

Gradients of Intercellular CO₂ Levels Across the Leaf Mesophyll¹

Received for publication July 6, 1987 and in revised form December 4, 1987

DAVID F. PARKHURST*, SUAN-CHIN WONG, GRAHAM D. FARQUHAR, AND IAN R. COWAN
School of Public and Environmental Affairs and Biology Department, Indiana University, Bloomington Indiana 47405 (D.F.P.); and Department of Environmental Biology, Research School of Biological Sciences, Australian National University, Canberra City, A.C.T. 2601, Australia (S-C.W., G.D.F., I.R.C.)

ABSTRACT

Most current photosynthesis models, and interpretations of many whole-leaf CO₂ gas exchange measurements, are based on the often unstated assumption that the partial pressure of CO₂ is nearly uniform throughout the airspaces of the leaf mesophyll. Here we present measurements of CO₂ gradients across amphistomatous leaves allowed to assimilate CO₂ through only one surface, thus simulating hypostomatous leaves. We studied five species: *Eucalyptus pauciflora* Sieb. ex Spreng., *Brassica chinensis* L., *Gossypium hirsutum* L., *Phaseolus vulgaris* L., and *Spinacia oleracea* L. For *Eucalyptus*, maximum CO₂ pressure differences across the leaf mesophyll were 73 and 160 microbar when the pressures outside the lower leaf surface were 310 and 590 microbar, respectively. Using an approximate theoretical calculation, we infer that if the CO₂ had been supplied equally at both surfaces then the respective mean intercellular CO₂ pressures would have been roughly 12 and 27 microbar less than the pressures in the substomatal cavities in these cases. For ambient CO₂ pressures near 320 microbar, the average and minimum pressure differences across the mesophyll were 45 and 13 microbar. The corresponding mean intercellular CO₂ pressures would then be roughly 8 and 2 microbar less than those in the substomatal cavities. Pressure differences were generally smaller for the four agricultural species than for *Eucalyptus*, but they were nevertheless larger than previously reported values.

there. Because no net CO₂ exchange occurred through that side, the CO₂ pressure measured in that cuvette would be close to the pressure in the substomatal chambers. The other chamber was operated normally, and p_i was calculated for that side using the methods of Caemmerer and Farquhar (1) (a list of abbreviations used in this paper can be found in Table I). The maximum differences in CO₂ pressure across the mesophyll were 10 to 14 μ bar. These results suggest that in a 'one-sided' leaf having internal structure similar to that in cotton and cocklebur, p_i should vary by no more than about 14 μ bar across the mesophyll.

Mott and O'Leary (4) performed a modified version of the Sharkey experiment using open gas exchange systems on both sides of sunflower and cocklebur leaves. They operated the system normally on one side, and adjusted the ambient CO₂ level at the other side until no net gas exchange occurred there. This technique allowed them to maintain small and equal positive pressures in both chambers. This method prevented room air (often high in CO₂) from leaking into either chamber, and also removed any pressure difference that might drive a bulk flow of air through the leaf.

In sunflower, Mott and O'Leary (4) found no measurable differences in the estimates of c_i at the two surfaces. In cocklebur, c_i at the surface not exchanging CO₂ was always lower than c_i at the other surface. The difference increased with increase in ambient CO₂ concentration at the 'fed' surface. For example (K Mott, personal communication, 1985), when the upper and lower surfaces of a leaf were exposed to ambient CO₂ concentrations

In recent years, one aim of much photosynthesis research has been to understand the factors limiting net carbon assimilation rates of whole leaves. This paper is concerned with one of those factors, *i.e.* whether gaseous diffusion of CO₂ through the intercellular air spaces (between the substomatal chamber and the chlorenchyma cell walls) may appreciably reduce CO₂ availability to the mesophyll cells. Actually, we deal more directly with a related question: How large are the gradients in CO₂ partial pressures in the intercellular air spaces of leaves? Knowledge of patterns of CO₂ levels within leaves will increase our understanding of the factors that limit CO₂ assimilation. In what follows, we consider previous measurements of CO₂ gradients across leaves, and then discuss the measurements we have made with leaves of five C3 species.

Two published studies present estimated gradients that are comparable with ours. Sharkey *et al.* (9) placed leaves of cotton and cocklebur (both amphistomatous species) in two-sided gas exchange chambers, and recycled the gas flow through one of the chambers until the CO₂ pressure reached an equilibrium

Table I. Abbreviations Used in This Paper

p_i	intercellular (air-space) partial pressure of CO ₂
c_i	intercellular (air-space) concentration of CO ₂
D	diffusivity of CO ₂ through air
L	mesophyll thickness
p	intercellular partial pressure of CO ₂
p_a	ambient partial pressure of CO ₂ in the cuvette
p_c	partial pressure of CO ₂ at the chloroplasts
p_{es}	partial pressure of CO ₂ at the evaporating surfaces of cells within the leaf mesophyll
$ \Delta p_i $	absolute value of the difference between the calculated " p_i " values at the top and bottom surfaces of the leaf
\bar{p}	mean intercellular CO ₂ pressure throughout the mesophyll
p_u	intercellular partial pressure of CO ₂ just inside the upper surface of a leaf
R	CO ₂ assimilation rate per unit volume of tissue
$RuBP$	ribulose 1,5-bisphosphate
z	distance into the mesophyll measured from the abaxial side

¹ Supported in part by grants from the Office of Research and Graduate Development, Indiana University, Bloomington.

of 348 and 515 $\mu\text{l L}^{-1}$, the corresponding estimated c_i values were 329 and 376 $\mu\text{l L}^{-1}$, respectively, a difference of 47 $\mu\text{l L}^{-1}$. Although Mott and O'Leary (4) measured several differences of magnitudes similar to this, they did not report those measurements because that paper had a different purpose.

Mott and O'Leary also estimated the resistance to intercellular gaseous diffusion of CO₂ across the cocklebur leaf. Their estimate of 3.0 $\text{m}^2 \text{s mol}^{-1}$ is close to the value 3.2 obtained in a different way, but for the same species, by Farquhar and Raschke (2). In contrast, Sharkey *et al.* (9) estimated the resistance for cocklebur to be 1.0 $\text{m}^2 \text{s mol}^{-1}$. It is possible that the equilibrium method used by these authors caused a bulk flow of air through the leaf; if such a flow occurred, it would lead to underestimates both of cross-mesophyll diffusion resistance and of estimated CO₂ gradients.

The measurements just described indicated a rather wide range of CO₂ pressure differences across amphistomatous leaves constrained to assimilate CO₂ through only one surface. Mathematical models for three-dimensional CO₂ diffusion in leaves also predict that CO₂ pressure differences may be quite large in some leaves (5, Table 2; 6, Figure 7.6). We now report further measurements performed with a number of species using the Mott and O'Leary (4) method described above.

MATERIALS AND METHODS

Plant Material. Measurements were made on leaves of several plant species, including seven leaves of the sclerophyllous snow gum (*Eucalyptus pauciflora* Sieb. ex Spreng.); one leaf each of three mesophytic agricultural species, *i.e.* radish (*Brassica chinensis* L.), bean (*Phaseolus vulgaris* L.), and spinach (*Spinacia oleracea* L.); and two leaves of cotton (*Gossypium hirsutum* L.). All plants were greenhouse grown. Most leaves were fully expanded, although the snow gum leaf of Figure 4B was about 80% expanded. The leaves of the snow gum seedlings varied from the juvenile form (leaf surface held horizontally) to the adult form (leaf surface held vertically). Leaf thicknesses are noted in Table II.

Gas Exchange System. Rates of CO₂ assimilation and transpiration were measured for attached leaves using a small (1 × 3 cm² projected area) double sided glass and aluminum chamber. The through-flow gas exchange system consisted of one CO₂-free air supply and one air humidifier. After passing through the humidifier, the airflow was split into two circuits, each having a gas mixing unit, an absolute infrared CO₂ analyzer (Beckman 315B or Binos 1), a differential infrared CO₂ analyzer, and a Humicap humidity sensor (Vaisala Co., Finland). All gases passed through ice traps before entering infrared gas analyzers; this avoids foreign gas broadening of the CO₂ absorption band at 4.3 μm (3). Each CO₂ mixing unit comprised two mass flow controllers (model FC260, Tylan Corp., Carson CA). We controlled partial pressure of CO₂ by mixing 10% CO₂ with CO₂-free air.

A 500 W air-cooled xenon arc lamp, equipped with an aspheric reflector (type Xenon 500, Optical Radiation Corp., Azusa, CA), illuminated the leaves. The focused beam passed through a concave lens and a multiple IR reflection mirror system (Schott type 115, Mainz, W. Germany). The transmitted light then passed via an optical fibre light guide into the leaf chamber. Details of the gas exchange methods were as described by Wong and Woo (13).

Calculations of Gas Exchange Parameters. The equations used to calculate assimilation rate, stomatal conductance, and p_i were taken from Caemmerer and Farquhar (1). See also Sharkey *et al.* (9, p. 657).

RESULTS

Figure 1 summarizes our results, shown as the absolute value $|\Delta p_i|$ of the difference in p_i across the two surfaces of a leaf,

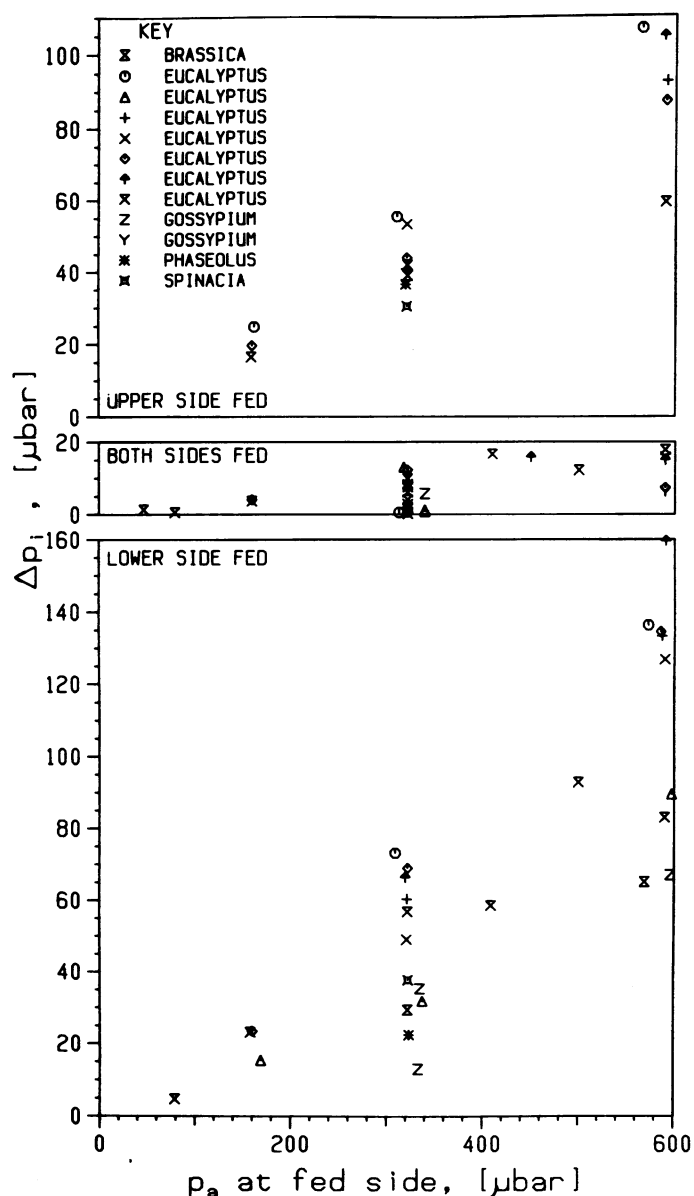


FIG. 1. Measured differences between estimates of CO₂ pressure p_i (actually p at the sites of evaporation) for the upper and lower surfaces of 12 leaves representing 5 plant species, when the upper, lower, or both surfaces were allowed to assimilate CO₂.

plotted against ambient CO₂ pressure (p_a) at the side exchanging CO₂. As in the experiments of Mott and O'Leary (4), p_i was always higher at the side allowed to assimilate CO₂. Differences were greatest with snow gum, the largest being 160 μbar with 600 μbar ambient pressure at the 'fed' side (bottom). When ambient pressure at the exchanging side (bottom) was 310 μbar , maximum Δp_i was 73 μbar . In a given leaf, p_i increased more or less linearly with ambient CO₂ pressure. The corresponding means and minima, classified according to which surface was fed CO₂, are listed in Table II.

In the early stages of our measurements, we were concerned that the large values of Δp_i found might be artifacts caused by differential errors in the two gas exchange systems. To ensure that the measured differences were not produced by such errors, we periodically exchanged the connections of inlet and outlet tubing to the two chambers. Figure 2 shows the differences in Δp_i measured before and after exchanging connections, plotted

Table II. Minimum, Average, and Maximum Values of CO_2 Pressure Differences Across Leaves ($|\Delta p_i|$), and Corresponding Estimated Differences (E.D.) between p_i in the Substomatal Cavity at the Fed Side and Mean p_i throughout the Mesophyll

These quantities are given for the cases when the leaves were fed CO_2 through the lower side only or the upper side only, with CO_2 partial pressures in the leaf chamber at the fed side ranging from 309 to 338 μbar (i.e., near normal ambient levels). The estimated differences are the $|\Delta p_i|$ values multiplied by the factor of 1/6 derived in the Appendix.

		Side Fed CO_2			
		Lower		Upper	
		$ \Delta p_i $	E.D.	$ \Delta p_i $	E.D.
A. All studied leaves					
	Minimum	13	2	24	4
	Mean	47	8	42	7
	Maximum	73	12	55	9
		Side Fed CO_2			
		Lower		Upper	
Plant	Leaf Thickness	$ \Delta p_i $	N	$ \Delta p_i $	N ^a
B. Means by species					
	μm				
<i>Brassica</i>	248	30	2 (1)	32	2 (1)
<i>Eucalyptus</i>	378–490	59	10 (7)	47	7 (5)
<i>Gossypium</i>	300–320 ^b	24	2 (1)		0
<i>Phaseolus</i>	210	22	1	36	1
<i>Spinacia</i>	480	38	1	30	1

^a Number of measurements (leaves) represented in the associated means. ^b Thickness of similar leaves of the same variety.

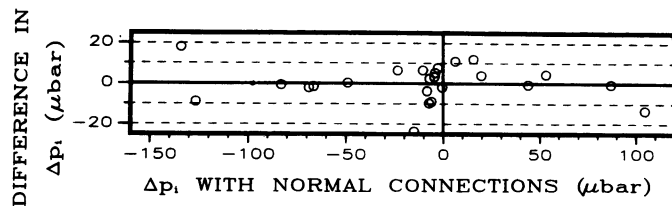


FIG. 2. Differences between (a) measurements of Δp_i made with the CO_2 supply and measurement circuitry to the two cuvettes reversed, and (b) measurements made with the normal gas circuitry.

against the magnitudes of Δp_i with the normal arrangement of the connections. All the differences lie between -25 to $+20$ μbar , with most being within 10 μbar .

Leaf Assimilation versus p_i . Figure 3 shows the variation of net leaf assimilation with p_i , corresponding with the data shown in Figure 1. The variation of A with p_i is similar for the different leaves when they were fed at the top (irradiated) surface only. The response curves for the various leaves were less similar when CO_2 was fed to the bottom surfaces only. When CO_2 was fed in the latter way, some (but not all) of the relatively thick-leaved eucalypts tended to assimilate less rapidly than when they were fed with similar p_i values at the top surface.

Two of the seven snow gum leaves were studied especially intensively, and curves relating assimilation to p_i for these leaves are plotted in Figure 4.

DISCUSSION AND CONCLUSIONS

Our results have several implications for modeling whole-leaf photosynthesis and for interpreting gas-exchange measurements of leaves; we discuss those implications below. However, our measurements were made with amphistomatous leaves that were constrained to operate in hypostomatous (or hyperstomatous)

states when fed through only one surface. It is thus important to estimate how large the variations in CO_2 pressure might be within normal amphistomatous and hypostomatous leaves.

In fact, lower CO_2 pressure variations are to be expected in ordinary amphistomatous leaves because there are two surfaces admitting CO_2 rather than one, and because average diffusion distances are cut by half. The analysis in the Appendix suggests that the differences between the substomatal and average internal partial pressures of CO_2 in the leaf will be roughly one-sixth of the differences in substomatal pressures measured when CO_2 is admitted through one surface. The factor of one-sixth is derived from a one-dimensional analysis that neglects any limitations to paradermal diffusion. A more correct three-dimensional analysis would yield a higher factor (e.g. perhaps one-fifth) but the exact value would depend on stomatal conductances, leaf thickness and structure, and enzyme activities. (We were not able to measure leaf structure and enzyme activities within the scope of this study). The estimated differences presented in Table II are derived using the factor of one-sixth obtained from the simplified one-dimensional analysis.

Variations in CO_2 pressure across amphistomatous leaves assimilating through similar stomatal conductances on both surfaces are likely to be much lower than most of those we have measured. Indeed, when the ambient CO_2 levels at the two surfaces were equal and both sides were assimilating, Δp_i varied from 0 to 20 μbar (Fig. 1). These differences are similar to those reported by Mott and O'Leary (4). However, one can only estimate p_i at the surfaces for such leaves—we know of no way to measure the actual internal gradients. Furthermore, conductances are often lower on the upper surfaces than on the lower in amphistomatous leaves (8), and this imbalance could make the gradients larger than they would be if conductances were equal on both surfaces.

The CO_2 pressure gradients may be large in hypostomatous leaves as well, but we know of no technique to measure pressure

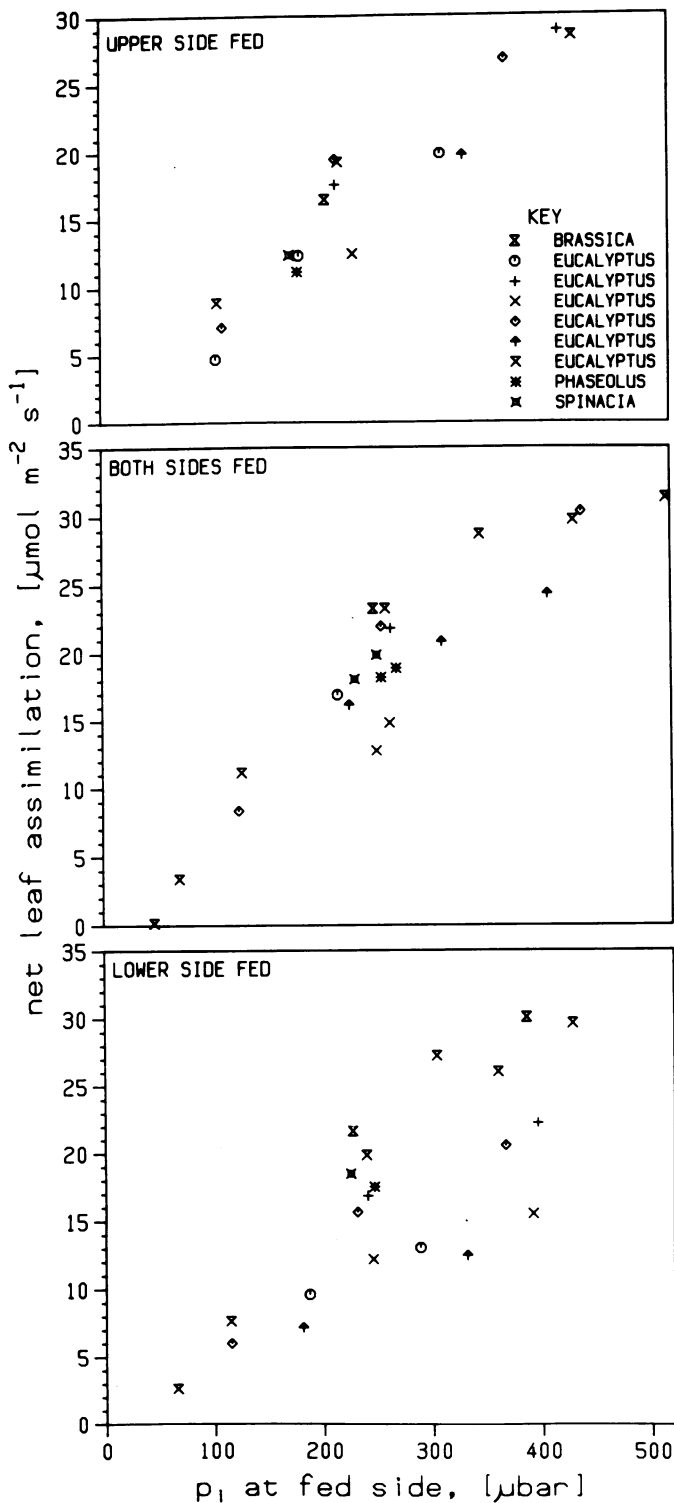


FIG. 3. Net CO₂ assimilation rates of leaves fed CO₂ through the upper surface only, both surfaces, or the lower surface only. The horizontal axis is the estimated p_i at the fed surfaces. Data are from the same experiments represented by Figure 1, but only those leaves fed CO₂ in all three modes are included.

variation in such leaves. One might infer that CO₂ assimilation in hypostomatous leaves that were otherwise similar to those studied here could be substantially limited by intercellular diffusion. It is likely that gradients in cell structure and in bio-

chemical activities have evolved differently in hypostomatous leaves to decrease the limitation somewhat.

In any case, one would expect the pressure gradients to be greatest in thick leaves with high cell density (low porosity), high assimilation rates, and small substomatal chambers (6).

The CO₂ pressure differences across the mesophyll were generally greater when CO₂ entry took place only at the lower surface than when it occurred only through the upper surface. This suggests that the CO₂ fixation capacity is greatest in tissue near the upper surface, as it should be if local light levels are higher there and if RuBP-carboxylase activities are high there. Evidence for such enzyme and light distributions (in *Camellia* and spinach) has been presented by Terashima *et al.* (10–12).

For the snow gum leaf represented in Figure 4A, one can see that net assimilation rates at a given p_i were similar when either the top surface or both surfaces were fed CO₂, but were only about three-fourths as great when only the bottom surface was fed. The second leaf (Fig. 4B) does not exhibit this effect to the same extent as the first, if at all—possibly it was a more porous leaf than the other.

Figure 2 shows that there were no consistent errors in our gas exchange measurements caused by the exchange of connections. Although the larger differences indicate a lower resolution than one would like to have, they are clearly not large enough to explain the many Δp_i values greater than 25 μbar shown in Figure 1. Further, some of the points shown in Figure 2 are comparisons of measurements made as much as 2 h apart; during that time the assimilation rates of the leaf might have changed substantially, thereby causing some of the discrepancy.

Implications. Our results have implications for many related phenomena. When modeling whole-leaf CO₂ assimilation, one should either treat p_i and p_c (CO₂ pressure at the chloroplasts) as variables that change with distance from the stomata (5, 6), or else consider p_i and p_c to be assimilation-weighted averages of the values throughout the mesophyll. With the latter choice, it must be borne in mind that the average p_i will be lower than the p_i values usually calculated from gas-exchange measurements. For amphistomatous leaves, the differences should be similar to those estimated in Table II. For hypostomatous leaves, the differences may be considerably greater (5, 6), but we know of no experimental confirmation of those modeled gradients for true hypostomatous leaves.

One focus of much photosynthesis research is to understand the factors that limit CO₂ assimilation rates. To the extent that intercellular air-space diffusion is one such limiting factor in a given leaf, then ignoring that factor will probably result in incorrectly apportioning its effects to other causes (stomatal or biochemical limitations, for example). We do not yet know how large the diffusional limitation may be in typical leaves. However, by combining a diffusion model (6) with the biochemical model of Kirschbaum and Farquhar (3), Parkhurst (7) estimated that limitation for the thick, hypostomatous leaf of *Arbutus menziesii* Pursh. When the gaseous diffusion limitation of the intercellular air spaces was removed mathematically in the model (by setting the CO₂ diffusivity, in the mesophyll air spaces only, to an artificially high value), calculated CO₂ assimilation increased by 24%. Thus, the limitation may be substantial in some leaves.

Finally, a reviewer enquired about the implications of CO₂ gradients for the distribution of RuBP carboxylase activity in leaves. As Terashima *et al.* have shown (10–12), the activity of that enzyme varies through the mesophyll thickness in leaves of some species. Although such variation may be a response to a light gradient within the leaf, it may also represent a balancing between local CO₂ pressures and local enzyme activities in a way that increases the overall assimilation rate of the leaf. We hope in the future to test this speculation using variations on the model described in Ref. (7).

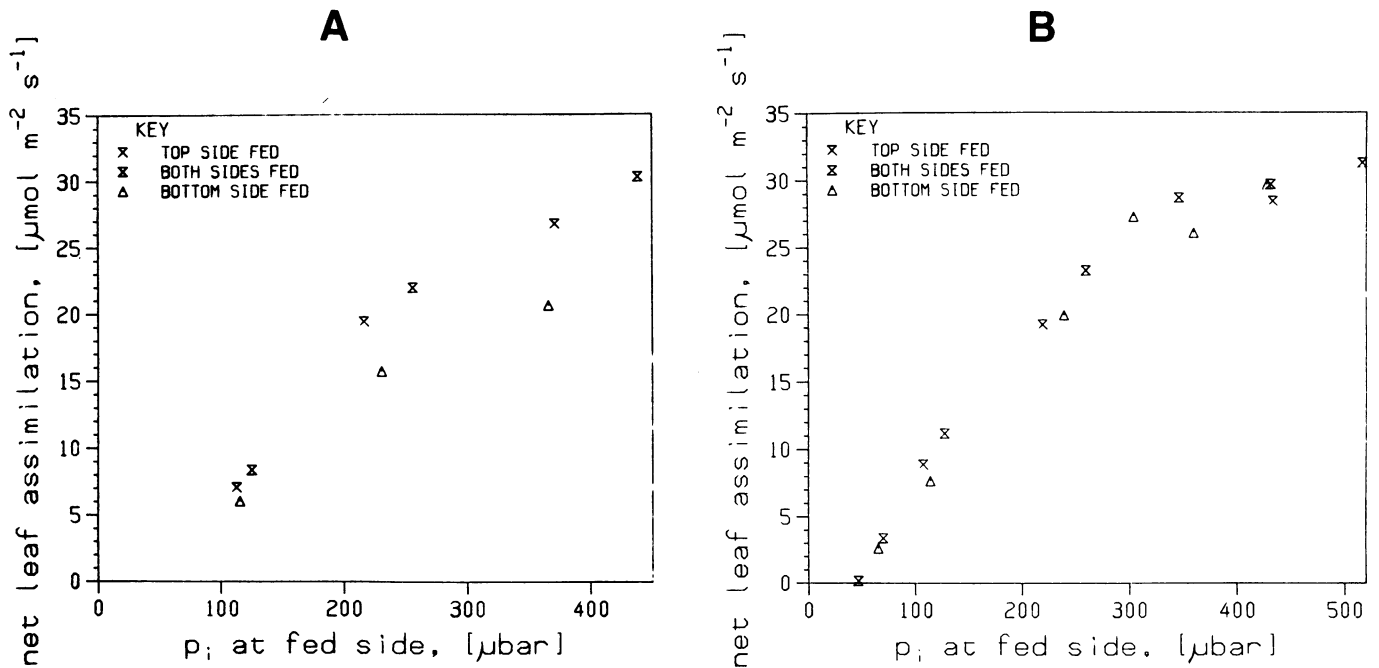


FIG. 4. A, Net CO₂ assimilation by a snow gum leaf with various p_i values at the upper, lower, or both surfaces; B, corresponding data for a second snow gum leaf.

APPENDIX

Comparison of CO₂ Pressure Differences in Leaves Exchanging CO₂ Through One versus Both Surfaces. The large measured CO₂ pressure differences reported in the main body of this paper were obtained with amphistomatous leaves that were allowed to exchange CO₂ through only one surface. The question arises how large the internal gradients would be in an amphistomatous leaf exchanging CO₂ normally through both surfaces. In particular, one would like to know how much the mean intercellular partial pressure, \bar{p} , differs from the p_{es} (p_i) measured by standard methods. (We use p_{es} in this section to emphasize that, as discussed above, the pressures usually denoted p_i are really the CO₂ pressures at the evaporating surfaces within the leaf.)

Assume, somewhat simplistically, a uniform volumetric CO₂ assimilation rate of R mol m⁻³ s⁻¹, for two leaves, each 2ℓ μ m thick. The first (A) is allowed to take up CO₂ through its lower surface only, while the second (B) assimilates equally through both surfaces. Let A have a CO₂ partial pressure of p_{es} just inside its lower surface. Then we can solve for the partial pressure just inside the upper surface, p_u , by solving the differential equation that equates the CO₂ diffusion across any paradermal plane to the total assimilation above that plane:

$$-D \frac{dp}{dz} = (2\ell - z)R, \quad p(0) = p_{es} \quad (\text{A1})$$

Here, z is the distance of the paradermal plane from the inside of the lower epidermis, and D is the diffusivity of CO₂ in air. The solution is

$$p(z) = p_{es} - \frac{R}{D} \left(2\ell z - \frac{z^2}{2} \right) \quad (\text{A2})$$

from which

$$p_u = p(2\ell) = p_{es} - \frac{2R\ell^2}{D} \quad (\text{A3})$$

Next consider leaf B. The assumption of uniform R allows us to treat this leaf as if it were two one-sided leaves, each of thickness ℓ . For one of the halves, Eq. A1 becomes

$$-D \frac{dp}{dz} = (\ell - z)R, \quad p(0) = p_{es} \quad (\text{A4})$$

From this we obtain

$$p(z) = p_{es} - \frac{R}{D} \left(\ell z - \frac{z^2}{2} \right) \quad (\text{A5})$$

The mean intercellular CO₂ pressure in the B leaf is thus

$$\bar{p} = \frac{1}{\ell} \int_0^\ell p(z) dz = p_{es} - \frac{R\ell^2}{3D} \quad (\text{A6})$$

Comparison with Eq. A3 shows that

$$p_{es} - \bar{p} = (p_{es} - p_u)/6. \quad (\text{A7})$$

Acknowledgments—D. F. P. thanks the members of the Research School of Biological Sciences, Australian National University, for their generous help and cooperation while he was a Visiting Fellow in the School, and Indiana University for sabbatical leave support. We thank an unidentified reviewer for helpful suggestions and questions.

LITERATURE CITED

1. CAEMMERER S VON, GD FARQUHAR 1981 Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* 153: 376–387
2. FARQUHAR GD, K RASCHKE 1978 On the resistance to transpiration of the sites of evaporation within the leaf. *Plant Physiol* 61: 1000–1005
3. KIRSCHBAUM MUF, GD FARQUHAR 1984 Temperature dependence of whole-leaf photosynthesis in *Eucalyptus pauciflora* Sieb. ex Spreng. *Aust Plant Physiol* 1984: 519–538
4. MOTT KA, JW O'LEARY 1984 Stomatal behavior and CO₂ exchange characteristics in amphistomatous leaves. *Plant Physiol* 74: 47–51
5. PARKHURST DF 1977 A three-dimensional model for CO₂ uptake by continuously distributed mesophyll in leaves. *J Theor Biol* 67: 471–488
6. 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, New York, pp 215–249
7. PARKHURST DF 1987 Photosynthesis in relation to internal leaf structure. XIV International Botanical Congress, West Berlin, FRG. Abstr. p 383
 8. POSPIŠILOVÁ J, J SOLAROVÁ 1980 Environmental and biological control of diffusive conductances of adaxial and abaxial leaf surfaces. *Photosynthetica* 14: 90–127
 9. SHARKEY TD, K IMAI, GD FARQUHAR, IR COWAN 1982 A direct confirmation of the standard method of estimating intercellular partial pressure of CO₂. *Plant Physiol* 69: 657–659
 10. TERASHIMA I 1984 Ecophysiological anatomy of a leaf photosynthetic system. PhD thesis. Botany Department, University of Tokyo, Hongo, Tokyo, 113 Japan
 11. TERASHIMA, I, Y INOUE 1985 Vertical gradient in photosynthetic properties of chloroplasts within a leaf of spinach. *Plant Cell Physiol* 26: 781–785
 12. TERASHIMA I, T SAEKI 1985 A new model for leaf photosynthesis incorporating the gradients of light environment and of photosynthetic properties of chloroplasts within a leaf. *Ann Bot* 56: 489–499
 13. WONG SC, KC Woo 1987 Simultaneous measurements of steady state chlorophyll *a* fluorescence and CO₂ assimilation in leaves: the relationship between fluorescence and photosynthesis in C₃ and C₄ plants. *Plant Physiol* 80: 877–883