) of \( V_{c,max} \) and \( I_{max} \)) were generated. Figure 1 shows sample distributions of \( V_{c,max} \) and \( I_{max} \) for three simulated portions of leaves of varying heterogeneity.

A leaf with homogeneous \( V_{c,max} \) and \( I_{max} \) (CV = 0%) and, by definition, perfect correlation (\( \Omega = 1.0 \)) showed an \( A-C_i \) response, with a clear inflection marking the transition between Rubisco-limited and RuBP regeneration-limited photosynthesis (Fig. 2). Raising CV(\( V_{c,max} \)) to 50% while keeping perfect correlation (\( \Omega = 1.0 \)) resulted in an \( A-C_i \) curve nearly identical to the \( A-C_i \) curve of the homogeneous leaf at low \( C_i \), but with a lower \( A \) at high \( C_i \) even though the underlying average \( V_{c,max} \) and \( I_{max} \) were unchanged from the first curve (Fig. 2). Decreasing the correlation between \( V_{c,max} \) and \( I_{max} \) while keeping the CV low (CV(\( V_{c,max} \)) = 10%; \( \Omega = 0.3 \)) likewise produced little change in the \( A-C_i \) curve from the homogeneous leaf. However, decreasing the correlation while also increasing the CV (CV(\( V_{c,max} \)) = 50%; \( \Omega = 0.3 \)) produced an \( A-C_i \) curve that deviated markedly from the curve for the homogeneous leaf and one that underestimated \( A \) at all \( C_i \) values, even though the average \( V_{c,max} \) and \( I_{max} \) were unchanged.

**Determining the Error in \( V_{c,max} \) and \( I_{max} \) Estimates for Biochemically Heterogeneous Leaves**

When the correlation between \( V_{c,max} \) and \( I_{max} \) was perfect (\( \Omega = 1.0 \)), there was practically no error in the estimation of \( V_{c,max} \) at all levels of CV (0%–50%), and only a 3% error in \( I_{max} \) at the highest CV used, 50% (Fig.
3, A and B). Similarly, when the correlation between $V_{c,max}$ and $I_{max}$ was decreased while keeping the CV low, the error in estimated $V_{c,max}$ was negligible (<1%; Fig. 3A). However, when the CV of $V_{c,max}$ and $I_{max}$ was increased while simultaneously lowering the correlation between the two, $V_{c,max}$ was underestimated by as much as 12.5%, where $CV_{VJ} = 50\%$ and $\Omega = 0.1$ (Fig. 3A). When the CV was kept below 10%, estimation error of $I_{max}$ was also near zero (Fig. 3B). In contrast to estimates of $V_{c,max}$, estimates of $I_{max}$ showed an error of up to 2.9% at $CV_{VJ} = 50\%$, even when $\Omega = 1.0$. When CV is increased and correlation decreased, $I_{max}$ was progressively underestimated, the error reaching 17.7% at $CV = 50\%; \Omega = 0.1$ (Fig. 3B).

Effect of Variation in TPU Limitation

Adding heterogeneity in $V_{TPU}$ produced a large effect on the whole-leaf CO$_2$ response curve (Fig. 4). A leaf with homogeneous $V_{c,max}$, $I_{max}$, and $V_{TPU}$ ($CV_{VJ} = 0\%; \Omega = 1.0, CV_{TPU} = 0\%$) shows the characteristic $A$-$C_i$ response with two clear inflections in the curve as control of photosynthesis progresses from Rubisco, through RuBP regeneration, to TPU, as predicted by the Farquhar model and the modifications from Sharkey (1985; Fig. 4, black squares). Estimating the parameters $V_{c,max}$, $I_{max}$, and $V_{TPU}$ by curve fitting the illustrated $A$-$C_i$ curve (Eqs. 1–7 in Supplemental Appendix S1) gave values of 90.8 $\mu$mol m$^{-2}$ s$^{-1}$, 170.1 $\mu$mol m$^{-2}$ s$^{-1}$, and 10.0 $\mu$mol m$^{-2}$ s$^{-1}$, respectively, essentially returning the exact values that were used to generate the data (see "Materials and Methods"). Increasing the heterogeneity in $V_{TPU}$ in the absence of heterogeneity in $V_{c,max}$ and $I_{max}$ ($CV_{VJ} = 0\%; \Omega = 1.0$; $CV_{TPU} = 50\%$) caused $A$ to decline very substantially relative to the curve without heterogeneity in $V_{TPU}$ even though the plateau considered characteristic of TPU limitation was lost (Fig. 4). Estimating the three biochemical parameters from this curve gave values of 86.5 $\mu$mol m$^{-2}$ s$^{-1}$, 141.1 $\mu$mol m$^{-2}$ s$^{-1}$, and 8.8 $\mu$mol m$^{-2}$ s$^{-1}$, respectively (a 3.9% underestimate of $V_{c,max}$, 17% underestimate of $I_{max}$, and 12% underestimate of $V_{TPU}$). Increasing variation in $V_{TPU}$ in the presence of heterogeneity in $V_{c,max}$ and $I_{max}$ ($CV_{VJ} = 50\%; \Omega = 0.3$; $CV_{TPU} = 20\%$) produced a greater decrease in $A$ than variation in $V_{c,max}$ and $I_{max}$ alone (Fig. 4). Parameter estimates for this combined heterogeneity were 70.0 $\mu$mol m$^{-2}$ s$^{-1}$, 110.9 $\mu$mol m$^{-2}$ s$^{-1}$, and 7.2 $\mu$mol m$^{-2}$ s$^{-1}$, underestimating the true means of $V_{c,max}$, $I_{max}$, and $V_{TPU}$ by 22.2%, 34.8%, and 28%, respectively.

Figure 1. Sample distributions of $V_{c,max}$ and $I_{max}$ in three simulated leaves exhibiting different degrees of within-leaf biochemical heterogeneity. Each pixel represents the $V_{c,max}$ or $I_{max}$ of a square section of a theoretical leaf, as, for example, might be enclosed in a gas exchange cuvette (400 sections/leaf). $CV_{VJ}$ is the coefficient of variation of both $V_{c,max}$ and $I_{max}$ and $\Omega$ is the correlation between $V_{c,max}$ and $I_{max}$ (1.0 is perfect correlation; $CV_{VJ}$ is expressed in percent.

Figure 2. The effect of heterogeneity in $V_{c,max}$ and $I_{max}$ on simulated leaf CO$_2$ uptake ($A$) to intercellular CO$_2$ concentration ($C_i$) curves. All curves were generated with the same average $V_{c,max}$ and $I_{max}$ (90 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$ and 170 $\mu$mol electrons m$^{-2}$ s$^{-1}$, respectively, taken from the approximate mean values from 109 $C_3$ species surveyed in Wullschleger (1993). $V_{TPU}$ was assumed to be nonlimiting. $CV_{VJ}$ and $\Omega$ are as described and defined in Figure 1.
Effect of Variation in $g_s$

There was a very minor effect of heterogeneity in $g_s$ on the whole-leaf CO$_2$ response curve (Fig. 5). The addition of heterogeneity in $g_s$ (CV of $g_s$ = 50%) caused a small, but visible, decrease in $A$ at all $C_i$ above 150 $\mu$mol mol$^{-1}$ (Fig. 5). Parameter estimates of $V_{c,max}$, $J_{max}$, and $V_{TPU}$ from this curve gave values of 90.5 $\mu$mol m$^{-2}$ s$^{-1}$, 167.0 $\mu$mol m$^{-2}$ s$^{-1}$, and 9.9 $\mu$mol m$^{-2}$ s$^{-1}$, respectively, resulting in a nearly perfect estimation of $V_{c,max}$ but very slight underestimation of $J_{max}$ and $V_{TPU}$ by 1.8% and 1.0%, respectively. Increasing variation in $g_s$ in the presence of heterogeneity in $V_{c,max}$ and $J_{max}$ (CV of $g_s$ = 50%; $\Omega = 0.3$; CV of $g_s$ = 50%) produced a similar result; the $A$-$C_i$ response curve showed a minimal decrease in $A$ at most $C_i$ values when compared to the curve generated with variation in $V_{c,max}$ and $J_{max}$ alone (Fig. 5).

Heterogeneity in Light Environment and Light Acclimation

Heterogeneity in within-leaf light environment, in the form of decreasing light from the upper to lower surface of the leaf, was simulated by dividing the theoretical leaf into three layers of equal thickness. Each layer was ascribed a photon flux according to the exponential decline observed in actual leaves (Vogelmann and Evans, 2002). In the three-layer leaf simulations, there was a significant difference in whole-leaf photosynthesis between simulated leaves with and without light acclimation (Fig. 6, B and A, respectively). The $A$-$C_i$ response of the three-layer leaf without light acclimation (i.e. uniform $V_{c,max}$, $J_{max}$, $V_{TPU}$, and $R_d$ across all layers; see Supplemental List of Abbreviations S1 for complete list of abbreviations and definitions) showed lower $A$ than the single-layer leaf at $C_i > 250$ $\mu$mol mol$^{-1}$. However, when parameters were assumed to diminish with depth into the leaf, in concert with the light gradient, the response was virtually identical to that of the single-layer leaf (Fig. 6, A and B). In the nonacclimated leaf, the contribution of each of the three layers to overall photosynthesis was relatively equal, with the top two layers producing nearly identical $A$-$C_i$ curves and the bottom layer producing lower $A$ at $C_i$ above 200 $\mu$mol mol$^{-1}$ (Fig. 6A). In contrast, in the acclimated leaf, each layer contributed significantly different amounts to the overall leaf photosynthesis, approximately propor-

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**Figure 3.** A, The percent underestimation of $V_{c,max}$ that would result from fitting the Farquhar model to $A$-$C_i$ curves generated from leaves with varying degrees of biochemical heterogeneity. Heterogeneity was generated by increasing the CV of both $V_{c,max}$ and $J_{max}$ on the one axis and decreasing the correlation ($\Omega$) of $V_{c,max}$ and $J_{max}$ on the other. CV and $\Omega$ are as described in Figure 1. B, As in A, except the z axis is the percent underestimation of $J_{max}$. $V_{TPU}$ was nonlimiting in the simulations to simplify estimation of $J_{max}$.

**Figure 4.** The effect of heterogeneity in $V_{TPU}$ on CO$_2$ response curves of theoretical leaves with and without heterogeneity in $V_{c,max}$ and $J_{max}$. The $y$ axis has been rescaled to reveal the differences at high $C_i$. $CV_{\Omega}$ is the coefficient of variation of both $V_{c,max}$ and $J_{max}$; $\Omega$ is the correlation between $V_{c,max}$ and $J_{max}$ (1.0 is perfect correlation), and CV of $V_{TPU}$ is the coefficient of variation of a normal distribution of $V_{TPU}$ values across the leaf; $CV_{\Omega}$ and CV of $V_{TPU}$ are expressed in percent.
tional to the amount of light absorbed by each layer (50%, 30%, and 10%, respectively; Fig. 6B).

Adding variability in $V_{c,max}$ and $J_{max}$ in the three paradermal layers produced a significantly lower $A$ at all $C_i$ values (Fig. 6C). The effects of heterogeneity in $V_{c,max}$ and $J_{max}$ on the CO2 response curve that were observed in Figure 2 were seen within each layer (i.e. a smoothing of inflection points).

**DISCUSSION**

Spatial heterogeneity in $V_{c,max}$ and $J_{max}$ within leaves was found to have an effect on the ability of the Farquhar model to accurately characterize and predict photosynthesis at the leaf level. The most apparent effect is that, while an $A$-$C_i$ curve derived from a leaf with homogeneous biochemical properties (i.e. constant $V_{c,max}$ and $J_{max}$) across the leaf shows a distinct inflection point, curves derived from heterogeneous leaves show a smoother transition from Rubisco-limited to RuBP-limited photosynthesis (Fig. 2). This smooth transition resembles many reported $A$-$C_i$ curves measured both in the field and in controlled conditions, suggesting that photosynthesis in real leaves is more often heterogeneous than not (e.g.

**Implications for Modeling Photosynthesis from Heterogeneous Leaves**

Figure 7 compares the $A$-$C_i$ response curve for a simulated leaf exhibiting heterogeneity in $V_{c,max}$ and $J_{max}$ ($CV_{VJ} = 50\%$; $\Omega = 0.3$) to the predicted curve generated by the Farquhar model based on the estimates of $V_{c,max}$ and $J_{max}$ fitted to the heterogeneous leaf. There was a significant difference between the initial $A$ and modeled $A$ that resulted from using parameters derived from the Farquhar model to describe the heterogeneous leaf (Fig. 7A). The fitted curve agreed well with the original curve at low $C_i$, but then progressively overestimated $A$ as $C_i$ increased, reaching a maximum overestimation of 12.5% at $C_i = 350 \mu$mol mol$^{-1}$, in the transition region from Rubisco-limited to RuBP-limited photosynthesis (Fig. 7B). With further increase in $C_i$, the overestimation diminished progressively and then underestimated $A$ at $C_i > 800 \mu$mol mol$^{-1}$ (Fig. 7A).
Wullschleger, 1993). Why does heterogeneity lead to a smoother A-Ci response curve? Theoretically, when the \( V_{c,max} \) to \( J_{max} \) ratio varies between patches in a leaf, the \( C_i \) at which the transition occurs must also vary (Farquhar et al., 1980). Thus, the observed overall A-Ci response of the leaf is the average of a range of photosynthetic CO2 response curves, each with inflection points at different \( C_i \) values. This could explain an apparent inconsistency between metabolic control predicted by the Farquhar model and that determined by control analysis of transgenic plants. Implicit in the Farquhar model is that, at low \( C_i \), metabolic control will reside entirely with Rubisco (i.e. a control coefficient of 1), whereas above the inflection point of the curve, control will reside entirely with regeneration of RuBP. However, transgenic alteration of the amount of individual photosynthetic proteins suggests that control is shared between Rubisco and proteins involved in RuBP regeneration (for review, see Raines, 2003).Incomplete coupling of spatial variation in the amount of Rubisco and proteins controlling RuBP regeneration could explain the apparent contradiction between the theory of Farquhar et al. (1980) and observed metabolic control.

Heterogeneity in biochemical properties of the leaf led to underestimation of \( V_{c,max} \) and \( J_{max} \), but only when the two were uncoupled (Fig. 3). This implies that, providing the two parameters vary in concert within actual leaves, estimates of \( V_{c,max} \) and \( J_{max} \) made from A-Ci curves will not be in error due to this heterogeneity. However, spatial variability in the two parameters (CV\(_{V_{c,i}}\)) interacts with decreased coupling to amplify the error (Fig. 3). Estimation of \( V_{c,max} \) was affected less by a given amount of heterogeneity than \( J_{max} \). This is because \( V_{c,max} \) is estimated from the initial slope of the A-Ci curve. As \( C_i \) approaches 0, \( dA/dC_i \) will be unaltered. Increasing heterogeneity will simply cause the initial slope of \( dA/dC_i \) to decline from that projected by Rubisco kinetics at a progressively lower \( C_i \). As long as \( V_{c,max} \) is estimated from the true initial slope, it will not be underestimated. However, heterogeneity causes \( A \) to be lower at all higher values of \( C_i \). As a result, \( J_{max} \) will be underestimated (Fig. 2). Lower \( A \) at high \( C_i \) occurs because, by simulating variation in \( V_{c,max} \) independent of \( J_{max} \), some of the patches contributing to the simulated average \( A \) will be limited by low amounts of Rubisco, even at high \( C_i \). Consequently, one practical application of this finding is that the data points chosen from the A-Ci curve to estimate \( V_{c,max} \) and \( J_{max} \) should avoid the transition area between Rubisco- and RuBP-limited photosynthesis to minimize estimation errors due to heterogeneity. However, in leaves exhibiting TPU limitation, finding points that are exclusively RuBP limited could be difficult, if not impossible. This task is made even more difficult when even a little variation in \( V_{TPU} \) is introduced (Fig. 4). The A-Ci response curve appears to be very sensitive to variation in TPU, showing significant decreases in \( A \) at even low \( CV_{TPU} \) (Fig. 4). Of greater concern, perhaps, is the loss of a clear plateau in the CO2 response curve in the presence of TPU heterogeneity because this makes it appear as if the leaf is not limited by TPU at all. Under these conditions, \( J_{max} \) would be underestimated and \( V_{TPU} \) might be assumed to not be limiting at any of the measurement values of \( C_i \).

Adding stomatal heterogeneity to the simulations did not alter the A-Ci response curve significantly (Fig. 5). A CV of 50% in \( g_s \) produced an A-Ci response that was virtually identical to the homogeneous case at lower \( C_i \) and caused a marginally lower \( A \) at higher \( C_i \). This minimal effect was the same regardless of variability in \( V_{c,max} \) and \( J_{max} \), indicating that there was no interaction between heterogeneity in \( V_{c,max} \) and \( J_{max} \) with \( g_s \). However, stomatal heterogeneity did visibly lower the \( C_i \) at each \( C_i \) compared to the uniform leaf. This should be considered a worst-case scenario because the leaf was assumed to be entirely heterobaric (i.e. the substomatal chambers were assumed to be not connected). In reality, heterogeneity in \( g_s \) would be partially offset by lateral diffusion between areas of high and low \( C_i \). The finding here should not be interpreted as a contradiction of the simulations of Cheeseman (1991) and Buckley et al. (1997), which ascribed a higher importance to heterogeneity in \( g_s \). These studies found significant effects of stomatal heterogeneity on the A-Ci curve, but only when either very low \( g_s \) values made up a large proportion of the distribution or the distributions were heavily skewed or bimodal (Cheeseman, 1991; Buckley et al., 1997). Here, use of a maximum CV of 50% showed the A-Ci response to be far less sensitive to heterogeneity in \( g_s \) than in \( V_{c,max} \) and \( J_{max} \) (Fig. 5).
The effects of varying the light environment within the leaf were in agreement with previous analyses (Fig. 6; Terashima and Hikosaka, 1995). When the chloroplasts within the cross-section of the leaf were acclimated to their respective light environments in the three-layer simulations, the nitrogen-use efficiency of the leaf was maximized (Fig. 6B). However, when all chloroplasts were assumed to be equal through the cross-section of the leaf (i.e. nonacclimated), the A-Ci response curve was lower than its potential maximum (Fig. 6A). This was due to the fact that the lower layers were not light saturated and, as a result, the actual mean $V_{c,max}$ and $J_{max}$ of the leaf was slightly underestimated by curve fitting the Farquhar model (Fig. 6A). This error could be significant for leaves that are naturally near vertical in orientation and sit from both surfaces. Such leaves are unlikely to show acclimation of photosynthetic capacity from the adaxial to abaxial surface, but, if artificially lit only from above, as in a conventional gas exchange chamber (Smith et al., 1998; Johnson et al., 2005), then the error shown in Figure 6A would apply. However, this effect was small compared to the effects of lateral heterogeneity in $V_{c,max}$ and $J_{max}$ on the overall CO$_2$ response curve (Fig. 6C).

Landscape and regional models of terrestrial carbon assimilation are commonly scaled from the Farquhar model of leaf photosynthesis (for review, see Cramer et al., 1999). This, in turn, is parameterized from leaf-level measurements of the A-Ci curve from which $V_{c,max}$ and $J_{max}$ are derived. Under conditions where the measured leaves exhibit biochemical heterogeneity, this could result in an overestimation of modeled CO$_2$ uptake (Fig. 7). The modeled response curve, generated here from the estimated values of $V_{c,max}$ and $J_{max}$ showed the greatest deviation from the actual curve in the transition area between the Rubisco-limited and RuBP regeneration-limited sections of the curve (Fig. 7). This could have substantial consequences because most photosynthesis in nature occurs near the inflection point of the A-Ci curve (Drake et al., 1997; Bernacchi et al., 2005).

How prevalent is heterogeneity of $V_{c,max}$ and $J_{max}$ and how well coupled are they in nature? The $V_{c,max}$ to $J_{max}$ ratio is generally well conserved within species, even under a variety of conditions such as variable nutrient availability and water stress (Wullschleger, 1993; Wohlfart et al., 1999; Medlyn et al., 2002; Nogues and Alegre, 2002; Bruck and Guo, 2006). To date, there have been no studies of within-leaf variability of both $V_{c,max}$ and $J_{max}$, so it is difficult to assess the degree to which heterogeneity in these parameters actually affects leaf-level photosynthetic studies that employ the Farquhar model. However, it is conceivable that certain environmental stresses could induce conditions where heterogeneity could be a significant factor. One example is the effect of tropospheric ozone stress. In species such as wheat (*Triticum aestivum*) and oak (*Quercus robur*), under both short- and long-term ozone exposure, $V_{c,max}$ is decreased to a greater extent than $J_{max}$ (Farage et al., 1991; Farage, 1996), possibly because decreased Rubisco activity is often the first symptom of ozone damage (Pell et al., 1997; Long and Naidu, 2002). Effects of ozone are known to be heterogeneous across the leaf surface, (Nie et al., 1993), suggesting that decrease in $V_{c,max}$ relative to decrease in $J_{max}$ would not be uniform across the leaf. Senescence within a leaf is often patchy and, because Rubisco protein is catabolized before the membrane proteins of the electron transport apparatus (Weng et al., 2005), uncoupled variation in $V_{c,max}$ and $J_{max}$ could result during the latter part of a leaf’s lifespan.

Of greater uncertainty is the variability in other photosynthetic parameters, such as $V_{TPU}$ or $R_d$ (mitochondrial respiration). There is currently no literature on the within-leaf variability of TPU; $V_{TPU}$ has always been considered as a whole-leaf parameter. Effects of variation in $R_d$ were not considered in this study outside the light acclimation simulations, but recent studies have suggested that $R_d$ could vary within leaves in a manner coupled to the photosynthetic capacity (Tcherkez et al., 2008).

In conclusion, this study found that leaf-level photosynthetic heterogeneity within the mesophyll could lead to underestimation of $V_{c,max}$, $J_{max}$ or $V_{TPU}$ calculated by fitting the Farquhar model to the A-Ci response of photosynthesis. Substantial error, though, would only result if the $V_{c,max}$ to $J_{max}$ ratio or $V_{TPU}$ itself was heterogeneous within the leaf (e.g. $\Omega < 0.8$ or $CV_{TPU} > 10\%$). Given that it would be expected that variation in $V_{c,max}$ and $J_{max}$ would normally be well coupled, we conclude that error caused by heterogeneity in the estimation of these parameters and error resulting in turn from their use in crop production and Earth system models will be small. Proof of this conclusion would require quantification of within-leaf heterogeneity of $V_{c,max}$, $J_{max}$ and $V_{TPU}$ in actual leaves.

**MATERIALS AND METHODS**

**Construction of the Model**

The equations in the Farquhar model of photosynthesis as implemented by Long and Bernacchi (2003) were coded into a graphical modeling environment (Stella 7.0.3; iSee Systems). The equations and constants used are given in Supplemental Appendix S1. The model was designed so that each run simulated a theoretical leaf with a specified range of stochastic variability in biochemical heterogeneity in the mesophyll from stomatal effects, biochemical heterogeneity in the mesophyll could be a significant factor. One example is the effect of tropospheric ozone stress. In species such as wheat (*Triticum aestivum*) and oak (*Quercus robur*), under both short- and long-term ozone exposure, $V_{c,max}$ is decreased to a greater extent than $J_{max}$ (Farage et al., 1991; Farage, 1996), possibly because decreased Rubisco activity is often the first symptom of ozone damage (Pell et al., 1997; Long and Naidu, 2002). Effects of ozone are known to be heterogeneous across the leaf surface, (Nie et al., 1993), suggesting that decrease in $V_{c,max}$ relative to decrease in $J_{max}$ would not be uniform across the leaf. Senescence within a leaf is often patchy and, because Rubisco protein is catabolized before the membrane proteins of the electron transport apparatus (Weng et al., 2005), uncoupled variation in $V_{c,max}$ and $J_{max}$ could result during the latter part of a leaf’s lifespan.

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In conclusion, this study found that leaf-level photosynthetic heterogeneity within the mesophyll could lead to underestimation of $V_{c,max}$, $J_{max}$ or $V_{TPU}$ calculated by fitting the Farquhar model to the A-Ci response of photosynthesis. Substantial error, though, would only result if the $V_{c,max}$ to $J_{max}$ ratio or $V_{TPU}$ itself was heterogeneous within the leaf (e.g. $\Omega < 0.8$ or $CV_{TPU} > 10\%$). Given that it would be expected that variation in $V_{c,max}$ and $J_{max}$ would normally be well coupled, we conclude that error caused by heterogeneity in the estimation of these parameters and error resulting in turn from their use in crop production and Earth system models will be small. Proof of this conclusion would require quantification of within-leaf heterogeneity of $V_{c,max}$, $J_{max}$ and $V_{TPU}$ in actual leaves.
Heterogeneity in \( V_{\text{TPU}} \) and \( g_s \)

Heterogeneity in \( V_{\text{TPU}} \), the limitation on carbon assimilation rate imposed by capacity for TPU, was added to the model for selected simulations by varying the CV of \( V_{\text{TPU}} \) (Eq. 1) across all sections while keeping the mean value constant (\( V_{\text{TPU}} = 10 \text{ mol m}^{-2} \text{s}^{-1} \)). \( V_{\text{TPU}} \) was varied independently of \( V_{\text{c,max}} \) and \( J_{\text{max}} \). In these selected simulations, \( V_{\text{TPU}} \) was estimated for each leaf from the whole-leaf CO2 response curve as defined in the equations in Supplemental Appendix S1.

Likewise, \( g_s \) was varied for selected simulations by controlling the CV of the population of \( g_s \) associated with the sections of the leaf. Mean \( g_s \) was 0.1 mmol m\(^{-2}\) s\(^{-1}\). When \( g_s \) was varied, the leaf was assumed to be perfectly heterobaric (i.e. there was no diffusion of CO2 between the intercellular spaces of different sections of the leaf). Consequently, \( C_i \) for each section at each amount of CO2 concentration \( (C) \) was calculated based on the intersection of the demand function (the A-C response curve) and the supply function (1/g). Overall \( C_i \) for each leaf was calculated as the mean of the \( C_i \) of each section, in the same manner as the calculation of A in the construction of the whole-leaf CO2 response curves.

Modeling Cross-Sectional Light Acclimation and Photosynthetic Heterogeneity

To investigate the effect of transdermal variation in light environment and photosynthetic capacity on the A-C response, the leaf model was replicated in triplicate to simulate three leaf layers of equal thickness, which might approximate to upper palisade, lower palisade, and spongy mesophyll. The light absorbed by each layer was 30%, 30%, and 10% of the incident photon flux, respectively, and was approximated from the data of Vogelmann and Evans (2002). For simplicity, the incident photon flux was 2,000 flux, respectively, and was approximated from the data of Vogelmann and Evans (2002) to estimate \( V_{\text{c,max}} \) and \( J_{\text{max}} \) for each layer, then summed to give the overall photosynthetic capacity of each leaf. The A for all three layers was also summed at each \( C_i \) to generate a whole-leaf CO2 response curve. Simulations were run with and without lateral variation in \( V_{\text{c,max}} \) and \( J_{\text{max}} \) to check for interaction between transdermal and paradermal heterogeneity.

Modeling Photosynthesis from a Heterogeneous Dataset

To determine the effect of heterogeneity on the ability of the Farquhar model to accurately predict photosynthetic performance, the modeled A for a given \( C_i \) was compared to the actual A from a leaf with a \( V_{\text{c,max}} \) and \( J_{\text{max}} \) CV of 50% and a \( \Delta \) of 0.3. The resulting A-C curve was then used to estimate \( V_{\text{c,max}} \) and \( J_{\text{max}} \) as if it had been generated from gas-exchange methods, following the procedures of Long and Bernacchi (2003). A further A-C curve was then generated with these estimates from the Farquhar model, assuming no heterogeneity. The difference between A generated by this modeled curve and actual A for the simulated leaf at any given \( C_i \) represents the error that results from assuming homogeneity of the A-C response across a leaf that in reality is heterogeneous.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Appendix S1. The equations of the Farquhar model as implemented here.

Supplemental List of Abbreviations S1. Definitions, units, and, where appropriate, values of all abbreviated terms in the text.

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