

***In vivo* temperature response functions for leaf steady-state photosynthesis models**

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Introduction

Predicting the responses of leaf photosynthesis to elevated atmospheric [CO₂] and increased temperature is fundamental to projecting the impact of global change on the biosphere. Describing temperature effects at the leaf level is equally critical to predicting community gas exchange as leaf temperature varies diurnally and seasonally. Farquhar *et al.* (1980) presented a steady-state mechanistic model of C₃ leaf photosynthetic carbon assimilation (*A*), modified by Harley & Sharkey (1991). This model has been widely validated and has provided the basis for scaling carbon uptake to canopies, ecosystems and landscapes. The original model of Farquhar *et al.* (1980) was parameterized for a leaf temperature of 25°C, however, the model's accuracy appears to decrease at higher and lower temperatures. Although other temperature functions have been proposed (McMurtrie & Wang 1993; Harley & Baldocchi 1995) these also produce modeled values for *A* that deviate strongly from observed measurements. The error associated with these temperature functions most likely results from their derivation from *in vitro* measurements made over a narrow temperature range.

Better predictions of Rubisco-limited photosynthesis are necessary because photosynthesis is commonly Rubisco-limited under natural conditions (Rogers & Humphries 2000). The difficulties associated with the *in vitro* estimations of kinetics might be overcome through *in vivo* measurements using anti-sense Rubisco small subunit (anti-*rbcS*) tobacco plants, which express low Rubisco concentrations. The photosynthetic rates of the anti-*rbcS* plants are Rubisco-limited over a large range of *C_i* in contrast to wild-types. This provides a more accurate basis for estimating Rubisco kinetic properties (von Caemmerer 2000).

The objective of this study was to determine *in vivo* temperature dependencies of Rubisco kinetic parameters over a range of biologically significant temperatures. Because the properties of Rubisco enzyme kinetics are assumed to be conserved among higher plants (Farquhar *et al.* 1980), the *in vivo* temperature functions developed with this research should provide increased accuracy of leaf, canopy, and global carbon uptake models.

Materials and methods

Plants were grown in greenhouses located at the University of Illinois, Urbana, IL USA. Seeds of a transformed line of tobacco (*Nicotiana tabacum*, L. cv W38) described by

Rodermal (1988) were sown in 0.9 L plastic containers and individually transplanted into 1.5 L pots two weeks after emergence. Nutrient additions were given weekly in the form of 300 $\mu\text{L L}^{-1}$ of NPK 15:5:15 (Peters Excel, The Scotts Co., Marysville, OH USA) to pot saturation. The reduction in Rubisco made these plants vulnerable to photoinhibition which was avoided by maintaining leaves at a PPFD of approximately 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ using shade cloth.

Leaf gas exchange rates were measured using an open gas exchange system (LI 6400, LI-COR, Inc., Lincoln, NE USA) on the two newest fully expanded leaves of each plant. The chamber was modified by replacing the peltier external heat sink with a metal block containing water channels, which in turn were connected to a circulating water bath allowing maintenance of any leaf temperature between 10–40°C. Photosynthesis was measured at a PPFD of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to prevent photoinhibition from occurring. The vapor pressure deficit in the cuvette was maintained between 0.5 and 2.0 kPa to prevent stomatal closure.

Three replicate individuals were measured at 5°C intervals between 10 and 40°C for each set of parameters (R_d and Γ^* , $V_{c,max}$ and K_c , K_o , $V_{o,max}$). The temperature responses of R_d and Γ^* were determined from measurements made following the procedure of Laisk (1977). We used gas-exchange measurements at differing C_i and O_2 concentrations to solve for the six parameters. Measurements of A/C_i curves in 2% O_2 , to minimize photorespiration, were used to estimate $V_{c,max}$ and K_c . The equation describing the Michaelis-Menten function approximating the response of A to C_i in the absence of O_2 was fit to these data by maximum-likelihood regression. Measurements of A over a range of $[\text{O}_2]$ were used to determine K_o using equation [4] with values of K_c and $V_{c,max}$ taken from the A/C_i curves. Values for K_o were then used to solve again for K_c and $V_{c,max}$, this time accounting for photorespiration. This was repeated until values for K_o , K_c , and $V_{c,max}$ were constant. Values of K_c , K_o , and $V_{c,max}$ and Γ^* were then used to solve for $V_{o,max}$ using equation [7]. Upon completion of these measurements the temperature responses of Γ^* , R_d , $V_{c,max}$, $V_{o,max}$, K_c , and K_o were determined. The temperature responses of $V_{c,max}$, $V_{o,max}$ and R_d were standardized to a value of 1.0 at 25°C. This allows for a temperature response curve to be extrapolated from any absolute values of $V_{c,max}$ and R_d obtained at 25°C.

The observed temperature responses for sugar maple (*Acer saccharum*) from Jurik *et al.* (1988) and lemon (*Citrus limon* L.) are compared with the newly determined temperature responses and with temperature responses of earlier studies (Farquhar *et al.* 1980, McMurtrie & Wang 1993, Harley & Baldocchi 1995). Values of A were normalized to unity at 25°C for each set of temperature responses. The modeled values were expressed as percentage deviation from the observed values.

Results and Discussion

Constants associated with the kinetic properties of Rubisco (i.e., K_o , K_c , Γ^*) are generally assumed to be conserved for most higher plants utilizing the C_3 photosynthesis pathway. Therefore, once these parameters are established, they may be incorporated into generic leaf, canopy and ecosystem photosynthesis models. Parameters that depend on enzyme concentration ($V_{c,max}$, $V_{o,max}$ and R_d) are not conserved even within an individual. On the other hand, relative changes in these parameters with temperature should be conserved since they depend on enzyme structure and not on the concentration. The temperature response of these parameters is expected to remain proportional for all species and can thus be normalized to 25 °C.

The derived temperature response functions for the various parameters are provided in Fig. 1. The temperature response of Γ^* is similar to values determined by previous studies at lower temperatures (Laik 1977; Brooks & Farquhar 1985; Fig 1.a). However, the temperature dependence is not linear, as evident from the data at higher temperatures. A possible explanation for the inconsistency with previous studies is that there are a limited number of replicate measurements made at higher temperatures. At a given temperature, $V_{c,max}$ is expected to differ between species and among individuals of a species based on enzyme content and activation state. The pattern and magnitude of variation in Rubisco-limited CO_2 assimilation with temperature should be conserved as it is a property of enzyme kinetics that should remain constant for all species and growth conditions (von Caemmerer 2000). The activation energy of $V_{c,max}$ presented in this paper ($65.33 \text{ kJ mol}^{-1}$) is similar to the value presented at temperatures above 15° ($64.85 \text{ kJ mol}^{-1}$) (Badger & Collatz 1977). Further, this value is within the range presented by previous studies (Jordan & Ogren 1984, $74.35 \text{ kJ mol}^{-1}$; Farquhar *et al.* 1980, $58.52 \text{ kJ mol}^{-1}$).

The temperature dependence of $V_{o,max}$ has been assumed to be a constant proportion of $V_{c,max}$ over a range of temperature at $0.21 \cdot V_{c,max}$ (Farquhar *et al.* 1980) though numerous values ranging from 0.19 to $0.77 \cdot V_{c,max}$ have been determined (Badger & Collatz 1977; Badger & Andrews 1974; Jordan & Ogren 1981, 1984; Makino *et al.* 1988; Whitney *et al.* 1999). One study provides temperature responses from *in vitro* measurements of $V_{c,max}$ and $V_{o,max}$ (Badger & Collatz 1977). The ratio, $V_{o,max}/V_{c,max}$, decreases with temperature (Fig. 1d) as observed previously (Badger & Collatz 1977).

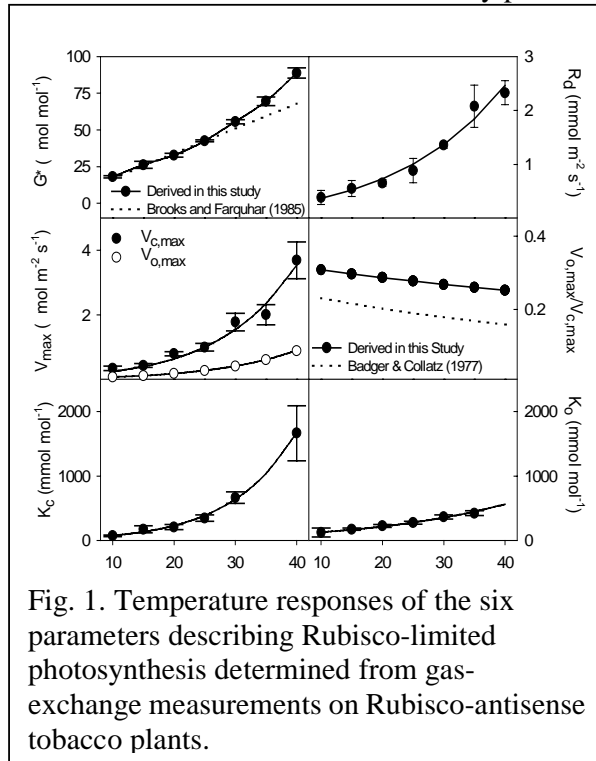


Fig. 1. Temperature responses of the six parameters describing Rubisco-limited photosynthesis determined from gas-exchange measurements on Rubisco-antisense tobacco plants.

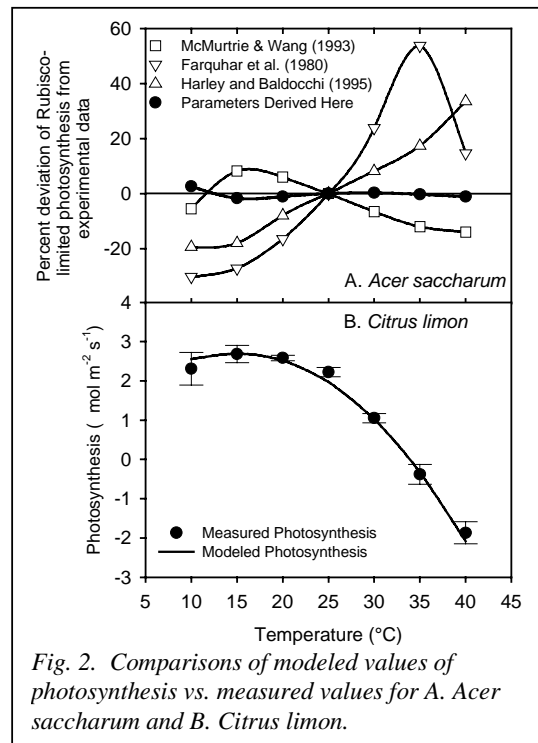


Fig. 2. Comparisons of modeled values of photosynthesis vs. measured values for A. *Acer saccharum* and B. *Citrus limon*.

However, our ratio is considerably higher. The differences between these data and those presented by Badger and Collatz (1977) might be attributable to *in vivo* vs. *in vitro* measurements.

Reported values for K_c and K_o vary considerably at a given temperature (Badger & Collatz 1977; Jordan & Ogren 1984; Harley *et al.* 1985), even though the values for these parameters are expected to remain similar among higher plants (von Caemmerer *et al.* 1994). The values determined in this study can only be compared with those of von Caemmerer *et al.* (1994) who provide *in vivo* values for K_c and K_o . Results from this study and those reported by von Caemmerer *et al.* (1994) are within 1% of each other at 25°C for K_c and within 10% for K_o (Fig. 1e), even though the two studies used different transgenic strains of tobacco.

The ability of these temperature functions derived from tobacco to predict temperature dependence of Rubisco-limited photosynthesis was compared, along with previously published temperature functions, to experimental data (Fig. 2; experimental data from Jurik *et al.* 1988). The temperature response of Rubisco-limited photosynthesis modeled using the data from this study shows stronger correspondence with the experimental data than previous temperature functions. In addition to the comparison of temperature responses, the ability of the temperature response functions to accurately predict photosynthesis at any temperature between 10 and 40°C was tested against data collected from lemon (Fig. 3). These comparisons suggest the temperature responses of the *in vivo* enzyme kinetics improves the ability to predict the rate of Rubisco-limited photosynthesis over the temperature range at which most carbon assimilation will occur in the terrestrial biosphere.

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