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Variable responses of mesophyll conductance to substomatal carbon dioxide concentration in common bean and soybean

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Abstract

Some reports indicate that mesophyll conductance (g_m) to carbon dioxide varies greatly with the substomatal carbon dioxide concentration (C_i) during the measurement, while other reports indicate little or no change in g_m with C_i. I used the oxygen sensitivity of photosynthesis to determine the response of g_m to C_i over the range of about 100 to 300 µmol mol^{-1} C_i at constant temperature in common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) grown over a range of temperatures and photosynthetic photon flux densities (PPFD). In soybean grown and measured at high PPFD there was only a slight, approximately 15% decrease in g_m with C_i over the range of 100 to 300 µmol mol^{-1}. With lower PPFD during the measurement of g_m, and especially with low PPFD during plant growth, there was a larger decrease in g_m with C_i in soybean. In common bean, the same range in C_i resulted in about a 60% decrease in g_m for plants grown and measured at high PPFD, with an even larger decrease for plants at low growth or measurement PPFD. Growth temperatures of 20 to 30°C had little influence on the response of g_m to C_i or its absolute value in either species. It is concluded that these two species differed substantially in the sensitivity of g_m to C_i, and that PPFD but not temperature during leaf development strongly affected the response of g_m to C_i.

Additional key words: *Glycine max*; light; *Phaseolus vulgaris*; temperature.

Introduction

With the recognition that there is a significant difference in the carbon dioxide concentration between the substomatal airspace and the site of carbon dioxide fixation within chloroplasts during photosynthesis, there has been renewed interest in estimating the "mesophyll conductance" (g_m) to the movement of carbon dioxide along this path and determining the structural, metabolic and environmental factors which affect g_m (Evans and von Caemmerer 1996, Flexas *et al.* 2008, Warren 2008a). Variation in g_m with the carbon dioxide concentration in the substomatal airspace (C_i) would greatly complicate the biochemical modeling of photosynthesis until the basis of such variation is understood. Variation in g_m with C_i has been observed in some species using both the carbon isotope and the fluorescence methods of estimating g_m (During 2003, Flexas *et al.* 2007a, Hassiotou *et al.* 2009, Vrabl *et al.* 2009), but did not occur in other studies (Loreto *et al.* 1992, Bunce 2008, 2009, Tazoe *et al.* 2009). Evans (2009) suggested that errors in the fluorescence methods could create the appearance of a change in g_m with C_i, although fluorescence methods have been implemented somewhat differently in various studies. It remains unclear whether the different responses of g_m to C_i result from species differences or from differences in measurement techniques.

The method of using the oxygen sensitivity of photosynthesis to estimate g_m (Bunce 2009) is well suited to determining the C_i response of g_m over the lower range of C_i, where photosynthesis is limited by carboxylation capacity. Because O_2 and CO_2 compete for RuBp at Rubisco, and this competition determines the rate of net photosynthesis as long as neither substrate is saturating, the sensitivity of CO_2-limited photosynthesis to a change in [O_2] provides information on the [CO_2] at Rubisco (C_c). The main assumptions of the method are that competition at Rubisco described by the Farquhar-type C_3 photosynthesis model fully explains [O_2] effects on CO_2 fixation and that respiration in the light is unchanged over the [O_2] range used. The resolution of the method is greatest when photosynthesis is limited by carboxylation

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Abbreviations: C_c – [CO_2] at Rubisco; C_i – [CO_2] in the substomatal (intercellular) airspace; g_m – mesophyll conductance to CO_2; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; Rubisco – ribulose-1,5-bisphosphate carboxylase/ oxygenase; RuBp – ribulose bisphosphate; V_{Cmax} – the maximum rate of carboxylation of Rubisco.

capacity rather than electron transport (Bunce 2009).

In some surveys of species (Evans and von Caemmerer 1996, Hassiotou *et al.* 2009), g_m was found to scale approximately with maximum P_N, but there is less information on whether g_m scales with maximum P_N within a species grown under conditions which produce a range of maximum P_N. A few studies have measured the response of g_m to temperature, and found the responses to vary among species (reviewed in Bunce 2008, Warren 2008b). However, there is little information on the response of g_m to measurement PPFD. In this study, I used the oxygen sensitivity of P_N to estimate g_m in soybean and common bean over a range of C_i values, for plants grown over a range of temperatures and PPFD levels and measured at different PPFD levels at constant temperature.

Materials and methods

Soybeans (*Glycine max* L. Merr., cv. Kent) and common beans (*Phaseolus vulgaris* L., cv. Dark Red Kidney) were grown in controlled environment chambers. Plants were grown singly in 20 cm diameter pots filled with vermiculite and flushed daily with a complete nutrient solution containing 14.5 mM nitrogen. Plants were grown at 1,000 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) for 12 h per day, with constant air temperatures of 20, 25, or 30°C. Plants were also grown at 25°C with a PPFD of 150 μmol m^{-2} s^{-1}. In all growth conditions, the [CO_2] was controlled at 370 μmol mol^{-1}, and the relative humidity was 60%. Light was supplied by a mixture of high-pressure sodium and metal halide lamps. Leaf gas exchange measurements were made on second trifoliolate leaves a few days after they reached maximum area.

The response of g_m to C_i over the range of 100 to 300 μmol mol^{-1} was measured at 25°C, 1500 μmol m^{-2} s^{-1} PPFD and a leaf to air vapor pressure deficit of 1.2 to 1.4 kPa for three or four leaves of both species grown under all of the environmental conditions given earlier. Leaf gas-exchange measurements were made using a *LI-6400* portable photosynthesis system (*LI-Cor, Inc.*, Lincoln, Nebraska). Steady-state rates of P_N in both 2% and 21% [O_2] were recorded at six different external [CO_2], ranging from about 100 to 800 μmol mol^{-1}. The system software was used to correct the output of the infrared analyzers for the background [O_2] and to calculate C_i. Gas-exchange measurements were conducted inside a controlled environment chamber in which the water vapor pressure was controlled to match (± 0.1 kPa) that inside the cuvette. This was found to eliminate the need to correct for water vapor leakage into or out of the cuvette (Rodeghiero *et al.* 2007). Corrections for CO_2 leakage through cuvette materials were made based on the difference between the chamber and cuvette [CO_2], using the manufacturer's protocol. Possible leakage of CO_2 between the leaf surface and the gasket was tested by increasing the [CO_2] around the cuvette to several thousand μmol mol^{-1} and observing changes in CO_2-exchange rate. No significant leaks were detected.

A Farquhar-type C_3 photosynthesis model with updated kinetic parameters (Sharkey *et al.* 2007) was used to estimate g_m from P_N and C_i at 2 and 21% [O_2], using the method in Bunce (2009). This was done from composite P_N *vs.* C_i curves from three or four leaves for each species and growth condition by determining by trial and error values for V_{Cmax} and g_m which fit the observed rates of P_N at both 21 and 2% [O_2] interpolated at C_i values of 100 to 300 μmol mol^{-1}, with steps of 50 μmol mol^{-1}. Estimates of g_m at higher C_i were often not feasible because P_N at 2% [O_2] became insensitive to increases in C_i above 300 μmol mol^{-1}. The composite P_N *vs.* C_i curves consisted of measurements on four leaves per species and growth condition. Interpolated values of P_N at the steps in C_i was determined by fitting curves for an exponential rise to a maximum of the form $P_N = P_{N\,0} + a\,(1 - e^{-bC_i})$, using the nonlinear regression utility of *SigmaPlot v. 11* software. Statistical tests were conducted using *JMP v. 5* (*SAS Institute*, Cary, North Carolina).

Results

In soybean grown and measured at high PPFD, g_m decreased only slightly with increases in C_i (Fig. 1). Values at 300 averaged about 85% of those at 100 μmol mol^{-1}. Growth temperatures from 20 to 30°C had little effect on the magnitude of g_m, or its response to C_i. Low PPFD during the measurement of g_m did not affect the g_m measured at low C_i, but reduced it by about 25% at the higher C_i values (Fig. 1). At low measurement PPFD, g_m decreased relatively more with C_i than at high PPFD, and the value of g_m at 300 μmol mol^{-1} was about 65% of that at 100 μmol mol^{-1} C_i. Low PPFD during plant growth resulted in lower g_m values over the whole range of C_i (Fig. 1), and increased the relative reduction in g_m with C_i. P_N at a C_i of 250 μmol mol^{-1} decreased slightly with growth temperature and was reduced by low growth or measurement PPFD (Table 1).

The decrease in g_m with C_i in beans grown and measured at high PPFD was larger than in soybean (Fig. 2), with values at 300 μmol mol^{-1} C_i about 40% of those at 100 μmol mol^{-1} for all three growth temperatures. Even larger decreases in g_m with C_i occurred in bean leaves either grown or measured at lower PPFD (Fig. 2). P_N

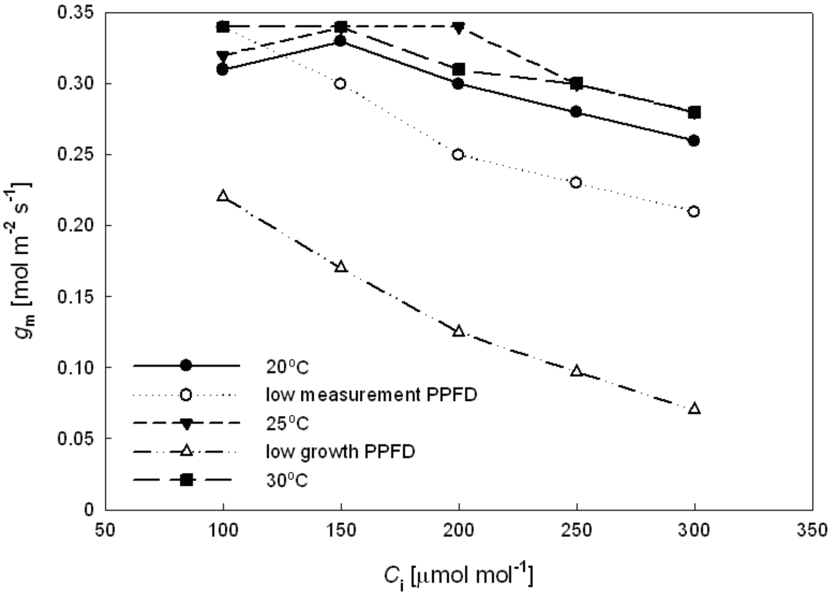

Fig. 1. Responses of g_m to C_i in soybean leaves measured at 25°C and 1,500 µmol m^{-2} s^{-1} PPFD for plants grown at 1,000 µmol m^{-2} s^{-1} PPFD at 20, 25, and 30°C, and for plants grown at 25°C at 150 µmol m^{-2} s^{-1}, measured at 1,500 µmol m^{-2} s^{-1} PPFD, and for plants grown at 25°C and 1,000 µmol m^{-2} s^{-1} PPFD but measured at 300 µmol m^{-2} s^{-1} PPFD. Estimates of g_m are based on gas-exchange measurements on three or four leaves per species and growth condition.

Table 1. Rates of P_N at 250 µmol mol^{-1} C_i, 25°C in bean and soybean leaves grown at a range of temperatures and PPFDs and measured at either 1,500 or 300 µmol m^{-2} s^{-1} PPFD. P_N values are means of three or four leaves.

Growth temperature [°C]	Growth PPFD [µmol m^{-2} s^{-1}]	Measurement PPFD [µmol m^{-2} s^{-1}]	P_N [µmol m^{-2} s^{-1}]	
			Bean	Soybean
20	1,000	1,500	24.8	26.3
25	1,000	1,500	25.7	25.1
30	1,000	1,500	24.0	23.8
25	1,000	300	11.4	12.3
25	150	1,500	15.8	15.2

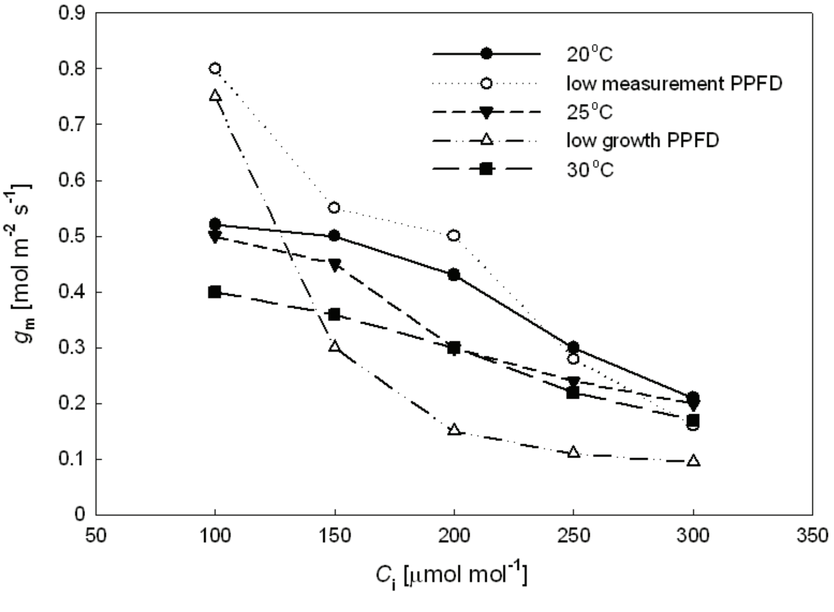

Fig. 2. Responses of g_m to C_i in common bean leaves measured at 25°C and 1,500 µmol m^{-2} s^{-1} PPFD for plants grown at 1,000 µmol m^{-2} s^{-1} PPFD at 20, 25, and 30°C, and for plants grown at 25°C at 150 µmol m^{-2} s^{-1}, measured at 1,500 µmol m^{-2} s^{-1} PPFD, and for plants grown at 25°C and 1,000 µmol m^{-2} s^{-1} PPFD but measured at 300 µmol m^{-2} s^{-1} PPFD. Estimates of g_m are based on gas-exchange measurements on three or four leaves per species and growth condition.

at a C_i of 250 µmol mol^{-1} in bean were similar to soybean for the same growth and measurement conditions (Table 1).

In both species the ratio of the CO_2 level at Rubisco compared to the substomatal air space (C_c to C_i) decreased with C_i for plants grown and measured at high

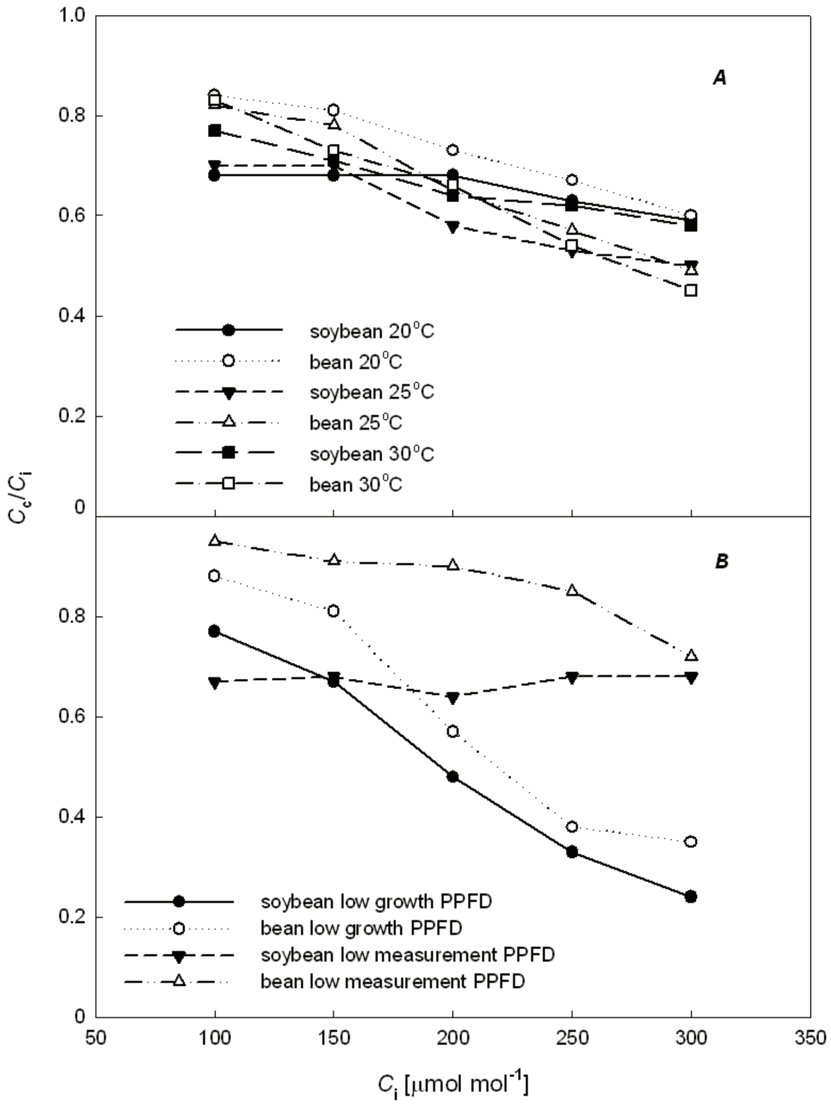

Fig. 3. The ratio of C_c to C_i at different C_i values for soybean and common bean *A*: grown at 20, 25, or 30°C at 1,000 μmol m^{-2} s^{-1} PPFD and measured at 1,500 μmol m^{-2} s^{-1} PPFD and *B*: for plants grown at 25°C and 150 μmol m^{-2} s^{-1} PPFD, measured at 1,500 μmol m^{-2} s^{-1} PPFD or grown at 1,000 μmol m^{-2} s^{-1} PPFD but measured at 300 μmol m^{-2} s^{-1} PPFD. Estimates of the ratio are based on gas-exchange measurements on three or four leaves per species and growth condition.

PPFD (Fig. 3*A*). On average the decrease in the C_c to C_i ratio was larger in bean than in soybean (Table 2), reflecting the larger decrease in g_m with C_i in bean. In bean, low measurement PPFD raised the C_c to C_i ratio at all C_i values (Fig. 3*B*). In both bean and soybean grown at low PPFD, the C_c to C_i ratio decreased much more strongly with increasing C_i than for plants grown at higher PPFD (Fig. 3*B*).

Discussion

These results indicate that bean and soybean, despite having similar leaf P_N under the same conditions, had contrasting responses of g_m to C_i when grown and measured at high PPFD, with very little change in g_m with C_i in soybean, but a large decrease in g_m with C_i in bean. The estimates of g_m in both cases used the same methodology and instrumentation, thus the differences in response were not caused by different measurement techniques in this case. The range of C_i over which g_m was determined covered the normal operating range for the current ambient atmospheric carbon dioxide concentration, including values typical of plants with low stomatal conductance because of water stress.

While several studies have tested for effects of water stress on g_m, effects of growth temperature and growth PPFD have been less studied. The small effect of growth temperature on g_m observed here in both species agrees with the few prior studies which have tested this (Yamori *et al.* 2005, Warren and Dreyer 2006, Bunce 2008, Warren 2008b), although minor changes were observed in some cases. Our results additionally indicate that a range of growth temperatures did not substantially affect the response of g_m to C_i, whether g_m was highly sensitive to C_i, as in bean or relatively insensitive, as in soybean.

The lower g_m in leaves developed at low PPFD observed here in both species is consistent with the lower

Table 2. Linear regression equations for the responses of the ratio C_c/C_i to C_i over the range of 100 to 300 μmol mol^{-1} C_i in leaves of bean and soybeans grown at three temperatures, measured at 1,500 μmol m^{-2} s^{-1} PPFD.

Species	Growth temperature [°C]	Intercept	Slope	r^2
Bean	20	0.98	–1,200	0.99
	25	1.01	–1,700	0.98
	30	1.02	–1,900	0.99
Soybean	20	0.74	–460	0.79
	25	0.83	–1,100	0.90
	30	0.85	–940	0.96

g_m in *Arabidopsis thaliana* grown at low PPFD (Flexas *et al.* 2007b) compared with the same species grown at high PPFD (Bunce 2008), and with the comparison of sun- and shade leaves of *Fagus sylvatica* (Warren *et al.* 2007). At least as importantly, low growth PPFD greatly increased the relative decrease in g_m with C_i in both species examined here, which makes comparisons of g_m sensitive to the measurement C_i (*e.g.* Fig. 2). Because the sensitivity of g_m to C_i was affected by growth PPFD, this could potentially account for some of the variation in responses of g_m to C_i in the literature. However, as shown here and in some other studies (*e.g.* Flexas *et al.* 2007a, and Hassiotou *et al.* 2009), high sensitivity of g_m to C_i can occur in some species even when grown at high light.

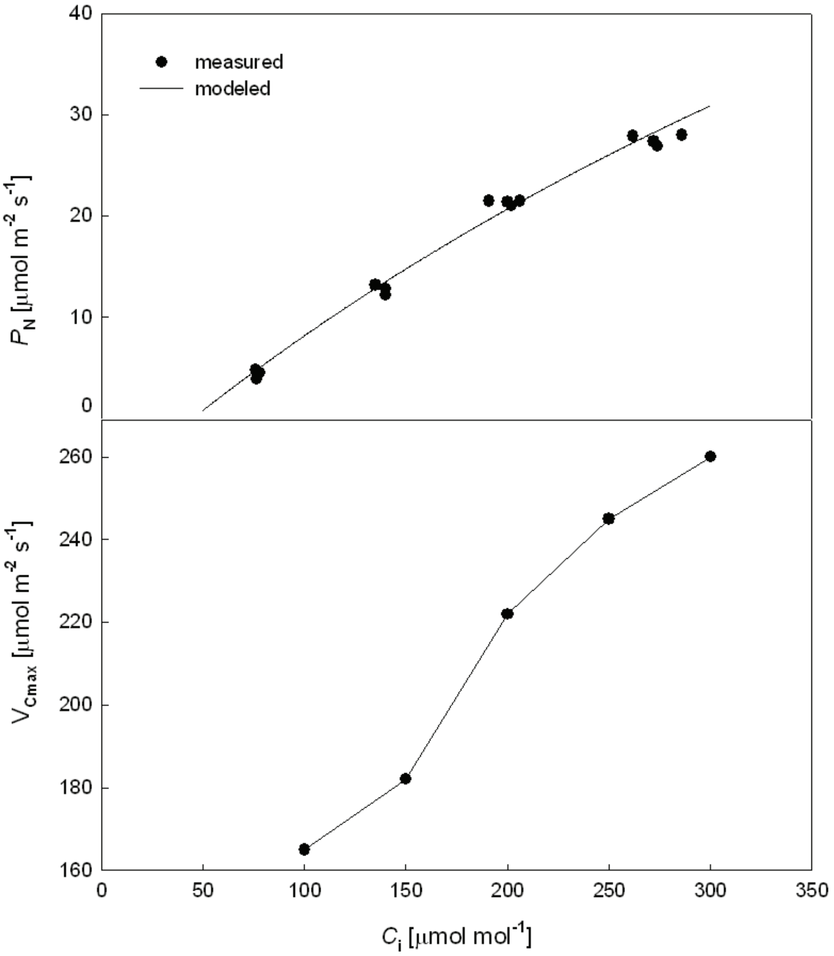

Fig. 4. Measured and modeled responses of P_N to C_i in common bean leaves, assuming infinite g_m and a single value of V_{Cmax}, and values of V_{Cmax} needed to predict measured P_N values of those leaves using measured values of g_m. Plants were grown at 25°C, 1,000 μmol m^{-2} s^{-1} PPFD, and leaves of four plants were measured at 25°C and 1,500 μmol m^{-2} s^{-1} PPFD.

Tazoe *et al.* (2009) reported no effect of measurement PPFD on g_m in wheat leaves measured over the range of 200 to 1,500 μmol m^{-2} s^{-1}, while Hassiotou *et al.* (2009) found a 22% decrease in g_m at 500 *vs.* 1,500 μmol m^{-2} s^{-1}. Our results for bean and soybean measured at 150 μmol m^{-2} s^{-1} similarly indicate only minor effects of measurement PPFD on the absolute value of g_m when averaged over the range of C_i measured. However, for both bean and soybean, low measurement PPFD substantially increased the sensitivity of g_m to C_i.

There has been debate whether g_m scales with maximum P_N such that the C_c to C_i ratio varies little among species (Evans and von Caemmerer 1996, Loreto *et al.* 2002, Warren and Adams 2006). The data for bean and soybean indicate that the C_c to C_i ratio often decreased with measurement C_i. The overall range in the ratio was reasonably small for plants grown at high PPFD, but quite large for both species when grown at low PPFD.

The value of g_m has been found to depend on chloroplast position (Tholen 2008) and on aquaporin activity (Terashima and Ono 2002, Flexas *et al.* 2006, Miyazawa *et al.* 2008), as well as being temperature-dependent in some cases (Yamori *et al.* 2005, Warren and Dryer 2006, Warren 2008b), but it is not known how those responses may relate to changes in g_m with C_i, or with effects of growth and measurement PPFD.

Simple biochemical models of C_3 photosynthesis which assumed infinite g_m have been extremely useful in diverse applications in agriculture and ecology. Farquhar-type biochemical models of C_3 photosynthesis can generally be fit to P_N *vs.* C_i data in the C_i range of 100 to 300 μmol mol^{-1} with a single value of V_{Cmax} or a single value of V_{Cmax} combined with a single value of g_m (Sharkey *et al.* 2007). However, because C_c to C_i ratio decreased with C_i in most cases in bean and soybean (Fig. 3), the V_{Cmax} values required to fit the model based on C_c with the data increased substantially with C_i. This is illustrated in Fig. 4 for bean grown and measured at 25°C and high PPFD, where a single value of V_{Cmax} gave a satisfactory fit to the data when C_c was assumed to be equal to C_i, but V_{Cmax} had to increase strongly with C_i when using the measured values of g_m. Because the ratio of C_c to C_i decreased with increasing C_i in this case (Fig. 3), a plot of V_{Cmax} against C_c would be even steeper than that shown in Fig. 4. While this increase in V_{Cmax} with C_i could reflect changing activation status of Rubisco (*i.e.* deactivation at low C_i), it substantially complicates the modeling of photosynthesis until the response of g_m to C_i becomes predictable.

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