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Estimation of Mesophyll Conductance to CO_2 Flux by Three Different Methods[1]

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ABSTRACT

The resistance to diffusion of CO_2 from the intercellular airspaces within the leaf through the mesophyll to the sites of carboxylation during photosynthesis was measured using three different techniques. The three techniques include a method based on discrimination against the heavy stable isotope of carbon, ^{13}C, and two modeling methods. The methods rely upon different assumptions, but the estimates of mesophyll conductance were similar with all three methods. The mesophyll conductance of leaves from a number of species was about 1.4 times the stomatal conductance for CO_2 diffusion determined in unstressed plants at high light. The relatively low CO_2 partial pressure inside chloroplasts of plants with a low mesophyll conductance did not lead to enhanced O_2 sensitivity of photosynthesis because the low conductance caused a significant drop in the chloroplast CO_2 partial pressure upon switching to low O_2. We found no correlation between mesophyll conductance and the ratio of internal leaf area to leaf surface area and only a weak correlation between mesophyll conductance and the proportion of leaf volume occupied by air. Mesophyll conductance was independent of CO_2 and O_2 partial pressure during the measurement, indicating that a true physical parameter, independent of biochemical effects, was being measured. No evidence for CO_2-accumulating mechanisms was found. Some plants, notably *Citrus aurantium* and *Simmondsia chinensis*, had very low conductances that limit the rate of photosynthesis these plants can attain at atmospheric CO_2 level.

Leaves have a finite conductance for CO_2 diffusion in the mesophyll (5, 13). This causes the pCO_2[2] at Rubisco to be lower than the pCO_2 in the intercellular airspace. The drop in pCO_2 limits photosynthesis under most conditions. Many reports (1, 4, 5, 9, 19, 22) indicate that g_m can be low enough to substantially limit CO_2 uptake, especially in leaves with low rates of photosynthesis. von Caemmerer and Evans (22) found a good correlation between the rate of photosynthetic CO_2 assimilation and g_m in several plants when photosynthetic capacity was varied by varying nitrogen nutrition. Mesophyll conductance decreased less than did photosynthesis, resulting in slightly higher pCO_2 at Rubisco in plants with low rates of photosynthesis. Lloyd and Syversten (9) found a similar correlation between the rate of photosynthesis and g_m in a number of citrus trees and found that the low pCO_2 inside the chloroplast substantially limited photosynthesis in *Citrus aurantium* trees.

The mesophyll conductance to CO_2 diffusion has a number of components. The diffusion through the intercellular airspace has been investigated by Parkhurst (15). Using helium instead of nitrogen to change the diffusivity of CO_2 in air, Parkhurst and Mott (16) were able to demonstrate an intercellular airspace effect on g_m in some plants but not in others. The intercellular airspace component of g_m will depend on where within the leaf water evaporates. If water is lost from near the guard cells, as suggested by Cowan (2) and Tyree and Yianoulis (20), then conductance through the intercellular airspace from the guard cells to the sites of CO_2 uptake within the leaf is a component of g_m. On the other hand, if water evaporates deep within the leaf, as is indicated by recent anatomical studies (14), then any intercellular airspace diffusion effect will be part of g_s. There is a finite conductance associated with the dissolution of CO_2 in the water in the cell wall and transport across the cell wall and cell membrane. To the degree that these components are important, it is useful to express photosynthetic CO_2 assimilation per unit of mesophyll cell area, rather than planar leaf area. The ratio of these two areas is called A_{mes}/A by Nobel (13). von Caemmerer and Evans (22) used the ratio of cell wall area with chloroplasts appressed divided by planar leaf area on the assumption that there is relatively little lateral diffusion of CO_2 in the cellular ground substance. Yet a third component is the flux of CO_2 across the chloroplast envelope. Mächler *et al.* (11) believe this to be an important component of g_m and have suggested that there is active uptake of CO_2 when the CO_2 level at the chloroplast envelope is low.

Determination of g_m has only recently been possible. Evans

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[2] Abbreviations: pCO_2, partial pressure of CO_2; g_m, mesophyll conductance to CO_2 diffusion; A, photosynthetic CO_2 assimilation; C_a, partial pressure of CO_2 in the air outside the leaf; C_c, partial pressure of CO_2 inside the chloroplast; C_i, partial pressure of CO_2 inside the airspaces inside leaves; g_s stomatal (plus boundary layer) conductance to CO_2 diffusion; J, rate of photosynthetic electron transport; F'_m, fluorescence with all PSII reaction centers closed in energized state.

et al. (5) describe a technique based on carbon isotope discrimination, and Harley *et al.* (7) describe two additional techniques based on analysis of the CO_2 responsiveness of photosynthesis. These three methods can be used to estimate g_m.

We have measured g_m of 15 species using three methods: (a) the stable carbon isotope fractionation method (5); (b) constant J method (7); and (c) variable J method (7). We made extensive measurements with leaves of *Quercus rubra* and *Xanthium strumarium* as examples of plants with low and high rates of photosynthesis, respectively. We compared the effect of low *versus* high g_m on O_2 sensitivity of photosynthesis, and we measured the g_m, g_s, and rate of photosynthesis to determine how g_m varies with other plant gas-exchange parameters.

MATERIALS AND METHODS

Isotopic and Constant J Methods

Plant Material and Experimental Conditions

The following species were used: *Arbutus unedo, Cucurbita pepo, Gossypium hirsutum, Nicotiana alata, Quercus ilex, Quercus rubra, Simmondsia chinensis,* and *Xanthium strumarium.* Plants were seedlings with the exception of *S. chinensis.* All plants were grown in pots in a greenhouse under temperature ranging from 20 to 30°C. Plants were watered daily and fertilized weekly with full-strength Hoagland solution. Experiments were carried out during the months of July and September 1990 in Madison, WI.

Gas-Exchange Measurements (Madison)

Leaves were enclosed in an aluminum cuvette with a glass window in the top. A uniform PFD of 500 μmol m^{-2} s^{-1} was maintained through the experiments. This was usually slightly less than saturating for photosynthesis. The light source was a 2.5-kW xenon arc lamp and PFD was measured by a Li-Cor quantum sensor 190SB. The leaf temperature was set at 25°C for all plants except Simmondsia, for which the experiments were run at 27°C. The temperature of the cuvette was controlled by water circulating within the aluminum and leaf temperature was monitored with a copper-constantan thermocouple appressed to the abaxial side of the leaf.

Air composition entering the cuvette was changed by mixing different proportions of N_2, O_2, and 5% CO_2 in air with Datametrics type 825 mass-flow controllers. Two small ozone-free fans moved the air across the leaf and then over a heat exchanger within the cuvette. A Li-Cor 6251 IR gas analyzer was used to measure the partial pressure of CO_2 before and after the cuvette, and air humidity was measured with a General Eastern Dew-10 hygrometer. Further details of this gas-exchange system are reported in Loreto and Sharkey (10). For calculations of photosynthetic parameters, we used the equations of von Caemmerer and Farquhar (23).

Measurements of Carbon Isotope Fractionation

When a steady leaf photosynthesis rate was reached, air leaving the gas-exchange system was passed through a vacuum line at a rate of 150 mL min^{-1} for 3 to 10 min. Carbon dioxide was collected in a liquid nitrogen trap consisting of three coils of glass. The coils were tall enough that the air passing through them was rewarmed on each pass out of the liquid nitrogen trap. After collection, the CO_2 was distilled into a small glass tube used to transport the CO_2 to a mass spectrometer. The $^{13}C/^{12}C$ ratio of CO_2 from samples of air entering and leaving the cuvette was analyzed with a Finnigan Delta E mass spectrometer. The equations described by Evans *et al.* (5) were used to calculate sequentially leaf discrimination against ^{13}C, C_c, and g_m. Usually the leaf removed approximately one-third of the CO_2 from the air stream as it passed through the chamber.

Chl Fluorescence Measurements

To measure Chl fluorescence, we used a modulated fluorometer (Heinz Walz PAM 101) equipped with the polyfurcated light probe described by Schreiber *et al.* (17). We followed nomenclature of van Kooten and Snel (21) and the protocol described by Loreto and Sharkey (10) for the determination of initial fluorescence, fluorescence with all PSII reaction centers closed in nonenergized state, steady-state fluorescence, F'_m, and fluorescence with all PSII reaction centers open in energized state.

Leaf Anatomy Determination

Leaf sections were fixed and embedded as described by Sharkey *et al.* (19). The mesophyll cell surface per unit leaf area (A_{mes}/A) and the percentage of air space in the mesophyll were determined from transverse sections of leaves as outlined by Nobel (12). Leaves from *N. alata, C. pepo, G. hirsutum, Q. ilex, A. unedo,* and *X. strumarium* were used.

Variable J Method

Plant Material and Experimental Conditions

Plants of *A. unedo, Citrus aurantium, Q. ilex, Cucumis sativus, X. strumarium,* and seedlings of *Triticum* spp. were grown in growth cabinets. The daylength was 16 h and light intensity was 400 μmol m^{-2} s^{-1}. Air temperature was 30/20 ± 1°C day/night. Plants were watered daily and a nutrient solution was added to the water once a week. Experiments on this set of plant material were carried out on attached leaves.

Plants of *Eucalyptus globulus, Nerium oleander, Hedera helix, Beta vulgaris,* and *Vicia faba* were grown outdoors. Single, fully expanded leaves were cut and used during the experiments maintaining the petiole under water. These experiments were carried out in the months of January through April 1991 in Rome, Italy. In addition, the method was used for some of the samples already tested for g_m in Madison by the isotopic and constant J methods.

Gas-Exchange Measurements (Rome)

A single leaf was clamped into an assimilation chamber supplied by Walz. Light was provided by an incandescent lamp (Osram HQR 250W). The light response of each plant was determined and then a level of light just saturating for

photosynthesis was chosen. This was usually near 1000 μmol m^{-2} s^{-1}. Air temperature was regulated with a Peltier cooling system. Leaf temperature was maintained at 25°C and monitored with an iron-constantan thermocouple firmly appressed to the abaxial surface of the leaf. Air coming into the chamber was mixed from N_2, O_2, and absolute CO_2 cylinders with mass flow controllers (Matheson). Leaf to air water vapor pressure difference was set by bubbling CO_2-free air through water and condensing excess water in a trap immersed in a thermostated water bath. Humidity of the air leaving the chamber was measured with a Vaisala chip. The concentration of CO_2 entering the chamber was monitored with an IR gas analyzer (Anarad). A water/CO_2 IR gas analyzer (Binos, Leybold-Hereaus) was used to measure differences between the water vapor contents and the CO_2 partial pressures of air entering and leaving the chamber. The equations of von Caemmerer and Farquhar (23) were used for gas-exchange calculations.

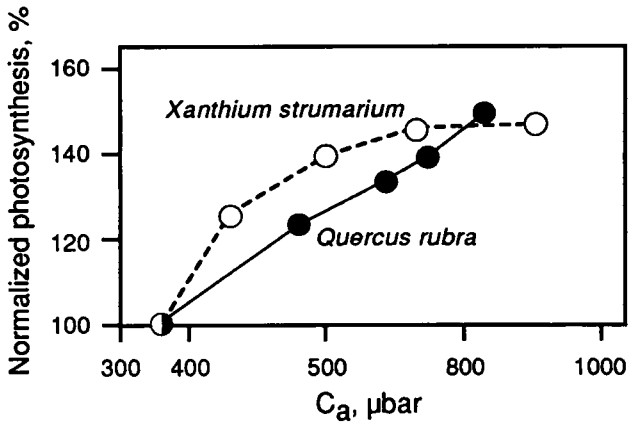

Figure 1. Response of photosynthetic CO_2 assimilation to ambient CO_2 for *Q. rubra* and *X. strumarium*.

Chl Fluorescence Measurements

Fluorescence of PSII was measured with a PAM 101 modulated fluorometer. The whole set of measurements was conducted as described for the constant J method. However, the polyfurcated optic fiber was inserted through a gas-tight hole into the chamber. This reduced the distance between fiber and leaf and maintained the fiber at a constant angle of 45° with the leaf. The same set of fluorescence parameters indicated for the constant J method was calculated. In addition, the quantum yield of PSII was estimated according to Genty *et al.* (6) from the ratio $\Delta F/F'_m$ with $\Delta F = F'_m$ − steady-state fluorescence. This parameter was used for calculating the electron transport rate as discussed in Harley *et al.* (7).

RESULTS

We used the isotopic method, the constant J modeling method, and the variable J modeling method to determine g_m of four leaves of *Q. rubra* (Table I). The isotopic method was carried out under four different gas compositions. There was no statistically significant difference among the means comparing the effect of gas composition on g_m determined by the isotopic method, or comparing the three methods of determining g_m. The variable J method failed for two of the four leaves because dC_c/dA was outside the limits chosen as described in Harley *et al.* (7). To examine the behavior of these methods with leaves expected to have a high g_m, we measured four leaves of *X. strumarium* (Table I). Only three gas compositions were used for *Xanthium*. The measure of g_m at high CO_2 was lower than at ambient CO_2 or ambient CO_2 and low O_2. For *Xanthium*, the constant J method failed because the variance in J did not reach a minimum. The variable J method worked for two of the leaves and agreed with the isotopic method used with ambient CO_2.

Plants with a low g_m have a greater CO_2 sensitivity (*i.e.* steeper slope) at high CO_2 than plants with a high g_m. This can be seen in Figure 1, where CO_2 response curves of *Q. rubra* and *X. strumarium* normalized to 100% at 360 μbar CO_2.

A low g_m implies that C_c is substantially lower than C_i. This could affect O_2 sensitivity of photosynthesis. To test this, we measured A and C_c in normal and low O_2 for the leaves of a

Table I. *Mesophyll Conductance Measured in Four Leaves of Q. rubra and Four Leaves of X. strumarium*

Missing values are for data sets in which dC_c/dA was not between 10 and 50 or where the variance did not reach a minimum.

Experimental Conditions	Replications				Mean
	g_m (mol m^{-2} s^{-1} bar^{-1})				
Q. rubra (isotopic method)					
Ambient CO_2	0.17	0.10	0.18	0.15	0.15
High CO_2 ($c_i \approx 750$ μbar)	0.12	0.14	0.11	0.08	0.11
Ambient CO_2 2% O_2	0.12	0.18	0.15	0.11	0.14
Ambient CO_2 38% O_2	0.16	0.19	0.10	0.10	0.14
Constant J	0.20	0.11	0.14	0.12	0.14
Variable J	0.18	0.14			0.16
X. strumarium (isotopic method)					
Ambient CO_2	0.60	0.37	0.52	0.51	0.50
High CO_2 ($c_i \approx 750$ μbar)	0.31	0.24	0.41	0.44	0.35
Ambient CO_2 2% O_2	0.56	0.42	0.70	0.76	0.61
Variable J	0.64	0.47			0.55

Table II. *O_2 Sensitivity of Photosynthesis in a High and a Low g_m Species*

The ambient CO_2 partial pressure was 350 μbar, all SEs were less than 10% of the measured value.

mbar O_2	A		Ratio 20/200	C_c	
	200	20		200	20
	μmol m^{-2} s^{-1}			μbar	
Xanthium	14.2	21.0	1.48	262	259
Quercus	8.9	11.4	1.28	188	147

high and low g_m species, reported in Table I. The response of A to switching to low O_2 was greater in *X. strumarium* than in *Q. rubra* (Table II). The reason for the greater O_2 sensitivity in the high g_m species was that C_c did not fall upon switching to low O_2 in *X. strumarium,* whereas it fell by over 40 μbar in *Q. rubra.*

The results for the variable J method reported in Table I are the average of estimates from one leaf over a range of CO_2. One of the advantages of the variable J method is that the effect of CO_2 on g_m can be determined. This is shown for *C. aurantium* and *Q. ilex* in Figure 2. The value of g_m found by the variable J method was nearly always independent of CO_2 except when dC_c/dA was outside the range of 10 to 50. In these cases, g_m could vary widely and have unrealistic values (*e.g.* negative values) (data not shown).

The g_m of a large number of species was determined using the variable J method (Table III). The values varied from a low of 0.023 mol m^{-2} s^{-1} bar^{-1} for *C. aurantium* to a high of 0.638 mol m^{-2} s^{-1} bar^{-1} for *Triticum* spp. There was a general correlation between CO_2 assimilation rate and g_m and between stomatal conductance and g_m.

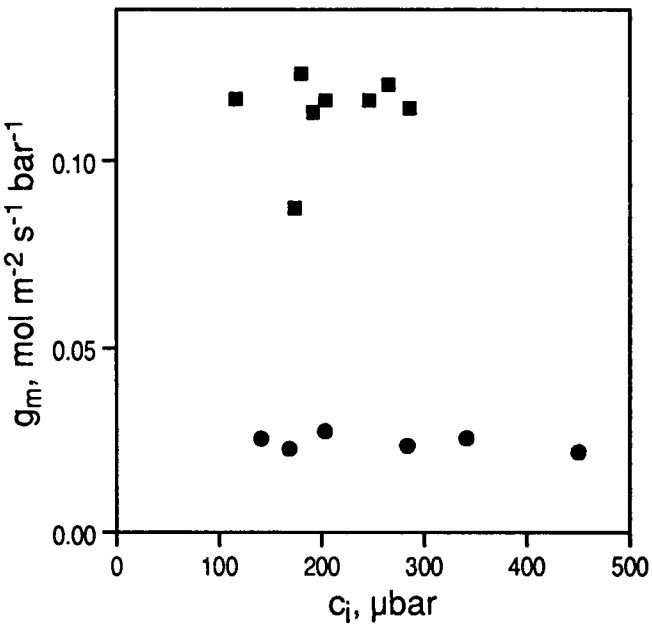

Figure 2. Estimate of g_m by the variable J method over a range of CO_2 for *C. aurantium* (●) and *Q. ilex* (■).

Table III. *Net A, g_s, and g_m of Leaves of Sclerophytic and Mesophytic Plants*

Estimation of g_m by quantitative modeling method as outlined in the text. All A and g_s data at atmospheric CO_2 partial pressure and saturating light intensity. Sclerophytes are indicated by (s) and mesophytes are indicated by (m).

Species	A	g_s	g_m
	μmol m^{-2} s^{-1}	mol m^{-2} s^{-1}	mol m^{-2} s^{-1} bar^{-1}
A. unedo (s)	9.7	0.080	0.161
B. vulgaris (m)	12.4	0.089	0.343
C. aurantium (s)	2.2	0.014	0.023
C. sativus (m)	13.0	0.128	0.448
E. globulus (s)	11.8	0.121	0.119
H. helix (s)	10.4	0.065	0.147
N. oleander (s)	5.7	0.045	0.215
Q. ilex (s)	7.2	0.046	0.113
Q. rubra (s)	10.8	0.160	0.142
Triticum spp. (m)	20.8	0.176	0.638
V. faba (m)	14.9	0.096	0.338
X. strumarium (m)	13.9	0.290	0.470

The relationship between photosynthetic CO_2 assimilation and g_m was explored further by plotting all g_m estimates, regardless of the method used, against A determined for that leaf at ambient CO_2 and saturating light (Fig. 3). The correlations between these two parameters did not differ between mesophytes and sclerophytes, nor by method of determining g_m, and so a regression of all data was performed. The value for g_m was roughly 0.025 times A when averaged over all species (r^2 = 0.76). (More correctly, $g_m/A = 0.0025 \cdot 10^{-6}$ · atmospheric pressure.)

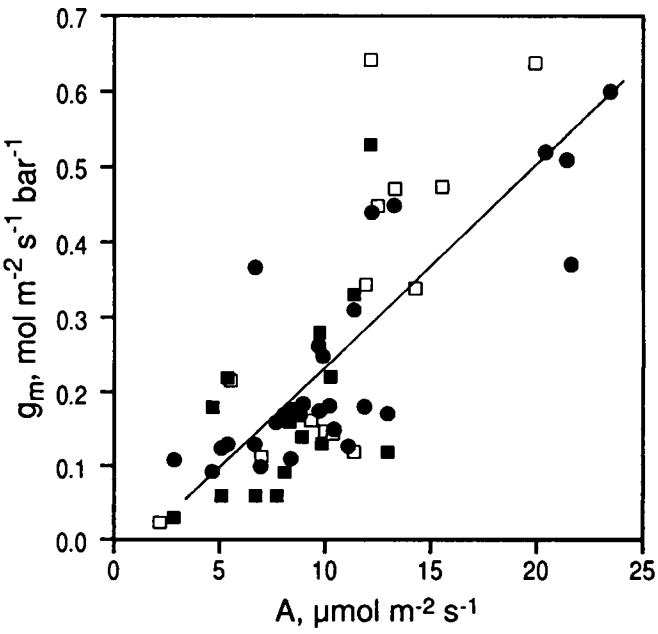

Figure 3. Relationship between net photosynthesis (A) and mesophyll conductance (g_m). Symbols describe method used for g_m determination: ● = isotope fractionation; ■ = constant J modeling; □ = variable J modeling.

The relationship between g_s and g_m is shown in Figure 4. Again, there was no difference among correlation coefficients and so all data were combined. The regression had a positive intercept and r^2 of 0.80. A line representing $g_m = 1.4\ g_s$ is shown in Figure 4. This line appears to fit the data well and was within the 95% confidence levels of the linear regression.

Six species covering a wide range of g_m were selected for further analysis by EM. No relationship between g_m and A_{mes}/A was apparent. In Figure 5, g_m is plotted against airspace. There appeared to be a slight association of g_m and relative airspace in the leaf with g_m greater with greater percentage airspace.

DISCUSSION

The modeling methods and the isotopic method for estimating g_m rely on unrelated properties of Rubisco. The isotopic method makes use of the discrimination between isotopes exhibited by Rubisco, whereas the constant and variable J methods make use of the fact that Rubisco will use O_2 when the CO_2 level is low. The data reported here are an important confirmation of the isotopic method of estimating g_m. The data also confirm that plant species with very high rates of photosynthesis, like those often used to test models of photosynthesis, tend to have such high values of g_m that it is hard to measure. All of the methods work best when g_m is low.

Each of the three methods tested can provide a reliable estimate of g_m. There was a relatively large amount of noise in the data and so averages of several estimates should be used whenever possible. The isotopic method is useful over a greater range of conditions than either of the other two methods. For example, the isotopic method is the only reliable method by which g_m can be estimated when leaves are in low O_2. However, the large amount of equipment, the cost, and the time required to estimate g_m by the isotopic method will likely restrict its use. The other two methods can be used by anyone with a gas-exchange system and modulated fluorometer.

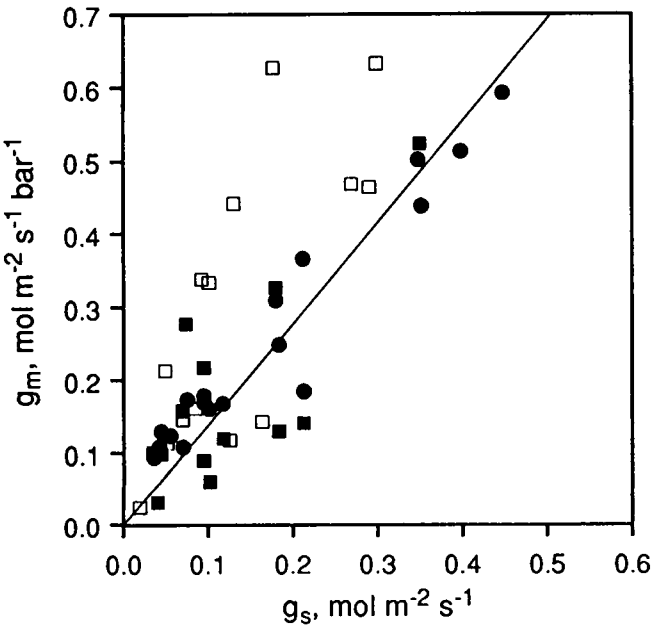

Figure 4. Relationship between stomatal (g_s) and mesophyll (g_m) conductance. Symbols describe method used for g_m determination: ● = isotope fractionation; ■ = constant J modeling; □ = variable J modeling.

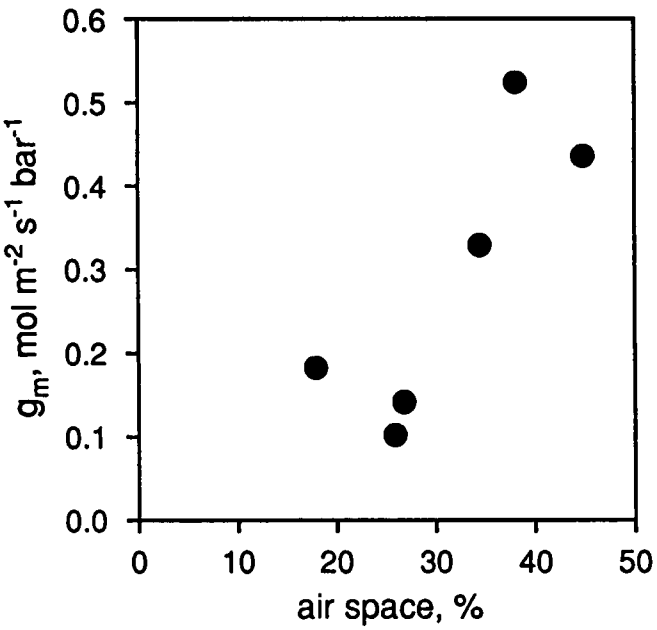

Figure 5. Relationship between percentage of airspace in the leaf mesophyll *versus* the mesophyll conductance (g_m) of leaves of six plants.

The pathway for CO_2 diffusion is likely to be variable across the leaf and even across the chloroplast. This makes it possible that local variability of g_m will be important in some cases, much like patchiness can sometimes affect the leaf average g_s. However, finding that the three methods used here give similar results helps justify using the leaf average g_m.

We found g_m to be correlated with A and g_s, as has been reported by von Caemmerer and Evans (22) and Lloyd and Syversten (9). From our data, we believe it is justified to incorporate g_m into models of photosynthesis assuming g_m to be 1.4 times g_s obtained under high light and unstressed conditions. Alternatively, g_m could be estimated as 0.025 times A at light saturation and ambient CO_2 when g_m and A are expressed in the same units as used here. Some of the plants reported here had lower rates of photosynthesis and correspondingly lower values of g_m than have been reported previously. Sclerophytic plants generally had low values of g_m and low rates of photosynthesis, but the relationship between photosynthesis and g_m did not vary between sclerophytes and mesophytes. Plants with particularly low g_m include *C. aurantium* (g_m = 0.02 mol m^{-2} s^{-1} bar^{-1}) and *S. chinensis* (g_m = 0.03 mol m^{-2} s^{-1} bar^{-1}).

It has been suggested that photosynthesis may be more O_2 sensitive in plants with low mesophyll conductances. In fact, the opposite effect was seen when we compared *Q. rubra* with *X. strumarium:* the O_2 sensitivity was lower in the low-mesophyll conductance leaves. This is because the increased

rate of photosynthesis caused by low partial pressure of O_2 caused C_c to drop more when g_m was low than when it was high. von Caemmerer and Evans (22) examined the predicted effect of g_m on the O_2 sensitivity of initial slopes and concluded that low g_m had no appreciable effect. Variation in g_m is another example of how the biophysics and biochemistry of photosynthesis in leaves can affect the apparent response to O_2 even though the specificity of Rubisco for CO_2 over O_2 is relatively invariant. A variation in O_2 response of photosynthesis cannot be taken as evidence for a change in the properties of photorespiration (18).

On the other hand, at high pCO_2 the CO_2 response of A was greater in plants with low g_m (Fig. 1). This fact is the basis of the constant J method of estimating mesophyll conductance and is one of the most striking indications of low g_m to be found in gas-exchange data. Because plants with a low g_m respond more to increases in CO_2, the increasing CO_2 level in the atmosphere could have more effect on low g_m plants than on high g_m plants. Because of the extremely low g_m of *C. aurantium* (see also ref. 9), we would expect that this plant would exhibit more response to elevated CO_2, and this has been reported (8). However, this is probably not a good plant from which to generalize about CO_2 responses (8) given that its g_m can be so low.

We were unable to gain any insight into which component of g_m is dominant. We did not find a relationship between g_m and A_{mes}/A, as would be expected if the cell wall or cell membrane were the major resistance. There was some association between g_m and relative airspace inside the leaf. This would support the intercellular airspace resistance as a significant component, but this would need confirmation using the helox techniques of Parkhurst and Mott (16) combined with estimates of g_m using methods used in these experiments.

If we are truly measuring a physical diffusion conductance, the estimate should be independent of gas composition. In nearly all cases where this was assessed, g_m was independent of gas composition and A (Table I, Fig. 2, see also Fig. 8 of Harley *et al.* [7]). For example, in *Q. rubra* (Table I) there was no difference in the g_m estimated with the isotopic method at either high CO_2 or high or low O_2. One exception is data of *X. strumarium* at high CO_2 (Table I). Whether this is a general phenomenon needs additional testing. However, a lowered g_m could occur in response to environmental conditions if substantial chloroplast rearrangement occurred. For example, Sharkey *et al.* (19) found that a transgenic tobacco plant with excess phytochrome had chloroplasts that had become cup-shaped, which prevented a close association between the chloroplast and the cell wall and caused g_m to be very small. Perhaps high CO_2 can cause a change in the shape of *X. strumarium* chloroplasts.

Mächler *et al.* (11) have suggested that the major site of resistance to CO_2 diffusion in the mesophyll is at the chloroplast envelope and that the chloroplast envelope has a high affinity, low capacity CO_2 pump. However, some of the data supporting this idea assume that CO_2 diffusing into the chloroplast from the air, and CO_2 coming to the chloroplast from photorespiration, both travel through the same section of the chloroplast envelope. A more realistic view is presented by Cowan (3). In his view, CO_2 from the atmosphere diffuses through that part of the chloroplast envelope nearest the cell wall, whereas CO_2 released in photorespiration is released on the side of the chloroplast away from the cell wall and can diffuse into the chloroplast through a different part of the chloroplast envelope. We also feel it is a mistake to not include a term for day respiration in the equations used to predict g_m. Estimates of g_m are difficult by any technique and we feel that they are not reliable enough to prove the existence of anomalous behavior without confirmation by several methods. Our measurements do not confirm the anomalous behavior upon which Mächler *et al.* (11) based their hypothesis of a chloroplast membrane CO_2 pump.

In summary, it is now possible to measure g_m by several methods. These methods depend upon different assumptions but give similar estimates. The mesophyll conductance can be surprisingly low and provide a substantial limitation to the rate of photosynthesis in plants such as *C. aurantium* and *S. chinensis*.

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LITERATURE CITED

1. **Bongi G, Loreto F** (1989) Gas-exchange properties of salt-stressed olive (*Olea europa* L.) leaves. Plant Physiol **90:** 1408–1416
2. **Cowan IR** (1977) Stomatal behavior and environment. Adv Bot Res **4:** 117–228
3. **Cowan IR** (1986) Economics of carbon fixation in higher plants. *In* TJ Givnish, ed, On the Economy of Plant Form and Function. Cambridge University Press, Cambridge, UK, pp 133–170
4. **Di Marco G, Manes F, Tricoli D, Vitale E** (1990) Fluorescence parameters measured concurrently with net photosynthesis to investigate chloroplastic CO_2 concentration in leaves of *Quercus ilex* L. J Plant Physiol **136:** 538–543
5. **Evans JR, Sharkey TD, Berry JA, Farquhar GD** (1986) Carbon isotope discrimination measured concurrently with gas exchange to investigate CO_2 diffusion in leaves of higher plants. Aust J Plant Physiol **13:** 281–292
6. **Genty B, Briantais J-M, Baker NR** (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta **990:** 87–92
7. **Harley PC, Loreto F, Di Marco G, Sharkey TD** (1992) Theoretical considerations when estimating mesophyll conductance to CO_2 flux by analysis of the response of photosynthesis to CO_2. Plant Physiol **98:** 1429–1436
8. **Idso SB, Kimball BA** (1991) Downward regulation of photosynthesis and growth at high CO_2 levels No evidence for either phenomenon in three-year study of sour orange trees. Plant Physiol **96:** 990–992
9. **Lloyd J, Syvertsen J** (1991) Mesophyll wall conductance and the partial pressure of CO_2 at chloroplasts of citrus and peach leaves (abstract No. 91). Plant Physiol **96:** 17
10. **Loreto F, Sharkey TD** (1990) A gas-exchange study of photosynthesis and isoprene emission in *Quercus rubra* L. Planta **182:** 523–531
11. **Mächler F, Müller JM, Dubach M** (1990) RuBPCO kinetics and the mechanism of CO_2 entry in C_3 plants. Plant Cell Environ **13:** 881–899
12. **Nobel PS** (1977) Internal leaf area and cellular CO_2 resistance: photosynthetic implications of variations with growth conditions and plant species. Physiol Plant **40:** 137–144
13. **Nobel PS** (1991) Physicochemical and Environmental Plant Physiology. Academic Press, San Diego

14. **Nonami H, Schulze E-D, Ziegler H** (1991) Mechanisms of stomatal movement in response to air humidity, irradiance and xylem water potential. Planta **183:** 57–64
15. **Parkhurst DF** (1986) Internal leaf structure: a three-dimensional perspective. *In* TJ Givnish, ed, On the Economy of Plant Form and Function. Cambridge University Press, Cambridge, UK, pp 215–249
16. **Parkhurst DF, Mott KA** (1990) Intercellular diffusion limits to CO_2 uptake in leaves. Studies in air and helox. Plant Physiol **94:** 1024–1032
17. **Schreiber U, Schliwa U, Bilger W** (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res **10:** 51–62
18. **Sharkey TD** (1988) Estimating the rate of photorespiration in leaves. Physiol Plant **73:** 147–152
19. **Sharkey TD, Vassey TL, Vanderveer PJ, Vierstra RD** (1991) Carbon metabolism enzymes and photosynthesis in transgenic tobacco (*Nicotiana tabacum* L.) having excess phytochrome. Planta **185:** 287–296
20. **Tyree MT, Yianoulis P** (1980) The site of water evaporation from substomatal cavities, liquid path resistances and hydroactive stomatal closure. Ann Bot **46:** 175–193
21. **van Kooten O, Snel JFH** (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth Res **25:** 147–150
22. **von Caemmerer S, Evans JR** (1991) Determination of the CO_2 pressure in chloroplasts from leaves of several C_3 plants. Aust J Plant Physiol **18:** 287–305
23. **von Caemmerer S, Farquhar GD** (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta **153:** 376–387