Journal of Experimental Botany, Vol. 55, No. 408, pp. 2581–2588, December 2004
doi:10.1093/jxb/erh260 Advance Access publication 22 October, 2004

Journal of Experimental Botany

RESEARCH PAPER

A/C_i curve analysis across a range of woody plant species: influence of regression analysis parameters and mesophyll conductance

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Received 1 July 2004; Accepted 21 July 2004

Abstract

The analysis and interpretation of A/C_i curves (net CO_2 assimilation rate, A, versus calculated substomatal CO_2 concentration, C_i) is dependent upon a number of underlying assumptions. The influence of the C_i value at which the A/C_i curve switches between the Rubisco- and electron transport-limited portions of the curve was examined on A/C_i curve parameter estimates, as well as the effect of mesophyll CO_2 conductance (g_m) values on estimates of the maximum rate of Rubisco-mediated carboxylation (V_{cmax}). Based on an analysis using 19 woody species from the Pacific Northwest, significant variation occurred in the C_i value where the Rubisco- and electron transport-limited portions of the curve intersect (C_{i_t}), ranging from 20 Pa to 152 Pa and averaging *c.* 71 Pa and 37 Pa for conifer and broadleaf species, respectively. Significant effects on estimated A/C_i parameters (e.g. V_{cmax}) may arise when preliminary estimates of C_{i_t}, necessary for the multiple regression analyses, are set either too high or too low. However, when the appropriate threshold is used, a significant relationship between A/C_i and chlorophyll fluorescence estimates of carboxylation is achieved. The use of the V_{cmax} parameter to describe accurately the Rubisco activity from the A/C_i curve analysis is also dependent upon the assumption that C_i is approximately equal to chloroplast CO_2 concentrations (C_c). If leaf mesophyll conductance is low, C_c will be much lower than C_i and will result in an underestimation of V_{cmax} from A/C_i curves. A large range of mesophyll conductance (g_m) values was observed across the 19 species (0.005±0.002 to 0.189±0.011 mol m^{-2} s^{-1} for *Tsuga heterophylla* and *Quercus garryana*, respectively) and, on average, g_m was 1.9 times lower for the conifer species (0.058±0.017 mol m^{-2} s^{-1} for conifers versus 0.112±0.020 mol m^{-2} s^{-1} for broadleaves). When this mesophyll limitation was accounted for in V_{cmax} estimates, considerable variation still existed between species, but the difference in V_{cmax} between conifer and broadleaf species was reduced from *c.* 11 μmol m^{-2} s^{-1} to 4 μmol m^{-2} s^{-1}. For example, A/C_i curve estimates of V_{cmax} were 31.2±6.2 and 42.2±4.4 μmol m^{-2} s^{-1}, and A/C_c curve estimates were 41.2±7.1 μmol m^{-2} s^{-1} and 45.0±4.8 μmol m^{-2} s^{-1}, for the conifer and broadleaf species, respectively.

Key words: A/C_i curve analysis, CO_2 assimilation, mesophyll CO_2 conductance, photosynthesis, Rubisco, V_{cmax}.

Introduction

A/C_i curve (net CO_2 assimilation rate, A, versus calculated internal CO_2 concentrations, C_i) analysis has become

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Abbreviations: A, net CO_2 assimilation rate; α_{leaf}, leaf absorptance; C_a, ambient CO_2 concentration; C_c, Rubisco catalytic site CO_2 concentration; C_i, internal CO_2 concentration; C_{i_t}, actual C_i value at which the A/C_i curve switches between the Rubisco- and electron transport-limited portions of the curve; F'_m, maximal fluorescence upon illumination with a 0.8 s 7000 μmol m^{-2} s^{-1} saturation flash; F_s, steady-state fluorescence; g_m, mesophyll CO_2 conductance; J, electron transport rate; J_c electron transport rate devoted to carboxylation measured by chlorophyll fluorescence; J_{max}, A/C_i curve estimate of the maximum rate of carboxylation limited by electron transport; J_o, electron transport rate devoted to oxygenation measured by chlorophyll fluorescence; K_c, Michaelis–Menten coefficient for CO_2 binding to Rubisco; K_o, Michaelis–Menten coefficient for CO_2 binding to Rubisco; *PPFD*, photosynthetic photon flux density; R_{day}, rate of respiration in the presence of light; S, specificity of Rubisco for O_2/CO_2; V_{cmax}, maximum rate of carboxylation limited by the amount, activity, and kinetics of Rubisco; W_c, rate of carboxylation limited by the amount, activity, and kinetics of Rubisco; W_j, rate of carboxylation limited by ribulose-1,5-*bis*phosphate regeneration supported by electron transport; W_p, rate of carboxylation limited by triose phosphate availability.

a common tool to estimate leaf photosynthesis under a wide variety of experimental conditions (Farquhar *et al*., 1980; Wullschleger, 1993, and references therein; Manter *et al*., 2000). The response function also represents the mechanistic basis behind many plant physiology models (Harley *et al*., 1992; Manter *et al*., 2003). While the acquisition of A/C_i response curves is relatively quick and easy to perform, inexpensive (after the initial equipment purchase), and non-destructive, verification of biochemical estimates and analysis assumptions has not been widely tested.

According to the Farquhar *et al*. (1980) model, carboxylation rates are limited by one of three processes: (i) the amount, activity, and kinetics of Rubisco (W_c), (ii) the rate of ribulose-1,5-*bis*phosphate regeneration supported by electron transport (W_j), and (iii) occasionally, triose phosphate availability (W_p). Each of these processes can be described mathematically and is expressed at different C_i values. Determination of leaf photosynthesis and gas exchange via A/C_i curve regression analysis necessitates the *a priori* designation of a C_i threshold at which the A/C_i curve switches between the Rubisco- and electron transport-limited portions of the curve (C_{i_t}*). It has been well documented that the W_c process occurs at the lowest C_i values (Farquhar *et al*., 1980) and common values of C_{i_t}* used for analysis range from 20–25 Pa (Harley *et al*., 1992; Wullschleger, 1993). However, the effect of C_{i_t}* values on A/C_i curve parameter estimates have not been well documented, and based on observations working with conifers (Manter *et al*., 2000; J Kerrigan and DK Manter, unpublished data), it was noted that actual C_{i_t} values may reach 50 Pa or more and differ markedly between plants.

A second inherent assumption in A/C_i curve analysis is that C_i is approximately equal to that of the catalytic site of Rubisco (C_c). However, limitations to mesophyll CO_2 conductance (g_m), which are not incorporated in A/C_i measurements, may result in a difference between C_i and C_c. Furthermore, of the limited number of plant species from which g_m has been quantified, considerable variation has been observed, ranging from *c*. 25 mmol m^{-2} $s^{-1}$to 400 mmol m^{-2} s^{-1} (von Caemmerer and Evans, 1991; Lloyd *et al*., 1992; Loreto *et al*., 1992; Epron *et al*., 1995). As a consequence of this difference in C_i and C_c, Epron *et al*. (1995) showed that estimates of the maximum rate of carboxylation limited by the amount, activity, and kinetics of Rubisco (V_{cmax}) from A/C_i curves may be lower than those determined from A/C_c curves (V_{cmax_ACc}). Finally, much of the variation in V_{cmax} values across species groups (i.e. broadleaves > conifers) (Wullschleger, 1993) may be associated with unaccounted differences in g_m and an underestimation of the actual Rubisco activity using the V_{cmax} parameter, since a similar pattern in g_m has also been reported (broadleaves > conifers) (Evans *et al*., 1986; von Caemmerer and Evans, 1991; Lloyd *et al*., 1992; Loreto *et al*., 1992; Epron *et al*., 1995). The purpose of this study was to examine specific parameters involved with leaf photosynthesis and gas exchange measurements to optimize A/C_i analysis and estimates of associated processes. The primary objectives were to (i) determine the influence of C_{i_t}* values on A/C_i curve parameter estimates and (ii) examine the effect of mesophyll CO_2 conductance (g_m) values on estimates of the maximum rate of Rubisco-mediated carboxylation (V_{cmax}) and compare A/C_i and A/C_c curve estimates. Nineteen woody plant species from the Pacific Northwest were used for measurements, and differences between coniferous and broadleaf species were noted.

Materials and methods

Plant material

One- and two-year-old potted seedlings of various Pacific Northwest species were obtained from local nursery stock in the spring and grown under ambient conditions in an outdoor cold-frame on the Oregon State University campus in Corvallis. Plants were irrigated as needed and fertilized with Osmocote Pro 18-8-8 (Scotts-Sierra Horticultural Products Co., Marysville, OH). The 19 species were *Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr. (white fir), *Abies grandis* (Douglas ex D. Don) Lindl. (grand fir), *Abies magnifica* Andr. Murray (California red fir), *Abies procera* Rehd. (noble fir), *Acer circinatum* Pursh (vine maple), *Acer macrophyllum* Pursh (big leaf maple), *Alnus rhombifolia* Nutt. (white alder), *Alnus rubra* Bong. (red alder), *Corylus cornuta* Marsh (beaked hazel), *Larix occidentalis* Nutt. (western larch), *Pinus lambertiana* Douglas (sugar pine), *Pinus monticola* Douglas ex D. Don (western white pine), *Populus trichocarpa×deltoides* (hybrid poplar), *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir), *Quercus garryana* Douglas (Oregon white oak), *Quercus rubra* L. (northern red oak), *Rhododendron macrophyllum* D. Don ex G. Don (Pacific rhododendron), *Rhododendron occidentale* (Torr. & A. Gray) A. Gray (western azalea), and *Tsuga heterophylla* (Raf.) Sarg. (western hemlock).

A/C$_i$ curves and fluorescence

Simultaneous measurements of A/C_i response curves and chlorophyll fluorescence were measured on current-year foliage (*c*. 2 cm^2 one-sided projected leaf area) from seedlings (*n*=2–3) of each species using a Li-Cor 6400 portable photosynthesis system (Open System Vers. 4.0, Li-Cor, Inc., Lincoln, NE) within a 10 d period in July. Cuvette conditions were maintained at a photosynthetic photon flux density (*PPFD*) of 1600 μmol m^{-2} s^{-1}, relative humidity >60%, and a leaf temperature of 25 °C. Leaf temperatures were measured directly for broadleaf species and calculated using the energy balance method for conifer species. Ambient CO_2 concentration (C_a) in the cuvette was controlled with a CO_2 mixer across the series of 30, 20, 10, 40, 50, 60, 80, 100, 160, and 200 Pa, and measurements were recorded after equilibration to a steady state (coefficient of variation ≤2%). CO_2 leakage into and out of the empty cuvette was determined at each reference C_a value and used to correct measured leaf fluxes using the equations provided in the Li-Cor operator's manual (see also Bernacchi *et al*., 2002). Following measurements, one-sided projected leaf area for conifers (broadleaf species' leaves were large enough to fill the entire cuvette) were estimated by placing needles between glass plates and digitally estimating leaf area (Agimage, Decagon Devices, Pullman, WA).

Non-linear regression techniques, based on the equations of Farquhar *et al*. (1980) and later modified by Sharkey (1985) and Harley and Sharkey (1991), were used to estimate V_{cmax}, J_{max} (the maximum rate of carboxylation limited by electron transport), and R_{day} (rate of respiration in the presence of light) for each A/C_i curve. In some cases, carboxylation may also be limited by triose phosphate availability (Sharkey, 1985; Harley and Sharkey, 1991); however, this was not observed in any of the plants measured in the study. As discussed elsewhere (Harley *et al.*, 1992; Wullschleger, 1993), it is necessary first to estimate the W_c curve (i.e. V_{cmax} and R_{day}) by selecting C_i values below some threshold (i.e. C_{i_t}*), where it is assumed that A is limited by the amount, activity, and kinetic properties of Rubisco, and then to use the remaining portion of the A/C_i curve to solve for the W_j curve and J_{max}. Typical C_{i_t}* values of 20–25 Pa have been suggested (Wullschleger, 1993), but the consistency with which C_{i_t}* values occur within this range and their influence on A/C_i curve parameter estimates have not received much attention.

To examine the influence of C_{i_t}* values, two V_{cmax} estimates were determined. The first estimate (V_{cmax_ACi1}) was calculated using a constant C_{i_t}*=25 Pa. The second estimate (V_{cmax_ACi2}) was calculated using a variable C_{i_t}* (i.e. increasing the C_{i_t}* value such that each successively higher C_a set-point was included in a new analysis), where the appropriate C_{i_t}* value resulted in the lowest regression mean square statistic. For each V_{cmax} estimate above, the carboxylation rate at C_a=40 Pa (W_{c_ACi}) was calculated from equation (1)

$$W_{c\_ACi} = \frac{V_{cmax} \times C_i}{C_i + K_c(1 + O/K_o)} \quad (1)$$

where K_c and K_o are the Michaelis–Menten coefficients for CO_2 and O_2, respectively, binding to Rubisco, and O is the intercellular partial pressure of O_2 (21 kPa). At each C_a concentration, chlorophyll fluorescence measurements of steady-state fluorescence (F_s) and maximal fluorescence (F'_m) upon illumination with a 0.8 s 7000 μmol m^{-2} s^{-1} saturation flash were also measured. Electron transport rate (J) was calculated according to equation (2)

$$J = \left(\frac{F'_m - F_s}{F'_m}\right) \times 0.5 \times PPFD \times \alpha_{leaf} \quad (2)$$

where α_{leaf} is leaf absorptance, which was assumed to be 85% (a typical value for C_3 plants; Ehleringer and Pearcy, 1983), and the 0.5 factor assumes equal distribution of electrons between PSII and PSI (actual range may vary between 0.4 and 0.6; Ögren and Evans, 1993; Laisk and Loreto, 1996; Albertsson, 2001). The carboxylation rate at C_a=40 Pa (W_{c_fluor}) was then calculated from equation (3)

$$W_{c\_fluor} = \frac{J \times C_i}{4(C_i + O/S)} \quad (3)$$

where S is the specificity factor of Rubisco for O_2 and CO_2 based on the equations of Harley *et al.* (1992).

Estimation of g_m *and* A/C_c *curve analysis*

Because of recent evidence that suggests V_{cmax} estimated from A/C_i curves may be underestimated when mesophyll conductance is low and C_c is less than C_i (Epron *et al.*, 1995; Centritto *et al.*, 2003), estimates of g_m and maximum Rubisco-mediated carboxylation (V_{cmax_ACc}) were also derived from A/C_c curves. For estimation of g_m the method outlined in Epron *et al.* (1995) was used. Briefly, an *in vivo* Rubisco specificity factor (S*) was determined as the slope of the linear regression, forced through the origin, between J_c/J_o and C_i/O, where J_c/J_o is the ratio of electron flow devoted to carboxylation and oxygenation measured by chlorophyll fluorescence and C_i/O is the ratio of the CO_2 and O_2 mole fractions in the intercellular space (Fig. 1A). In some cases, this relationship was not linear, so the initial slope (C_a <60 Pa) was used to calculate S* (Fig. 1B). Next, g_m was calculated from equations (4) and (5)

$$C_c = C_i \frac{S^*}{S} \quad (4)$$

$$g_m = A/(C_i - C_c) \quad (5)$$

where S was assumed to be 2560 mol mol^{-1}. This value of S is a typical value for C_3 plants which, based on *in vitro* measurements, has been shown to range from 2100 to 2950 mol mol^{-1} (Epron *et al.*, 1995, and references therein). Over this range of S values, estimates of C_c values from equation 4 will be underestimated by 21.9% if S is actually 2100 mol mol^{-1}, and overestimated by 13.2% if S is actually 2950 mol mol^{-1}; estimates of g_m from equation 5 will be underestimated by 26.4% if S is actually 2100 mol mol^{-1}, and overestimated by 11.2% if S is actually 2950 mol mol^{-1}. Finally, V_{cmax_ACc} was determined from the newly derived A/C_c curve using the same variable threshold technique (C_{c_t}*) technique outlined above for estimation of V_{cmax_ACi2}.

Analysis

The Marquardt estimation technique was used for all non-linear regression analysis of A/C_i and A/C_c curves using the PROC NLIN module of SAS (SAS Institute, Cary, NC). Differences in the various V_{cmax} estimates between conifer (n=9) and broadleaf (n=10) species were tested using the PROC GLM module in SAS, assuming a completely randomized design. All equations describing the relationship between parameters were determined using the curve-fitting tools found in Sigmaplot 2000 (SPSS, Inc., Chicago, IL).

Results

Three distinct A/C_i curve patterns (Fig. 2) reoccurred throughout the analysis of the dataset. The first two patterns were typified by increasing estimates of V_{cmax} as the C_{i_t}* value was raised (Fig. 2A, B). Typically any underestimation of the Rubisco-limited portion of the curve was readily

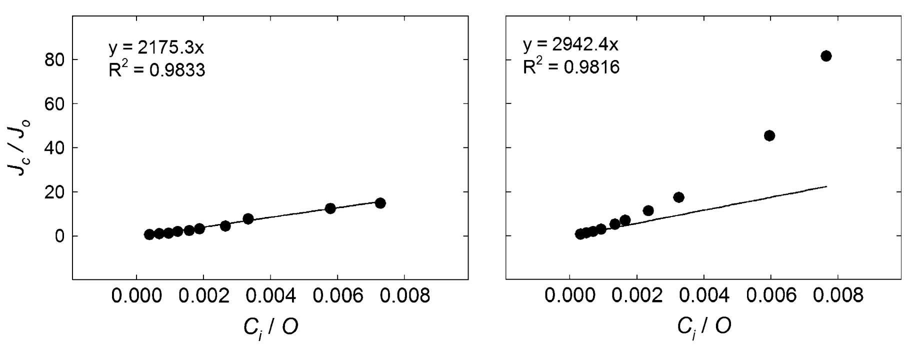

Fig. 1. Typical relationships between J_c/J_o and C_i/O used for estimation of S* showing a linear (A) and curvilinear (B) response.

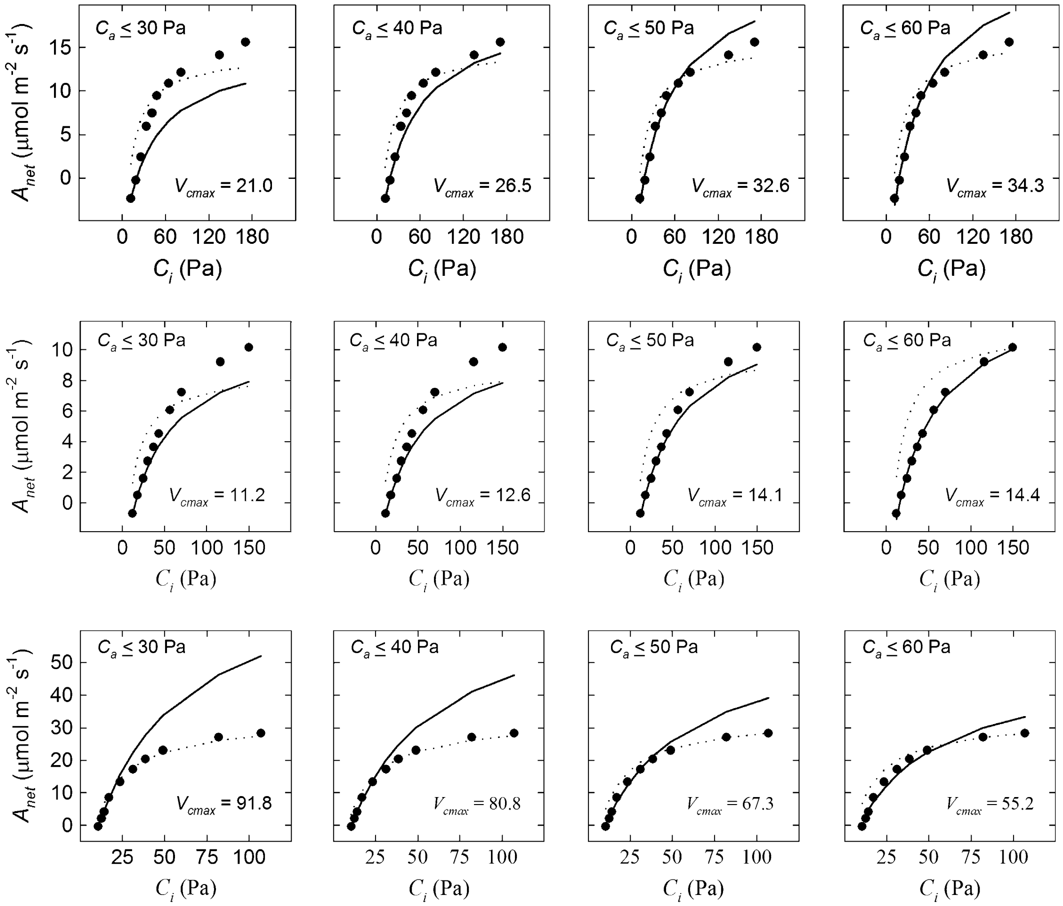

Fig. 2. Three representative A/C_i curve patterns (A, B, C) selected from measurements of 19 woody plant taxa. Each panel contains graphs of four different analyses of a single A/C_i curve derived from one plant using increasing C_i cut-off (C_{i_t}*) values (i.e. inclusion of increasing C_a set-point values). Closed circles are the measured rates of assimilation (A), solid lines are the estimated rate of carboxylation limited by Rubisco kinetics (W_{c_ACi}), and dotted lines are the estimated rate of carboxylation limited by electron transport (W_{j_ACi}).

apparent upon visual inspection in these two patterns; however, they differed as to whether an electron transport-limited portion of the A/C_i curve was present over the range of C_i values used in this study (cf. Fig. 2A, B). The third general pattern was typified by a decrease in the estimate of V_{cmax} as C_{i_t}* values were increased (Fig. 2C). The three pattern types were not limited to any particular species, although conifers appeared to be more sensitive to changes in the C_{i_t}* values (Fig. 3). It should be noted that the greatest changes (>100% change in V_{cmax} over the range of C_{i_t}* values) were easily seen by visual inspection (Fig. 2A), but the average change in V_{cmax} (*c.* 40.5% and 25.6% for the conifers and broadleaves, respectively) could not be visually differentiated (Fig. 2C).

Because of the difficulty in visually selecting the most appropriate A/C_i curve analysis, the regression mean square statistic was used to aid in selection. No apparent pattern in the 'best-fit' C_{i_t}* value was noted either among or within species, and values ranged from 28 to 79 Pa and from 22 to 63 Pa, averaging 46 and 39 Pa, for the conifer and broadleaf species, respectively (Table 1). Similarly, the actual intersection point between the Rubisco- and electron transport-limited portions of the A/C_i curve (C_{i_t}) varied greatly between plants, ranging from 25 to 152 Pa (average *c.* 71.Pa) for conifers and from 20 to 78 Pa (average *c.* 37 Pa) for broadleaves (Table 1).

The average V_{cmax} estimates from the two A/C_i curve analysis techniques are shown in Fig. 3. Species estimates spanned a considerable range with both analysis techniques. For example, using the constant C_{i_t}* technique V_{cmax} estimates ranged from 8.6 µmol m^{-2} s^{-1} (*Tsuga heterophylla*) to 53.4 µmol m^{-2} s^{-1} (*Pinus monticola*) for the conifers and from 20.0 µmol m^{-2} s^{-1} (*Rhododendron macrophyllum*) to 52.0 µmol m^{-2} s^{-1} (*Corylus cornuta*) for the broadleaves. The range of V_{cmax} estimates using the variable C_{i_t}* technique was 10.4 µmol m^{-2} s^{-1} (*Tsuga heterophylla*) to 70.0 µmol m^{-2} s^{-1} (*Pinus monticola*) and 28.2 µmol m^{-2} s^{-1} (*Acer circinatum*) to 68.1 µmol m^{-2} s^{-1} (*Alnus rubra*) for the conifer and broadleaf species, respectively. The percentage change in V_{cmax} between the two techniques averaged 25.1% and ranged from −19.8% (*Pseudotsuga menziesii*) to 73.8% (*Pinus monticola*). V_{cmax}

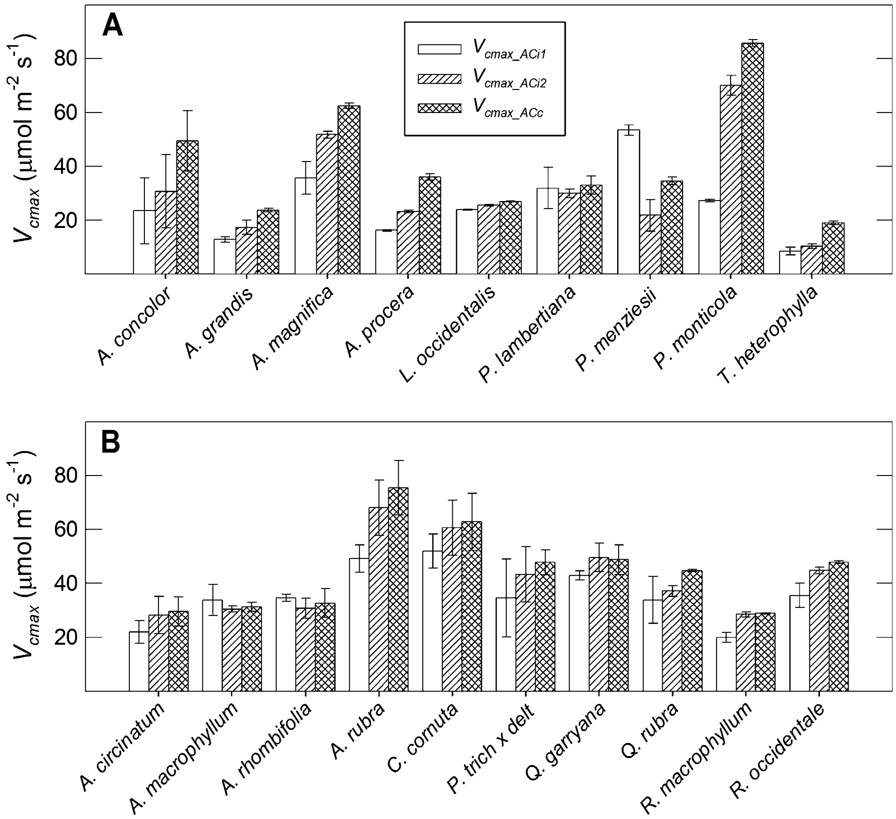

Fig. 3. Maximum rate of carboxylation limited by the amount, activity, and kinetics of Rubisco (V_{cmax}) for nine conifer (A) and ten broadleaf (B) species. V_{cmax} was estimated from (i) A/C_i curves using the constant C_{i_t}* (V_{cmax_ACi1}), (ii) A/C_i curves using the variable C_{i_t}* method (V_{cmax_ACi2}), or (iii) A/C_c curves using the variable C_{c_t}* method (V_{cmax_ACc}).

estimates between conifers and broadleaves were not statistically different (Table 3) despite a difference of *c*. 10 µmol m^{-2} s^{-1}. For example, mean values were 26.0±3.9 and 35.9±3.7 µmol m^{-2} s^{-1} for the constant C_{i_t}* technique, and 31.2±6.2 and 42.2±4.4 µmol m^{-2} s^{-1} for the variable C_{i_t}* technique, respectively. Similar results were also observed with other conifer (25±12 µmol m^{-2} s^{-1}) and broadleaf (47±33 µmol m^{-2} s^{-1}) species (Wullschleger, 1993).

Carboxylation rates derived from the two A/C_i curve analyses (constant or variable C_{i_t}*) were compared against estimates of carboxylation derived from chlorophyll fluorescence (W_{c_fluor}) (Fig. 4). Of the two A/C_i curve analysis techniques, the variable C_{i_t}* technique yielded the greatest similarity to the fluorescence-derived estimates (Fig. 4). The constant C_{i_t}* technique resulted in greater W_c errors for the conifers; particularly those plants that had lower V_{cmax} and higher C_{i_t} values (Fig. 4A).

In all, nine plants (eight conifers and one broadleaf) exhibited the second analysis pattern described above (Fig. 2B), and as a result J_{max} was not determinable. These plants were typified by relatively low values of V_{cmax_ACi2} (16.2±2.2 µmol m^{-2} s^{-1}) and included the following species: *Acer macrophyllum* (*n*=1), *Abies concolor* (*n*=1), *Abies grandis* (*n*=2), *Abies procera* (*n*=1), *Pseudotsuga menziesii* (*n*=1), and *Tsuga heterophylla* (*n*=3). Despite the wide range in values of V_{cmax_ACi2} and J_{max_ACi2}, a single, consistent linear relationship was observed (Fig. 5).

Epron *et al*. (1995) noted that the presence of low g_m values would result in an underestimation of V_{cmax} from A/C_i curve analyses because C_i is greater than C_c. They also suggested that conifers have, on average, lower g_m values compared with broadleaf species, which may account for some of the frequently observed differences in V_{cmax} between conifer and broadleaf species (Wullschleger, 1993). Estimates of g_m and V_{cmax} from A/C_c curves suggest that both of these hypotheses are correct. Like the V_{cmax} estimates, a wide range in g_m was observed across species, ranging from 0.005 mol m^{-2} s^{-1} (*Tsuga heterophylla*) to 0.145 mol m^{-2} s^{-1} (*Pinus monticola*) and 0.024 mol m^{-2} s^{-1} (*Populus trichocarpa*×*deltoides*) to 0.189 mol m^{-2} s^{-1} (*Quercus garryana*) for the conifer and broadleaf species, respectively (Table 2). On average g_m was 1.9-times lower for the conifer (0.058±0.017 mol m^{-2} s^{-1}) species as compared with the broadleaf (0.112±0.020 mol m^{-2} s^{-1}) species.

For all species, accounting for g_m limitations and the difference between C_i and C_c often resulted in a large increase in estimates of V_{cmax}. As shown in Fig. 4, V_{cmax_ACc} estimates increased *c*. 25.5% when averaged across all species, ranging from –1.6% (*Quercus garryana*) to 92.1% (*Abies concolor*), when compared with V_{cmax_ACi2} estimates. Once the effect of g_m limitations was taken into account, the difference in V_{cmax} between conifer and broadleaf species was reduced from *c.* 10 μmol m^{-2} s^{-1} to

Table 1. *Preliminary* (C_{i_t}*) *and actual* (C_{i_t}) C_i *values where the Rubisco- and electron transport-limited portions of the* A/C_i *curve intersect for nine conifer and ten broadleaf species*

C_{i_t}* was determined as the C_i value that resulted in the best-fit A/C_i curve analysis (i.e. lowest regression mean square), and C_{i_t} was determined algebraically as the intersection of the Rubisco (W_{c_ACi}) and electron transport (W_{j_ACi}) rates of carboxylation. Exact estimates of C_{i_t} for some plants (shown in parenthesis) could not be determined due to the entire A/C_i curve being limited by Rubisco kinetics; reported values are minimum estimates (i.e. observed C_i value when C_a=200 Pa).

Conifers	Rep	C_{i_t}*	C_{i_t}	Broadleaves	Rep	C_{i_t}*	C_{i_t}
A. concolor	1	33	31	*A. circinatum*	1	22	34
	2	53	(138)		2	43	50
	3	31	27		3	47	38
A. grandis	1	48	(152)	*A. macrophyllum*	1	36	36
	2	52	(103)		2	35	31
	3	44	43	*A. rhombifolia*	1	51	45
A. magnifica	1	63	70		2	48	47
	2	32	37	*A. rubra*	1	38	34
	3	29	35		2	47	42
A. procera	1	63	(99)		3	33	31
	2	38	46	*C. cornuta*	1	36	30
L. occidentalis	1	35	48		2	41	37
	2	32	56		3	42	36
P. lambertiana	1	52	55	*P. trchcrp.×dltds*	1	35	31
	2	28	25		2	41	37
	3	36	49		3	46	43
P. monticola	1	39	35	*Q. garryana*	1	42	50
	2	48	(81)		2	63	78
	3	35	38		3	48	36
P. menziesii	1	59	(70)	*Q. rubra*	1	29	27
	2	36	55		2	33	20
T. heterophylla	1	72	(141)	*R. macrophyllum*	1	24	22
	2	63	(125)		2	27	29
	3	79	(146)		3	22	27
				R. occidentale	1	45	47
					2	38	27

4 μmol m^{-2} s^{-1} (Table 3). For example, the mean values of V_{cmax_ACc} were 41.2±7.1 μmol m^{-2} s^{-1} and 45.0±4.8 μmol m^{-2} s^{-1} for conifers and broadleaves, respectively.

Discussion

It has been demonstrated that using the variable C_{i_t}* method is a more appropriate analysis method than using a constant C_i value. A/C_i curve analysis is dependent upon an *a priori* determination of the C_i value where the Rubisco and electron transport limitations to photosynthesis intersect. A detailed discussion of this parameter's influence on A/C_i curve analysis results has not been previously presented in the scientific literature, although examples of the use of the constant C_{i_t}* (Wullschelger, 1993) and variable C_{i_t}* (Manter *et al.*, 2000) methods may be found. Based on the better fit with chlorophyll fluorescence measurements, which do not depend upon presumptive determinations of C_{i_t}, the variable C_{i_t}* method is recommended for analysis of A/C_i curves. The impact of C_{i_t}* values on A/C_i curve estimates was not always visually apparent, and, on average, the constant C_{i_t}* method underestimated V_{cmax} by 25.1% compared with the variable C_{i_t}* technique.

Despite the wide range of photosynthesis and gas exchange estimates across the 19 woody plant species, some notable relationships were observed. A significant linear relationship occurred between J_{max} and V_{cmax}, and although the relative activity of these two processes appears to be constant across plants, C_{i_t} values varied greatly. As a result, actual rates of carbon assimilation, when light is saturating, will be limited by the amount, activity, and kinetics of Rubisco more often than electron transport rates ($C_i < C_{i\_t}$). Since Rubisco may serve as a storage protein and exist in an inactivated form (Cheng and Fuchigami, 2000; Warren *et al.*, 2000, 2003), it is likely that Rubisco activation is being limited in these plants by some other factor.

General differences between conifers and broadleaves were notable. For example, C_{i_t} was approximately two-times greater (*c.* 71.0 Pa and 37.1 Pa) and A/C_i curve

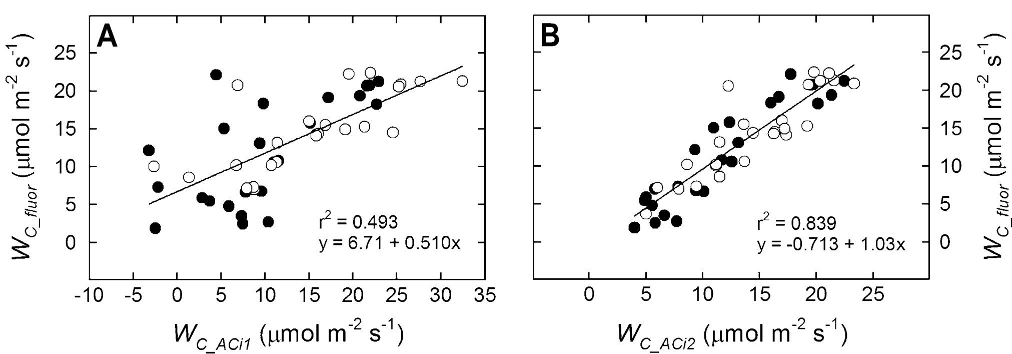

Fig. 4. Relationship between the rates of Rubisco-mediated carboxylation (C_a=40 Pa) estimated from A/C_i curve analyses (W_{c_ACi}) and chlorophyll fluorescence for 19 woody plant species. W_{c_ACi} was estimated from A/C_i curves analysed using either the constant (W_{c_ACi1}), (A) or variable C_{i_t}* method (W_{c_ACi2}), (B). Filled circles, conifers; open circles, broadleaves.

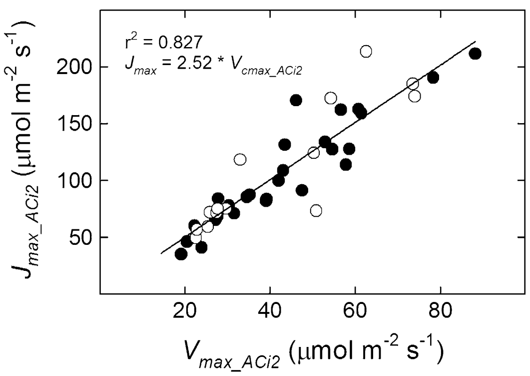

Fig. 5. Relationship between the maximum rates of carboxylation limited by the amount, activity, and kinetics of Rubisco (V_{cmax}) and electron transport (J_{max}) for 19 woody plant species. V_{cmax} and J_{max} were estimated from the A/C_i curve using the variable C_{i_t}* method. Filled circles, conifers; open circles, broadleaves

Table 2. *Average mesophyll (g_m) CO_2 conductance values (mol m^{-2} s^{-1}) and apparent Rubisco specificity (S*) for 19 woody plant species (n=2–3)*

Parameters estimates were based on the methods outlined in Epron *et al.* (1995).

	g_m		S*	
	Mean	SE	Mean	SE
Conifers				
A. concolor	0.039	0.025	793.9	250.4
A. grandis	0.013	0.007	907.0	123.7
A. magnifica	0.132	0.034	1670.9	268.5
A. procera	0.034	0.000	971.1	46.5
L. occidentalis	0.051	0.020	1738.4	76.0
P. lambertiana	0.074	0.049	2183.7	430.4
P. monticola	0.145	0.023	1611.0	72.4
P. menziesii	0.027	0.008	1114.5	221.5
T. heterophylla	0.005	0.002	629.0	43.8
Broadleaves				
A. circinatum	0.038	0.002	1956.0	517.5
A. macrophyllum	0.095	0.006	2257.6	437.4
A. rhombifolia	0.112	0.009	2032.3	247.7
A. rubra	0.169	0.028	2227.6	463.4
C. cornuta	0.184	0.014	2211.0	279.0
P. trchcrp.×dltds	0.024	0.017	2342.3	700.7
Q. garryana	0.189	0.011	2488.8	194.0
Q. rubra	0.175	0.016	2070.0	27.0
R. macrophyllum	0.084	0.032	2535.2	236.8
R. occidentale	0.054	0.008	1898.0	55.0

estimates of V_{cmax} were 10 μmol m^{-2} s^{-1} lower for conifers compared with broadleaves. The lower V_{cmax} in coniferous species may arise from a variety of other factors, such as less resource allocation to photosynthetic processes (Rubisco and chlorophyll per unit nitrogen), greater amounts of inactivated Rubisco, or lower g_m values. Based on simultaneous gas exchange and chlorophyll fluorescence measurements, conifers have, on average, significantly lower g_m values as compared with broadleaf species, and once this effect on C_c is accounted for the difference in

Table 3. *Comparison of conifer and broadleaf gas exchange maximum rates of carboxylation, limited by the amount, activity, and kinetics of Rubisco, derived from CO_2 response curves*

V_{cmax_ACi1} was calculated using a constant C_{i_t}*=25 Pa, V_{cmax_ACi2} was calculated using a variable C_{i_t}* where the appropriate C_{i_t}* value resulted in the lowest regression mean square statistic, and V_{cmax_ACc} was calculated from the A/C_c curve using the variable C_{c_t}* method. Values are the arithmetic means and individual standard errors calculated from Fig. 3.

	n	V_{cmax_ACi1}		V_{cmax_ACi2}		V_{cmax_ACc}	
		Mean	SE	Mean	SE	Mean	SE
Conifers	9	26.0	3.9	31.2	6.2	41.2	7.1
Broadleaves	10	35.9	3.7	42.2	4.4	45.0	4.8
P-value		0.0886		0.1612		0.6628	

V_{cmax} between conifers and broadleaves is reduced to 4 μmol m^{-2} s^{-1}. Similar results documenting the effect of g_m on V_{cmax} estimates have been found with other species (von Caemmerer *et al.*, 1994; Epron *et al.*, 1995) and conditions (Flexas *et al.*, 2002; Medrano *et al.*, 2002; Centritto *et al.*, 2003). After accounting for this limitation, V_{cmax} estimates still spanned a wide range across all species (19.1±0.7 for *Tsuga heterophylla* to 85.7±1.3 μmol m^{-2} s^{-1} for *Pinus monticola*).

The data presented here showed high inter- and intra-species variation for the intersection point at which the A/C_i curve switches from being limited by Rubisco to electron transport processes. Major errors in A/C_i curve analyses may arise from designating this threshold too high or too low and, as a general rule, species with low V_{cmax} values are more sensitive to estimation errors and exhibit higher C_{i_t} values. Considerable variation in g_m values among species may have contributed to perceived differences in Rubisco kinetics when determined solely from A/C_i curve analyses. Finally, it should be noted that all species were analysed with a set of constant values (e.g. *S*, α_{leaf}, K_c, K_o, and the distribution of electrons between photosystem I and II). These factors have all been shown to vary between species and, as such, will result in unknown errors in estimates of the various parameters reported (e.g. V_{cmax}, J_{max}, and g_m). While these assumptions may be increasing the variability of parameter estimates, it does not interfere with the general conclusions that across a range of 19 woody plant species (i) the selection of C_{i_t}* influences A/C_i curve estimates; (ii) g_m is correlated with V_{cmax}; and (iii) g_m limitations may result in substantially lower estimates of V_{cmax} from A/C_i curves as opposed to A/C_c curves.

Acknowledgements

The authors wish to thank Wendy Sutton and Dr Everett Hansen for supplying plant material and Dr Barbara Bond for use of the Li-Cor 6400.

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