

Analysis of CO₂ Fluxes: Inclusion of Wall Conductance (G_w) on the Estimation of Rubisco Activity, V_{cmax} of Soybean Leaves

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In global change research, where modelling of CO₂ fluxes from plants is an important component in determining vegetation capacity to protect the climate, mechanistic-based modelling is needed when projection of future CO₂ absorption dynamics need to be estimated more accurately. Rubisco is the world's most abundant protein in plants and has the job of uniquely preparing CO₂ for chemical reduction. Rubisco activity in the leaf, described by V_{cmax} , can be estimated from gas exchange measurements of the initial slope of the response of CO₂ assimilation rate, A , to intercellular [CO₂]. This technique of estimation is favourable because it can avoid the uncertainties and difficulties when V_{cmax} is obtained directly by extraction and biochemical assay in artificial media. Rate of assimilation of soybean plants grown at different temperature (20/15, 25/20, and 32/27 °C day/night temperature) and [CO₂] (350 and 700 μmol mol⁻¹), were measured using gas exchange. The effect of wall conductance (g_w) on the parameterization of assimilations rate was observed. The temperature dependence of V_{cmax} depends strongly on wall conductance, where the shape of the curve would change significantly if finite wall conductance were included in the analysis. The implication is that it changes the values and interpretation of the temperature response of assimilation rate.

Key words: wall conductance, V_{cmax} , gas exchange, temperature response, assimilation rate

INTRODUCTION

There is a resistance across a leaf for diffusion of CO₂ from the air surrounding the leaf (at ambient concentration, c_a) to the substomatal cavities (c_i) and to the sites of CO₂ fixation within the leaf (c_c), which results in reducing the concentration of CO₂ inside a leaf compared to the concentration in the air outside the leaf. In the photosynthesis model of Farquhar and von Caemmerer (1982), c_c is considered to be equal to c_i by assuming an infinite wall conductance ($g_w = \infty$), i.e. no barrier to CO₂ diffusion from the substomatal cavity to the site of carboxylation. Evans *et al.* (1986) and von Caemmerer and Evans (1991), using the stable isotope method and Di Marco *et al.* (1990) using a combination of leaf gas exchange and chlorophyll fluorescence, were able to estimate the CO₂ concentration at the catalytic site of Rubisco (c_c). This estimate was lower than that of c_i , and so enabled them to estimate a finite value of g_w .

Direct measurement of g_w has also been done by Loreto *et al.* (1992), Lloyd *et al.* (1992), Epron *et al.* (1995), and Maxwell *et al.* (1997) for different plants. The value of g_w reported for wheat and other herbaceous plants were 0.4 mol m⁻² s⁻¹ bar⁻¹ (Evans *et al.* 1986; 1994), 0.14 mol m⁻² s⁻¹ bar⁻¹ for *Quercus rubra* and 0.12 for *Eucalyptus globulus* (Loreto *et al.* 1992; Lloyd *et al.* 1992) and for *Fagus sylvatica* and *Castanea sativa* it was 0.1- 0.15 mol m⁻² s⁻¹ bar⁻¹ (Epron *et al.* 1995). The difference between c_i and c_c (as affected by wall resistance) was relatively similar

between herbaceous species examined by von Caemmerer and Evans (1991) and woody plants studied by Epron *et al.* (1995), 80 and 78 μbar respectively.

Restricted diffusion of CO₂ within a leaf makes CO₂ assimilation less "efficient". The interpretation of the gas exchange measurements, especially of rubisco activity (V_{cmax}), will differ depending on whether c_i or c_c was used for analysis. This is particularly true at very low [CO₂] where the effects of diffusion become highly significant. Hence, the use of c_c is more important for the estimation of V_{cmax} , which is fitted at low CO₂ concentration from the CO₂ response curve of assimilation rate (A) (June 2005). Epron *et al.* (1995) showed that the estimated value of V_{cmax} in *Fagus sylvatica* and *Castanea sativa* using c_c was 2.4 times higher than the value estimated from using c_i .

The discussion above implies that high internal resistances to CO₂ diffusion require large values of V_{cmax} to fit a particular value of A . Neglect of these resistances would lead to an overestimation of the assimilation rate, both at leaf and canopy level if V_{cmax} was known from independent biochemical assays. Wrong estimation of the assimilation rate leaf level would become a problem when scaling up is conducted to canopy and ecosystem level; for instance in the case of investigating the effect of climate change (rising CO₂ concentration and temperature) on the dynamics of CO₂ absorption by plants.

The study presented here applies the model of Farquhar *et al.* (1980) and Farquhar and von Caemmerer

(1982) to experimental data representing soybean plants grown under different temperatures and CO₂ concentrations, in order to be able to identify change in the model parameters, that is its rubisco activity V_{cmax} , due to the inclusion of wall conductance on model analysis. The implication on temperature dependence of assimilation rate was analysed.

MATERIALS AND METHODS

Plant Material. Seeds of soybean (*Glycine max* [L.] Merr.) were sown in 12 litre plastic pots containing a mixture of sand and vermiculite (1:1, v/v). Plants were grown in a controlled environment chamber with a 14 hour photoperiod of around 700 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, 60/70% relative humidity day/night and three different temperature regimes: 20/15, 25/20, 32/27 day/night °C under ambient [CO₂], 350 $\mu\text{mol mol}^{-1}$ and 700 $\mu\text{mol mol}^{-1}$. The lowest and highest temperature regimes were repeated with atmospheric [CO₂] enrichment to 700 $\mu\text{mol mol}^{-1}$.

The source of light used in the growth chamber was a metalarc lamp (General Electric Lighting), MVR 1000/U. Plants were well spaced (30 cm apart at sowing, two plants per pot) to avoid mutual shading. Each pot was flushed every second day with full-strength Herridge's solution (Herridge 1977) and watered twice daily on days when nutrients were not given. Measurements of gas exchange were made on expanded leaves of the third trifoliate (about 14-16 days after emergence).

Gas Exchange Measurements. Rates of CO₂ assimilation by the soybean leaves were measured over a wide range of CO₂ concentrations (50-900 $\mu\text{mol mol}^{-1}$), photon flux densities (0-1650 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and leaf temperatures (15-35 °C). Leaf to air vapour pressure difference was maintained at about 12.5 mbar. Irradiance at the leaf surface for all CO₂ exchange measurements was maintained at 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, except during measurement of the light response curve. Each different CO₂ concentration was maintained for at least 30 minutes to reach a steady state gas exchange, while each light intensity was maintained for at least 20 minutes before the measurements were recorded. Measurements were made on expanded leaves of the third trifoliate, 14-16 days after emergence of the leaves.

During gas exchange measurement, the leaf was clamped by a double-sided aluminium cuvette with a glass window such that 2.4 cm² of the leaf area was exposed to light and air flow. Leaf temperature was regulated by circulating water between the water jackets of the leaf chamber and a temperature controlled water bath. Calculated fluxes from the upper and lower leaf surfaces were combined to give whole leaf gas exchange. The leaf was illuminated by a 250 W metal halide lamp. Variations in light intensity were obtained by varying the distance between the lamp and the leaf and by putting in a copper wire screen under the light source. Ambient air was scrubbed of CO₂ by two 0.8 m soda-lime columns connected in series with an activated charcoal column. CO₂-free air flow was controlled by a mass flow controller.

Air was saturated with water vapour through a series of two sintered bubblers in distilled water and then passed through a glass condenser column to set the humidity level. A temperature regulated water bath was used to circulate cooling water through the water jacket of the condenser to set the dew point of the air. A mass flow controller was used to inject 2 or 10% CO₂ in the air to give the desired CO₂ concentration. Manual flow valves regulated the air flow to each side of both chambers at a rate of 0.8 l min⁻¹. The CO₂ concentration of air before entering the chamber was measured with an absolute infrared gas analyser (IRGA) (Fuji Electric, model ZAR), while the change in CO₂ concentration after passing over the leaf was measured with a differential IRGA (Beckman Instruments, model 865).

Leaf temperature was measured with a 0.1 mm copper/constantan thermocouple pressed against the lower leaf surface. Additional thermocouples measured the condenser and humidity sensor temperatures. Thermocouple signals were amplified using signal conditioning modules (model 3012 Advantech Inc. USA). Sensors were scanned by an analog to digital board in a standard IBM compatible PC. Calculations of gas exchange photosynthetic variables and parameters were based on those given by Farquhar and von Caemmerer (1982).

Models of Leaf Photosynthesis and Temperature Dependence of Parameters. Leaf photosynthesis was controlled by the amount of activated enzyme *RuBP carboxylase-oxygenase (Rubisco)*, the rate of regeneration of RuBP, and the relative partial pressures of CO₂ (c_i) and O₂ inside leaves. Therefore, under a given set of environmental conditions, the net CO₂ assimilation rate, A , was taken as being either the Rubisco-limited rate, A_v , or the predicted RuBP-regeneration limited rate of photosynthesis, A_j , whichever was the lower at a particular c_i . A had units of $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Farquhar *et al.* 1980; Farquhar & von Caemmerer 1982; June 2002; June 2005). The model equations used were as followed:

$$A_j = \frac{J}{4} \left(\frac{c_i - \Gamma^*}{c_i + 2\Gamma^*} \right) - R_d \quad (1)$$

$$A_v = V_{\text{cmax}} \left(\frac{c_i - \Gamma^*}{K_c \left(1 + \frac{O}{K_o} \right) + c_i} \right) - R_d \quad (2)$$

$$A = \min(A_j, A_v) \quad (3)$$

where c_i = partial pressure of CO₂ in the leaf (μbar); Γ^* = CO₂ compensation partial pressure in the absence of dark respiration (μbar); R_d = dark respiration by the leaf which continues in the light ($\mu\text{mol m}^{-2} \text{s}^{-1}$); O = ambient partial pressure of oxygen (mbar); K_c and K_o are Michaelis-Menten constants for carboxylation and oxygenation by Rubisco (μbar and mbar, respectively); V_{cmax} is the

maximum rate of Rubisco activity in the leaf ($\mu\text{mol m}^{-2} \text{s}^{-1}$); and J is the actual electron transport rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$).

The temperature dependence of K_c and K_o follows an Arrhenius function, using the following equations:

$$K_c = K_{c,25} \exp \left[\frac{E_c}{298.2 R} \left(1 - \frac{298.2}{T + 273} \right) \right] \quad (4)$$

$$K_o = K_{o,25} \exp \left[\frac{E_o}{298.2 R} \left(1 - \frac{298.2}{T + 273} \right) \right] \quad (5)$$

where R was the universal gas constant, $8.3144 \text{ J mol}^{-1} \text{ K}^{-1}$, and T was temperature in $^{\circ}\text{C}$. E_c and E_o were the apparent activation energies and the 25 subscript refers to the value at 25°C .

The effect of temperature on the CO_2 compensation point of photosynthesis in the absence of mitochondrial respiration followed the equation of von Caemmerer *et al.* (1994):

$$\Gamma^* = 36.9 + 1.88(T - 25) + 0.036(T - 25)^2 \quad (6)$$

The parameters K_c and K_o indicated the intrinsic kinetic properties of Rubisco. They were relatively constant, varying only with temperature for all C_3 species (Berry & Björkman 1980; Jordan & Ogren 1984), and hence in this experiment the values presented by Badger and Collatz (1977) and von Caemmerer *et al.* (1994) were used. The values for K_c , K_o and Γ^* (Pa) at 25°C are 40.4, 24800, 3.69 and the activation energies (Joule mol^{-1}) for K_c and K_o were 59400, 36000, respectively, assuming wall conductance $g_w = \infty$ (Badger & Collatz 1977; von Caemmerer *et al.* 1994).

There were two parameters that needed to be estimated for this particular investigation: V_{cmax} and R_d . The values of V_{cmax} and R_d were vary greatly between species and growth conditions (Farquhar & von Caemmerer 1982), and hence they were estimated for all treatments at three leaf temperatures. From the measurement of the light response curves, where the incident light ranged from 0 to $1650 \mu\text{mol m}^{-2} \text{s}^{-1}$, R_d was determined by extrapolation of a linear regression at the lower end of the response curve (at 0 – $150 \mu\text{mol m}^{-2} \text{s}^{-1}$). V_{cmax} was estimated from the lower end of the c_i response curve at c_i up to around $200 \mu\text{bar}$. Temperature dependence of K_c and K_o follows Eqs. (4) and (5). The temperature dependence of Γ^* follows Eq. (6).

Estimation of g_w . According to von Caemmerer *et al.* (1994) the CO_2 internal partial pressure in the site of carboxylation in the chloroplast, c_c , was calculated from the following equation:

$$c_c = c_i - \frac{A}{g_w} \quad (7)$$

where c_i was the CO_2 partial pressure in the substomatal cavities, A was assimilation rate and g_w was wall conductance. At 25°C and CO_2 concentration of $350 \mu\text{mol}$

mol^{-1} , von Caemmerer and Evans (1994) measured that

$$c_i - c_c = \frac{A}{g_w} = 80 \mu\text{bar}$$

Measurement by June (2002) on the assimilation light response curve at 25°C and CO_2 concentration of $350 \mu\text{mol mol}^{-1}$, resulted in light saturated CO_2 -assimilation rate (maximum assimilation rate) of $29.4 \pm 2.2 \mu\text{mol m}^{-2} \text{s}^{-1}$. Therefore, g_w for soybean would be roughly calculated

$$\text{as followed: } g_w = \frac{A(25^{\circ}\text{C}, 350 \text{ ppm})}{80 \mu\text{bar}} = \frac{29.4}{80} = 0.368$$

$\text{mol m}^{-2} \text{s}^{-1} \text{ bar}^{-1}$.

Taking into account the importance of g_w , V_{cmax} was then calculated using the following equation:

$$V_{\text{cmax}} = \frac{\frac{dA}{dc_i} g_w \left(\Gamma^* + K_c \left(1 + \frac{O}{K_o} \right) \right)}{g_w - \frac{dA}{dc_i}} \quad (8)$$

and by fitting to Eq. (2) at 15, 25 and 35°C , changing c_i to c_c (partial pressure of CO_2 at the site of carboxylation) and using the appropriate K_c , K_o and Γ^* , assimilation rate temperature dependence was simulated. The result is shown in Table 1 and Figure 2.

Temperature Response of g_w . Evans *et al.* (1994) and Terashima *et al.* (1995) found that the main limiting step for CO_2 diffusion from the intercellular space into the chloroplast may be water in the cell wall, and therefore Hikosaka (1997) used the temperature dependence of the diffusion coefficient of CO_2 in water, as found by Hesketh *et al.* (1983), for the calculation of the temperature dependence of g_w as follows:

$$g_w = g_{w,25} (-6.81 + 0.0262T) \quad (9)$$

where T is the absolute temperature (K). The calculated g_w for soybean plants used in this experiment at 25°C and $[\text{CO}_2]$ and at $350 \mu\text{mol mol}^{-1}$ was $0.368 \text{ mol m}^{-2} \text{s}^{-1} \text{ bar}^{-1}$. Using Eq. (9), g_w at 15, 25, and 35°C would be 0.27, 0.37, and $0.47 \text{ mol m}^{-2} \text{s}^{-1} \text{ bar}^{-1}$, respectively. The effect of using a finite value of g_w on V_{cmax} was examined, both by holding g_w constant and by increasing g_w with temperature as suggested by Hikosaka (1997).

RESULTS

CO_2 Response Curve. In general, the response curves of relationship between c_i and the CO_2 assimilation rate at different measurement temperatures (15, 25, and 35°C) for plants grown at low ($20/15^{\circ}\text{C}$) and high temperature ($32/27^{\circ}\text{C}$) with both 350 and $700 \mu\text{mol mol}^{-1}$ CO_2 concentration showed a typical crossing over due to increases in the CO_2 -compensation point and the RUBP-regeneration rate with increasing temperature (Figure 1). The initial slopes, dA/dc_i at c_i up to around $200 \mu\text{bar}$ was used to estimate

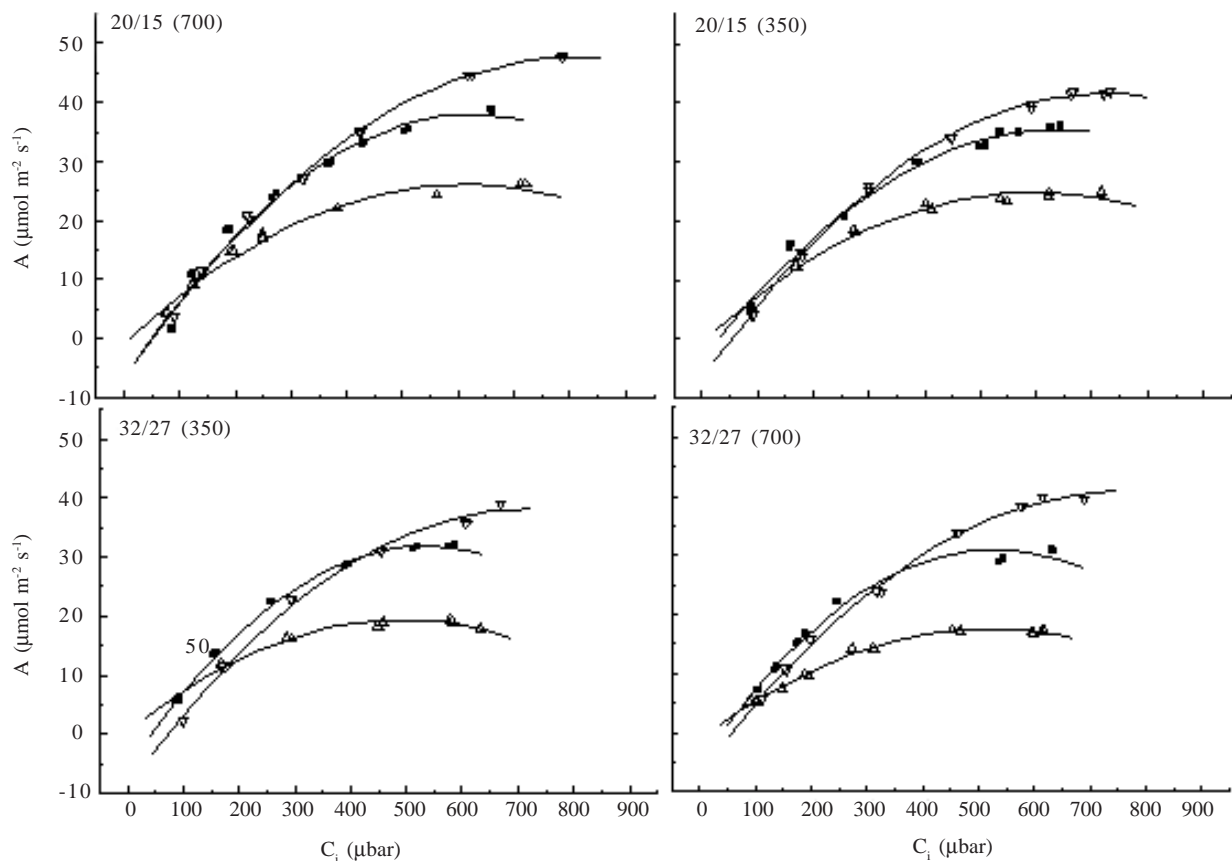


Figure 1. Relationship between net CO₂ assimilation rate (A) and intercellular concentration of CO₂ (c_i) obtained at three temperatures (Δ: 15 °C; ■: 25 °C, and ▽: 35 °C) of soybean leaves grown at different day/night temperatures and [CO₂]. Measurement was done with light intensity of 1200 μmolm⁻²s⁻¹.

Table 1. V_{cmax} temperature dependence equations with different assumptions in wall conductance (all equations have R² > 0.95)

Growth conditions	Day/night °C ([CO ₂])	Wall conductance (mol m ⁻² s ⁻¹ bar ⁻¹)	Temperature response of V _{cmax}
20/15 (350)		$g_w = \infty$	$V_{cmax} = 110.53 (1 + 0.083 (T-25) + 2.4 \times 10^{-3} (T-25)^2)$
		$g_w = 0.368$	$V_{cmax} = 209.79 (1 + 0.043 (T-25) - 3.5 \times 10^{-3} (T-25)^2)$
		g_w increases with T	$V_{cmax} = 211.17 (1 + 0.036 (T-25) - 3.9 \times 10^{-3} (T-25)^2)$
25/20 (350)		$g_w = \infty$	$V_{cmax} = 111.14 (1 + 0.061 (T-25) + 9.9 \times 10^{-4} (T-25)^2)$
		$g_w = 0.368$	$V_{cmax} = 157.07 (1 + 0.034 (T-25) - 2.7 \times 10^{-3} (T-25)^2)$
		g_w increases with T	
32/27 (350)		$g_w = \infty$	$V_{cmax} = 99.35 (1 + 0.066 (T-25) + 5.0 \times 10^{-4} (T-25)^2)$
		$g_w = 0.368$	$V_{cmax} = 122.31 (1 + 0.071 (T-25) + 3.3 \times 10^{-4} (T-25)^2)$
		g_w increases with T	
20/15 (700)		$g_w = \infty$	$V_{cmax} = 108.49 (1 + 0.084 (T-25) + 3.8 \times 10^{-3} (T-25)^2)$
		$g_w = 0.368$	$V_{cmax} = 123.83 (1 + 0.070 (T-25) + 3.7 \times 10^{-3} (T-25)^2)$
		g_w increases with T	
32/27 (700)		$g_w = \infty$	$V_{cmax} = 96.28 (1 + 0.088 (T-25) + 2.8 \times 10^{-3} (T-25)^2)$
		$g_w = 0.368$	$V_{cmax} = 113.07 (1 + 0.069 (T-25) + 7.1 \times 10^{-4} (T-25)^2)$
		g_w increases with T	

the value of V_{cmax}. The slopes changed with short-term temperature measurements and also with different growth conditions and was calculated by rearrange equation (2) to the following equation assuming infinite wall conductance with temperature dependence following equations (4), (5), and (6).

$$V_{cmax} = \frac{dA}{dc_i} \left(\Gamma^* + K_c \left(1 + \frac{O}{K_o} \right) \right) \quad (10)$$

The form of the equation is V_{cmax} = a [1 + x(T-25) + y(T-25)²], where a was V_{cmax} at 25 °C; x and y were intrinsic

dependencies on temperature. x is the slope of the curve at 25 °C and y is the curvature at 25 °C. It was shown that the inclusion of finite g_w in the analysis changes the temperature dependence of V_{cmax} (Table 1). All data show that V_{cmax} at 25 °C increased when using g_w = 0.368 mol m⁻² s⁻¹ bar⁻¹ compared to using g_w = ∞. For plants grown at lower temperature and ambient [CO₂], V_{cmax} already showed saturation at 35 °C, resulting in a changed temperature response curve. This saturation is shown by the negative value of y (Figure 2).

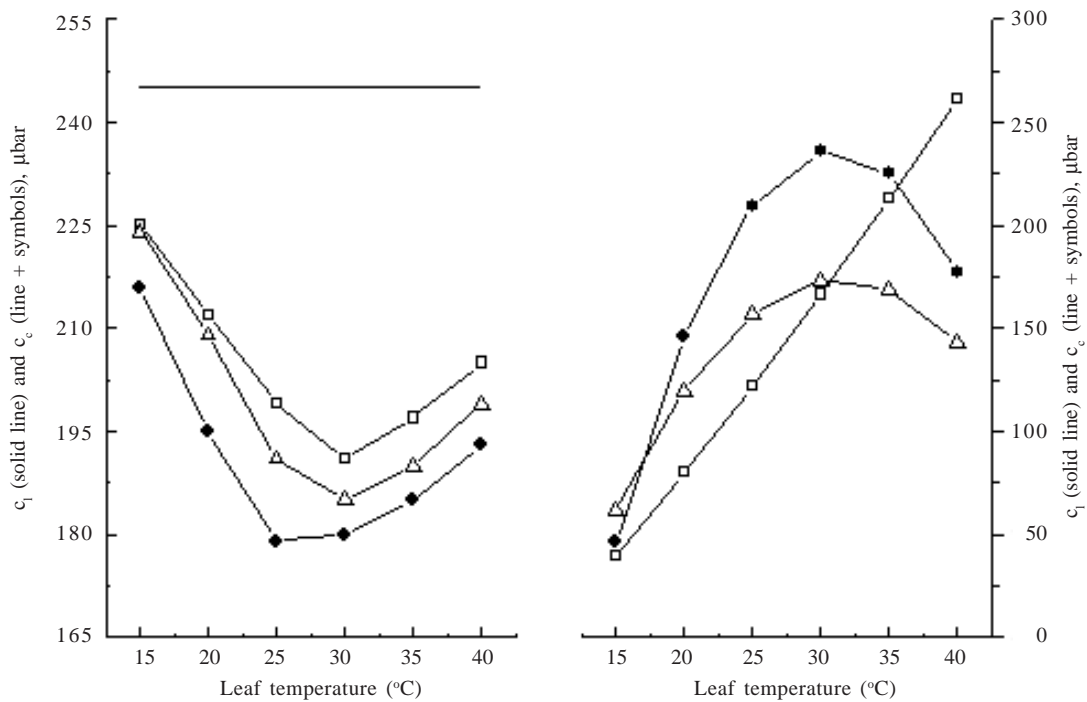


Figure 2. Temperature dependence of $[\text{CO}_2]$ at the site of carboxylation (c_i) (assuming c_i does not change with temperature but stays constant at $0.7 c_a$) and V_{cmax} with the inclusion of wall conductance, $g_w = 0.368 \text{ mol m}^{-2} \text{ s}^{-1} \text{ bar}^{-1}$, for plants grown at different temperatures: 20/15 °C (●), 25/20 °C (Δ), and 32/27 °C (□).

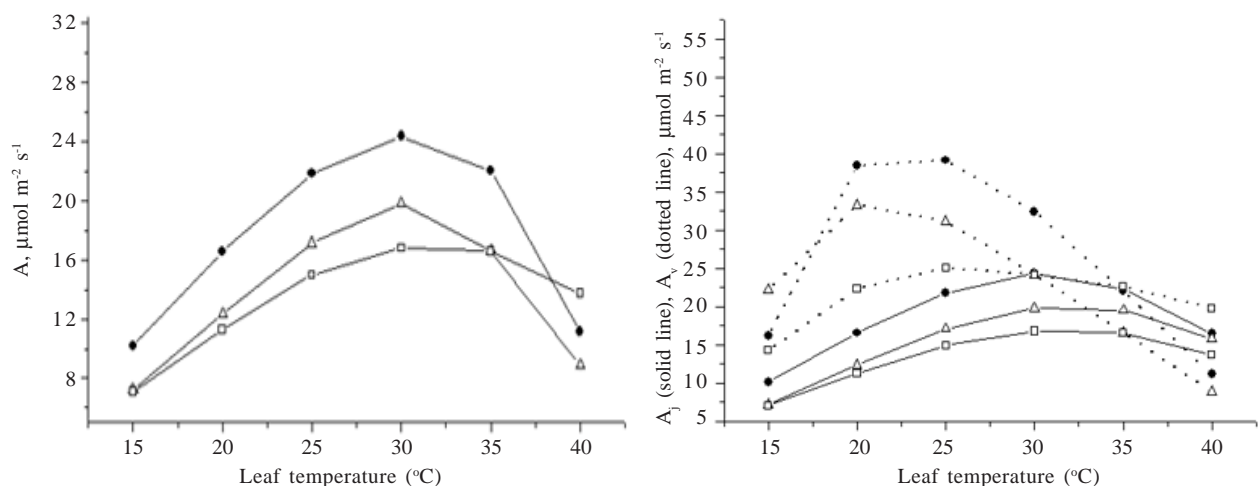


Figure 3. Simulated temperature dependence of CO_2 -assimilation rate (A), assimilation rate limited by RuBP regeneration (A_v) (solid line) and limited by Rubisco (A_r) (dotted line) of plants grown at different temperatures: 20/15 °C (●), 25/20 °C (Δ) and 32/27 °C (□), using $g_w = 0.368 \text{ mol m}^{-2} \text{ s}^{-1} \text{ bar}^{-1}$.

Using a g_w that increased with temperature, V_{cmax} increased more at 15 °C (because wall conductance was lower at this temperature) and decreased at 35 °C (wall conductance was higher) with the same amount of increase at 25 °C than when $g_w = \infty$ was used, and hence the responses of V_{cmax} to temperature were more curved when g_w varied with temperature.

DISCUSSION

Effect of Inclusion of Wall Conductance on Assimilation Rate and its Optimum Temperature. The inclusion of wall conductance (g_w) in the analysis of assimilation rate result in reduced V_{cmax} at higher measurement temperature (Table 1 & Figure 2) when plants

were grown at 20/15 and 25/20 °C day/night temperature but not when plants were grown at 32/27 °C. This will have an impact of the temperature dependence of assimilation rate (Figure 3). The temperature dependence of assimilation rate, A (which is the minimum of A_v and A_r , see Eq. (3) (using associated K_o , K_c and Γ^* for $g_w = 3.68 \text{ mol m}^{-2} \text{ s}^{-2} \text{ bar}^{-1}$) and using CO_2 concentration at the site of carboxylation (c_i) given by Eq. (7) can be simulated as shown in Figure 2 and Figure 3. The CO_2 concentration at the site of carboxylation (c_i) was lower than the CO_2 concentration in the intercellular spaces (c_a) (Figure 2). But even when c_i is assumed to be constant with temperature, c_c has a temperature dependence with the lowest values coinciding with the highest A (Figure 2 & Figure 3).

With $g_w = \infty$, V_{cmax} increases with short-term temperature measurement regardless of growth temperature but with $g_w = 0.368 \text{ mol m}^{-2} \text{ s}^{-1} \text{ bar}^{-1}$, V_{cmax} of plants grown at the two lower temperatures drops after around 30 °C. The higher temperature grown plants do not show saturation of V_{cmax} up to a measurement temperature of 35 °C. The implication of this response of V_{cmax} to temperature with a finite g_w is that it will change the interpretation of the temperature dependence of A_v (Rubisco-limited rate) and A_j (the predicted RuBP-regeneration limited rate) of photosynthesis, and hence A , as shown in Figure 3.

Now, the temperature where co-limitation (A_v or A_j) occurs changes. For plants grown at 20/15 and at 25/20 °C, below 33-35 °C the assimilation rate is limited by A_j (electron transport) while at higher temperature it is limited by rubisco, while plants grown at 32/27 °C, were always limited by A_j (electron transport in the thylakoid membrane). In June (2005), where analysis we conducted using $g_w = \infty$ the temperature where co-limitation occurs was happening at lower level (hence lower optimum temperature) and the assimilation of plants grown at 32/27 °C do not always limited by electron transport. Figure 3 also shows that for conditions in which electron transport becomes limiting (that is, growth at high temperature), the electron transport rate will dominate the behaviour of the CO_2 -assimilation rate, so the optimum temperature of A should shift towards the optimum temperature of rate of electron transport (June 2005).

Examining the Effect of Wall Conductance. Leaf photosynthesis can be described in terms of the CO_2 and O_2 partial pressure in the chloroplast, and the model used considers that chloroplast assimilation of CO_2 can be limited by either activity of Rubisco (V_{cmax}) or chloroplast capacity for electron transport. The partial pressure in the chloroplast and in the intercellular air spaces were assumed to be equal ($c_i = c_c$), due to absence of measurements, and the internal resistance to diffusion was assumed to be zero. Evans *et al.* (1986), von Caemmerer and Evans (1991), Evans *et al.* (1994), von Caemmerer *et al.* (1994), and Brugnoli *et al.* (1998), using carbon isotope discrimination techniques, have estimated the internal conductance. Di Marco *et al.* (1990) and Harley *et al.* (1992) used another method to estimate internal conductance, that is the combination of fluorescence and gas exchange techniques. The importance of this finite internal conductance is that it lowers the partial pressure of CO_2 in the chloroplast and thus affects the estimation of the Rubisco parameters from gas exchange measurements. Hence, when using a finite internal conductance in the model, it is important to use the parameter values appropriate to the assumptions. von Caemmerer *et al.* (1994) estimated K_c , K_o and Γ^* assuming either infinite ($g_w = \infty$) or finite ($g_w = 0.3 \text{ mol m}^{-2} \text{ s}^{-1} \text{ bar}^{-1}$) wall conductance and found the values to be different: 259 μbar , 179 mbar, and 38.6 μbar , respectively, with $g_w = 0.3 \text{ mol m}^{-2} \text{ s}^{-1} \text{ bar}^{-1}$, instead of 404 μbar , 248 mbar, and 36.9 μbar at $g_w = \infty$. De Pury (1995) examined the effect of including a finite internal conductance and found that it had very little effect (< 1%) on the diurnal course of canopy photosynthesis, provided

the parametrisation was internally consistent. de Pury (1995) used $g_w = 0.5 \text{ mol m}^{-2} \text{ s}^{-1} \text{ bar}^{-1}$ at $V_{cmax} = 100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ for his analysis and assumed that g_w does not change with temperature. Based on this experiment, the inclusion of wall conductance is significantly change the behaviour of the temperature dependence of V_{cmax} .

Equations in Table 1 show that when wall conductance is not taken into account ($g_w = \infty$), there is no indication of V_{cmax} (from all growth conditions) approaching saturation, or becoming lower when measurement temperature was increased, as seen by the positive values of y . However, when wall conductance is finite ($g_w = 0.368 \text{ mol m}^{-2} \text{ s}^{-1} \text{ bar}^{-1}$), plants grown at low temperature and low ambient $[\text{CO}_2]$ show saturation at 35 °C, but not plants grown at high temperature or plants grown at double $[\text{CO}_2]$.

Effect of Growth Condition on V_{cmax} . According to the model theory, the value of V_{cmax} indicates the maximum RuBP saturated rate of carboxylation, which is proportional to the amount or activity of RuBP carboxylase in the leaves. Under long term treatment of high $[\text{CO}_2]$, V_{cmax} has been reported to change. A decrease in V_{cmax} at elevated $[\text{CO}_2]$ has been found in gas exchange measurements for a number of species (Sage *et al.* 1989; Harley *et al.* 1992; June 2002). Decreased Rubisco activity and protein content in response to high CO_2 concentration have also been reported in trees (Nie & Long 1992; Van Oosten *et al.* 1992). However, Cambell *et al.* (1988) found that although photosynthetic capacity (maximum rate of photosynthesis) of soybean increased under elevated $[\text{CO}_2]$ treatment, the Rubisco activity was not affected. The data in Table 1 also show little effect of growth $[\text{CO}_2]$ on V_{cmax} in soybean.

For the soybean plants used in this experiment, V_{cmax} is constantly increasing with temperature, in relatively good agreement with Wang *et al.* (1996) and other estimates (Wullschlegel 1993). However, Ferrar *et al.* (1989) who investigated several species of *Eucalyptus* grown at contrasting temperatures found that in leaves grown at high temperature, V_{cmax} increased with short-term temperature measurement, but in leaves grown at low temperature, V_{cmax} did not increase as measurement temperature increased. They speculated that Rubisco may be inactivated or damaged at measurement temperatures higher than the growth temperature. In this experiment, although V_{cmax} increases with short-term temperature measurements for all growth conditions, plants grown at higher temperature have a slightly lower V_{cmax} than plants grown at lower temperature. This changes for measurement temperature higher than optimum temperature when wall conductance is included in the analysis (Table 1).

In conclusion, the use of c_c is important for the estimation of V_{cmax} , which is fitted at low CO_2 concentration from the CO_2 response curve of assimilation rate (A). This experiment have shown that the inclusion of finite g_w in the photosynthesis model used changes the temperature dependence of V_{cmax} and therefore a different conclusion (shift in optimum temperature) can be drawn when wall conductance is included in the analysis of temperature response of assimilation rate, in terms of which processes control assimilation rate at a certain temperature, whether rubisco or electron transport.

V_{cmax} was higher when determined using $g_w = 0.368 \text{ mol m}^{-2} \text{ s}^{-1} \text{ bar}^{-1}$ compared to using $g_w = \infty$. When $g_w = \infty$, V_{cmax} never gives any sign of saturation with increasing temperature while under finite g_w plants grown at lower temperature (20/15 and 25/20 °C) and ambient $[\text{CO}_2]$, its V_{cmax} shows saturation at around 30 °C, and plants grown at 32/27 °C do not show saturation with increasing temperature. This response of V_{cmax} with finite g_w results in changing the temperature dependence of assimilation rate through changing the controlling factor. The result is important when modelling the effect of climate change (change in temperature, CO_2 and radiation) on plant physiological activities.

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