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Estimation of Bundle Sheath Cell Conductance in C_4 Species and O_2 Insensitivity of Photosynthesis[1]

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Low conductance to CO_2 of bundle sheath cells is required in C_4 photosynthesis to maintain high $[CO_2]$ at the site of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Elevated $[CO_2]$ allows high CO_2 assimilation rates by this enzyme and prevents Rubisco oxygenase activity and O_2 inhibition of carboxylation. Bundle sheath conductance to CO_2 was estimated by chemically inhibiting phospho*enol*pyruvate carboxylase and calculating the slope of the linear response of leaf CO_2 uptake to $[CO_2]$. The inhibitor 3,3-dichloro-2-dihydroxyphosphinoylmethyl-2-propenoate was supplied to detached leaves of *Panicum maximum*, *Panicum miliaceum*, and *Sorghum bicolor* at 4 mM. Uptake of CO_2 was measured at 210 mL L^{-1} O_2 over the CO_2 concentration range of 0.34 to 28 mL L^{-1}. Without the inhibitor, CO_2 uptake increased steeply at low $[CO_2]$ and saturated at about 1 mL L^{-1}. After inhibition, CO_2 uptake was a linear function of $[CO_2]$ over much of the range tested. The slope of this CO_2 response, taken as bundle sheath conductance, was 2.35, 1.96, and 1.13 mmol m^{-2} s^{-1} for *P. maximum*, *P. miliaceum*, and *S. bicolor*, respectively, on a leaf area basis. Conductance based on bundle sheath area was 0.76, 0.93, and 0.54 mmol m^{-2} s^{-1}, respectively. Uptake of CO_2 by leaves of *P. maximum* supplied with the inhibitor was not affected by reduction of $[O_2]$ from 210 to 20 mL L^{-1} over the range of $[CO_2]$ used. Because $[CO_2]$ in bundle sheath cells of inhibited leaves is likely to be much lower than ambient, the lack of O_2 sensitivity of CO_2 uptake cannot be ascribed to lack of O_2 reaction with ribulose bisphosphate and is probably due to the low conductance of bundle sheath cells, especially at low ambient $[CO_2]$. The likely result of reducing $[O_2]$ from 210 to 20 mL L^{-1} is to stimulate carboxylation of ribulose bisphosphate, thus further reducing $[CO_2]$ in bundle sheath cells and increasing CO_2 diffusion to these cells from the mesophyll. However, the increase in diffusion is greatly limited by low conductance of the bundle sheath cell walls. Calculations based on estimated bundle sheath conductance show that changes in bundle sheath $[CO_2]$ of 0.085 to 0.5 mL L^{-1}, which might be associated with reduced $[O_2]$, would have a negligible effect on CO_2 uptake.

Photosynthesis of C_3 species under saturating irradiance is inhibited 20 to 40% by atmospheric $[O_2]$ (Björkman, 1966; Hesketh, 1967; Loreto et al., 1992). The inhibition is due both to competition of O_2 with CO_2 in the Rubisco carboxylation reaction and to release of CO_2 from oxidation of glycolate formed by oxygenation of RuBP (Laing et al., 1974; Chollet and Ogren, 1975). Thus, photorespiratory CO_2 loss and inhibition of photosynthesis are increased by high $[O_2]$ and reduced by high $[CO_2]$.

Photosynthesis of C_4 plants is nearly insensitive to $[O_2]$ between 20 and 210 mL L^{-1} (Hesketh, 1967; Chollet and Ogren, 1975). This insensitivity has been suggested to result from high $[CO_2]$ surrounding Rubisco in BSC, since high $[CO_2]$ has been shown to overcome the O_2 inhibition of C_3 photosynthesis (Chollet and Ogren, 1975). In C_4 species, however, photosynthesis is insensitive to O_2 at low $[CO_2]$, even down to Γ (Troughton, 1971), at which $[CO_2]$ in BSC may not be high enough to keep O_2 from reacting with RuBP. Furbank and Hatch (1987) estimated $[CO_2]$ in BSC of *Urochloa panicoides* (C_4) to be about 140 μM (approximately 5 mL L^{-1} depending on the proportion of inorganic C made up by HCO) at a C_a of 0.098 mL L^{-1}. Extrapolation of their plot of leaf inorganic C pool size versus C_a indicates, however, that the pool would be very low at Γ.

Another possible explanation for the lack of O_2 sensitivity of C_4 species, especially at low C_a, is the low conductance of BSC. The low conductance of BSC is assumed to be an essential feature of C_4 photosynthesis, required for maintenance of high C_{BS}. Low conductance also effectively limits CO_2 exchange so that CO_2 assimilation in BSC requires C_4 acid import and decarboxylation (Hatch, 1987).

The low BSC conductance is illustrated by a 79 to 98% reduction of **A** by inhibition of PEPcase (Jenkins, 1989). Jenkins et al. (1989) estimated the CO_2 permeability coefficient for BSC in intact leaves of seven C_4 species to range from 1.6×10^{-3} to 5.6×10^{-3} cm s^{-1}, which is equivalent to a g_{BSL} of 0.65 to 2.3 mmol m^{-2} s^{-1}. This low conductance of BSC means that direct fixation of CO_2 is very low at atmospheric $[CO_2]$, and C_a had to be raised to about 15 mL L^{-1} to recover **A** rates of uninhibited C_4 leaves at near atmospheric $[CO_2]$ (Jenkins et al., 1989).

The likely effect of low conductance of BSC on O_2 inhibition of photosynthesis is to minimize exchange of CO_2 between the air and BSC and thus minimize O_2 effects on such

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Abbreviations: **A**, CO_2 assimilation rate on a leaf area basis; BSC, bundle sheath cells; C_a, C_i, C_{BS}, $[CO_2]$ external to the leaf, in the leaf intercellular spaces, and in the vascular bundle sheath, respectively; DCDP, 3,3-dichloro-2-dihydroxyphosphinoylmethyl-2-propenoate; g_{BS}, g_{BSL}, conductance of bundle sheath to CO_2 based on area of the bundle sheath and the leaf, respectively; Γ, CO_2 compensation concentration; PEP, phospho*enol*pyruvate; PEPcase, phospho*enol*-pyruvate carboxylase; RuBP, ribulose bisphosphate.

exchange. Because both Rubisco and the CO_2 release reactions of photorespiration are restricted to BSC in C_4 species (Huber et al. 1976; Ohnishi and Kanai, 1983), low conductance of these cells is an effective barrier to CO_2 exchange between the atmosphere and reactions affected by $[O_2]$. Although the high $[CO_2]$ in BSC may be the main reason for O_2 insensitivity of C_4 plants at atmospheric or higher $[CO_2]$, low BSC conductance may be the more important factor at low $[CO_2]$.

In the research reported here we have attempted to estimate BSC conductance using a technique similar to that of Jenkins et al. (1989). They estimated BSC conductance on PEPcase-inhibited leaves using a diffusion model. Calculations of permeability were based on photosynthesis measured at a single high C_a (16 mL L^{-1}) and estimates of C_{BS} and C_i; it was assumed that the CO_2 response was linear up to 16 mL L^{-1} of CO_2. In addition, **A** at 16 mL L^{-1} CO_2 was measured as O_2 evolution. We have estimated bundle sheath conductance by chemically inhibiting PEPcase and measuring **A** over a C_a range of atmospheric to 28 mL L^{-1} and have examined the effect of O_2 on **A** in the absence of the C_4 acid-based CO_2 pump (i.e. in the presence of the PEPcase inhibitor DCDP).

MATERIALS AND METHODS

C_4 grasses representing the three decarboxylation groups were used: *Panicum maximum* (PEP carboxykinase-type), *Sorghum bicolor* (NADP-malic enzyme-type), and *Panicum miliaceum* (NAD-malic enzyme-type). Plants were grown in a greenhouse in 4-L pots containing a mixture of soil, peat, and perlite (1:1:1, v/v). Supplemental lighting by 400-W multivapor lamps was provided to ensure minimum midday PPFD of 1500 μmol photons m^{-2} s^{-1}. Temperatures in the greenhouse were maintained at 30 to 35°C during the day and 20 to 25°C at night. Plants were watered daily and fertilized three times weekly with full-strength Hoagland solution.

For CO_2 exchange, young, fully expanded leaves were detached and the bases quickly recut underwater. The leaf bases were placed in containers of 0.1 mM EDTA and a portion of the laminae (60–80 cm^2) was sealed in a 1.65-L acrylic chamber. Leaves were exposed to $[C_a]$ ranging from 0.34 to 28 mL L^{-1}. The initial measurement of **A** was made by flowing air containing 0.34 mL L^{-1} CO_2 through the chamber and calculating a rate from the depletion of $[CO_2]$ between the intake and exhaust. For measurements at higher CO_2 the intake and exhaust of the chamber were sealed after flushing for at least 10 min with the desired gas and **A** was determined from the rate of CO_2 depletion. Gas samples were withdrawn with a hypodermic syringe at intervals after sealing the chamber, and CO_2 was determined by injection into a N_2 stream, which passed through the sample cell of an IRGA. The size of the samples withdrawn ranged from 0.2 to 3.0 mL and was reduced as $[CO_2]$ in the chamber increased. By using this analysis technique the insensitivity of the IRGA at high $[CO_2]$ was avoided. The calculation of **A** was based on decreases in $[CO_2]$ of less than 2.5% of the initial concentration except at $[CO_2]$ less than 2 mL L^{-1}, in which cases decreases ranged from 10 to 15% of the initial concentrations.

For some leaves the response of **A** to $[CO_2]$ was determined without inhibition of PEPcase by DCDP (controls). For others, 0.1 mM EDTA supplied to leaf bases was replaced by 4 mM DCDP in 0.1 mM EDTA after the initial measurement in the open system (usually about 30 min) at 0.34 mL L^{-1} CO_2. When maximum inhibition of **A** was attained in this system, the chamber was flushed with the desired $[CO_2]$ and **A** was measured as described before.

Because high $[CO_2]$ and high humidity in the closed chamber during measurement reduce transpiration and thus reduce uptake of DCDP, the chamber was flushed with low $[CO_2]$ (about 0.34 mL L^{-1} CO_2) between measurements. Repeated measurements of **A** at 0.34 mL L^{-1} CO_2 were made occasionally to confirm the inhibition by DCDP. Measurements of transpiration over the range of $[CO_2]$ used were also made on *P. maximum* using the open system to examine the effects of high $[CO_2]$ and DCDP on leaf conductance. Measurements of **A** and transpiration were made at 30°C and an irradiance of 1260 μmol photons m^{-2} s^{-1} (PPFD). About 3 to 4 h were required to obtain measurements for each leaf.

To estimate effects of O_2, **A** was measured over the same range of C_a and under the same conditions described earlier, but at each C_a **A** was determined at 20 and 210 mL L^{-1} O_2. Measurements at the two $[O_2]$ for a given C_a were made as close together in time as possible to minimize other effects on **A** (drift with time, etc.).

Leaves similar to those used in the CO_2-exchange experiments were removed from the plants and small pieces (approximately 5–10 mm^2) were cut from the central part of the lamina and fixed in cold glutaraldehyde (30 mL L^{-1}). Leaf pieces were postfixed, dehydrated, sectioned, and stained for light microscopy as described by Brown and Hattersley (1989). Measurements of the perimeter of vascular bundle sheaths were made and the ratio of bundle sheath perimeter to leaf width was taken to represent bundle sheath area:leaf area ratio. This area ratio was used to convert g_{BSL} to g_{BS}.

RESULTS AND DISCUSSION

Estimation of Bundle Sheath Conductance

The response of **A** to C_a for control leaves was steep at low C_a values, and **A** was saturated at about 1 mL L^{-1}, but on the scale shown in Figure 1 and because of variability among replicate leaves, the saturation value of C_a cannot be determined precisely. Soybean (Fig. 1D), a C_3 species, responded in a manner similar to the C_4 species, except **A** saturated at somewhat higher C_a, as expected, and declined slightly at higher C_a. Maximum **A** was between 50 and 70 μmol m^{-2} s^{-1} for all of the species.

When C_4 leaves were treated with DCDP, **A** was very low at C_a near atmospheric, but increased with C_a in a linear fashion over most of the range examined. For *P. miliaceum* (Fig. 1B) and *S. bicolor* (Fig. 1C) the increase was steeper below a C_a of about 2 mL L^{-1}. The reason for this steeper response at low C_a in these two species is not known, but it is not due to the technique used in the experiments, since the response of *P. maximum* was linear over the entire range of C_a (Fig. 1A). It is possible that PEPcase was not completely inhibited in *P. miliaceum* and *S. bicolor*, but nearly complete

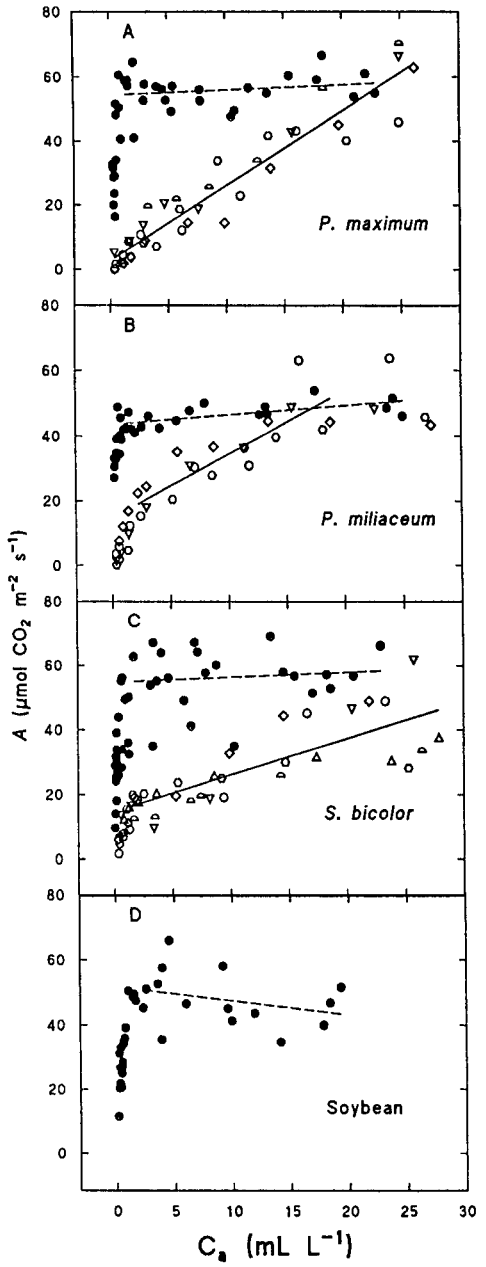

Figure 1. Response of **A** to C_a for *P. maximum* (A), *P. miliaceum* (B), and *S. bicolor* (C) before (filled symbols) and after (open symbols) DCDP treatment and for soybean without DCDP (D). Each open symbol represents a separate leaf. Measurements were made at 30°C, 1260 μmol photons m^{-2} s^{-1}, and 210 mL L^{-1} O_2. Regression (continuous) lines indicate the fit to all data for *P. maximum*, above 2 mL L^{-1} C_a for *S. bicolor*, and between 2 and 20 mL L^{-1} C_a for *P. miliaceum*. Regressions for DCDP-treated leaves were: for *P. maximum*, $\mathbf{A} = 2.68 + 2.35\ C_a$ ($r = 0.96$, $n = 40$); for *P. miliaceum*, $\mathbf{A} = 14.7 + 1.96\ C_a$ ($r = 0.86$, $n = 20$); for *S. bicolor*, $\mathbf{A} = 14.8 + 1.13\ C_a$ ($r = 0.77$, $n = 29$). Dashed lines were fitted by eye to indicate approximate CO_2-saturated values of **A**.

inhibition of **A** at atmospheric [CO_2] indicates that it was. Maximum **A** of control leaves was recovered in DCDP-treated leaves of *P. maximum* and *P. miliaceum* at C_a of 20 to 25 mL L^{-1}, but in *S. bicolor*, **A** was lower in DCDP-treated than in control leaves at the highest C_a used.

To estimate g_{BSL} of BSC, the response of the linear portions of the **A** versus C_a curves was used. Therefore, in *S. bicolor* C_a values above 2 mL L^{-1} were used and for *P. miliaceum* values between 2 and 20 mL L^{-1} were used, because higher C_a gave **A** values no higher or only slightly higher than those near 20 mL L^{-1}. All data for DCDP-treated *P. maximum* were used in determining g_{BSL}. If it is assumed that CO_2 is being taken up only by BSC and that the uptake is diffusion limited, then the slope of the **A** versus C_i response represents g_{BSL}.

We used the slope of the linear portion of the **A** versus C_a response (plotted in Fig. 1) as g_{BSL} because we could not measure transpiration (and thus could not determine C_i) in the closed system due to condensation. However, using C_i calculated from transpiration at the various [CO_2] in an open system for two replicates of *P. maximum*, the relationship is very similar to that of **A** versus C_a. The value of g_{BSL} using the calculated C_i was 2.57 mmol m^{-2} s^{-1} compared with 2.35 mmol m^{-2} s^{-1} from Figure 1A (Table I). Thus, the error in g_{BSL} caused by using C_a is not large.

Conductance on a leaf area basis was about 20% higher for *P. maximum* than for *P. miliaceum*, and about twice as great as for *S. bicolor* (Table I). Because the bundle sheath area:leaf area ratio was about 50% greater in *P. maximum* than in *P. miliaceum* (Table I), g_{BS} (conductance based on bundle sheath area) was higher for *P. miliaceum* than for *P. maximum*. Thus, g_{BS} of the species rank in the same way as was inferred for their respective photosynthetic types based on ^{13}C discrimination (Hattersley, 1982) and quantum yield (Ehleringer and Pearcy, 1983).

The range of values for g_{BS} presented in Table I (0.54–0.93 mmol m^{-2} s^{-1}) is similar to the range observed for seven C_4 species (0.65–2.27 mmol m^{-2} s^{-1}) by Jenkins et al. (1989). The two species common to both investigations, *P. maximum* and *P. miliaceum*, exhibited a g_{BS} of 0.76 and 0.93 mmol m^{-2} s^{-1}, respectively, in our experiments, and 1.54 and 2.27, respectively, in theirs. The higher values calculated by Jenkins et al. (1989) may result from expression of CO_2 exchange on a Chl basis and conversion to a bundle sheath area basis

Table I. *Values of bundle sheath conductance for P. maximum, P. miliaceum, and S. bicolor determined from* **A** *versus* C_a *in DCDP-inhibited leaves, and calculated on the bases of leaf* (g_{BSL}) *and bundle sheath* (g_{BS}) *area*

Species	C_4 Type[a]	Bundle Sheath/ Leaf Area Ratio	Conductance	
			g_{BSL}	g_{BS}
			mmol m^{-2} s^{-1}	
P. maximum	PEP-CK	3.1 ± 0.3	2.35	0.76
P. miliaceum	NAD-ME	2.1 ± 0.1	1.96	0.93
S. bicolor	NADP-ME	2.1 ± 0.1	1.13	0.54

[a] These C_4 types are characterized by decarboxylase enzymes in BSC; PEP-CK, PEP carboxykinase; NAD-ME, NAD-malic enzyme; NADP-ME, NADP-malic enzyme.

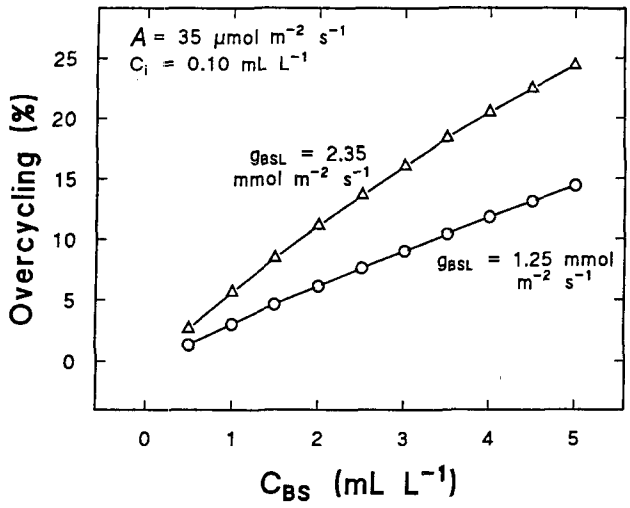

Figure 2. Overcycling of CO_2 in BSC calculated as a percentage of **A** plus leakage [$L = (C_{BS} - C_i)\, g_{BSL}$] for a range of C_{BS} (0.5–5 mL L^{-1}). **A** is assumed to be 35 μmol m^{-2} s^{-1}, C_i to be 0.1 mL L^{-1}, and g_{BSL} to be either 2.35 or 1.25 mmol m^{-2} s^{-1}. Overcycling (%) = $(L/A + L)$ 100.

using a Chl:bundle sheath area ratio from other work that did not include these two species. When g_{BSL} is calculated from the slope of the CO_2 response using their **A** data at 0.35 and 16.3 mL L^{-1} of CO_2, values of 2.48 and 2.46 mmol m^{-2} s^{-1} are obtained for *P. maximum* and *P. miliaceum*, respectively, which are not much higher than in Table I. If the response to C_a is not linear over the range tested for *P. miliaceum*, as indicated in Figure 1B, then g_{BSL} as determined from data by Jenkins et al. (1989) may be overestimated. Nevertheless, our more complete results based on CO_2 uptake measurements over a wide range of C_a are similar to the bundle sheath permeability values estimated by Jenkins et al. (1989) from O_2 evolution measurements at a single, high C_a value.

Low bundle sheath conductance is a central feature of C_4 photosynthesis because it allows high C_{BS} and minimal leakage of CO_2 from BSC. Diffusion of CO_2 from BSC represents a loss of energy expended in mesophyll cells to initially fix and then recycle CO_2. This overcycling of CO_2 has been calculated to range from about 10 to 50% of the PEP carboxylation rate at atmospheric [CO_2] (Farquhar, 1983; Furbank and Hatch, 1987). The g_{BSL} estimated in this work allows overcycling to be calculated by assuming values for C_{BS}. Calculations of overcycling are shown in Figure 2 as a percentage of **A** (taken as 35 μmol m^{-2} s^{-1}) plus leakage from bundle sheaths for g_{BSL} values of 1.25 (Fig. 3) and 2.35 mmol m^{-2} s^{-1} (Fig. 1A). For leaves with g_{BSL} of 1.25 mmol m^{-2} s^{-1}, overcycling is ≤15% up to C_{BS} of 5 mL L^{-1}. For g_{BSL} of 2.35 mmol m^{-2} s^{-1}, overcycling increases to 24% at 5 mL L^{-1}. This highest value for overcycling is similar to recent estimates for several C_4 species from short-term ^{13}C discrimination experiments (Henderson et al., 1992).

Effects of O_2 on CO_2 Uptake

Experiments similar to those described above were conducted with *P. maximum* on DCDP-treated leaves except that at each C_a, **A** was measured at 20 and 210 mL L^{-1} of O_2. Prior to DCDP-treatment, leaves had rates of 28 to 35 μmol m^{-2} s^{-1} at 0.34 mL L^{-1} of CO_2 and 210 mL L^{-1} O_2 (Fig. 3). After treatment with DCDP, **A** was a linear function of C_a up to the highest values used (approximately 28 mL L^{-1}). Conductance (g_{BSL}) estimated from the slopes of the CO_2 response in Figure 3 was only about one-half of the value for *P. maximum* estimated from Figure 1 and shown in Table I (1.25 versus 2.35 mmol m^{-2} s^{-1}). The reason for this lower g_{BSL} is not known. Although the plants were grown under similar conditions for all experiments, the [O_2] experiment was conducted 2 to 3 months later than the others. It may be that g_{BSL} varies with environment and other factors, and a systematic study of environmental and developmental effects is needed.

The slopes and zero-CO_2 intercepts of the lines representing 20 and 210 mL L^{-1} O_2 did not differ. Thus, from regression analysis and from inspection of paired data points in Figure 3, it is concluded that O_2 did not affect CO_2 uptake by DCDP-treated leaves. Although in the absence of the C_4 "CO_2-pump" O_2 would be expected to inhibit **A** more at low than at high C_a, there is no evidence in Figure 3 of an O_2 effect, even at low C_a.

The high [CO_2] required to recover control rates of **A** and the linear response of **A** over a wide range of C_a (Figs. 1 and 3) indicate that CO_2 uptake by DCDP-treated plants is predominately diffusion limited. Under such limitation increased carboxylation capacity caused by reducing [O_2] is likely to lower C_{BS}. Because of the diffusion limitation, increased carboxylation can result in higher **A** only if the gradient of [CO_2] from the air to BSC is increased (i.e. C_{BS} is decreased) sufficiently to cause a measurable increase in diffusion of CO_2 across the BSC walls. The low conductance values in Table I mean that large changes in the gradient are necessary to

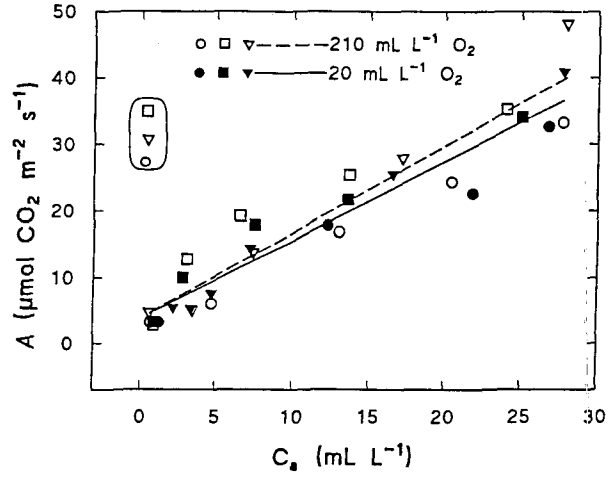

Figure 3. Response of **A** to C_a for *P. maximum* at 20 and 210 mL L^{-1} O_2. Conditions were the same as for Figure 1. Regressions were: for 210 mL L^{-1} O_2, $A = 3.74 + 1.30\ C_a$ ($r = 0.94$, $n = 15$); for 20 mL L^{-1} O_2, $A = 3.72 + 1.18\ C_a$ ($r = 0.96$, $n = 15$). Each different symbol represents a separate leaf. The three circled points represent control measurements at 0.34 mL L^{-1} CO_2 and 210 mL L^{-1} O_2 before application of DCDP.

effect measurable changes in **A**. The effect of $[CO_2]$ gradient changes on **A** was calculated using equations 1 and 2 as follows:

$$C_a = (\mathbf{A}/g_{BSL}) + C_{BS} \quad (1)$$

The first step was to calculate C_a using C_{BS} similar to $[CO_2]$ estimated for chloroplasts in C_3 plants (Caemmerer and Evans, 1991). They estimated that chloroplast $[CO_2]$ is about 0.5 C_a. Using this C_{BS} (0.17 mL L^{-1}), g_{BSL} of 1.25 mmol m^{-2} s^{-1} (Fig. 3), and **A** = 20 μmol m^{-2} s^{-1} (a value typical for C_3 species at a C_a of 0.34 mL L^{-1}), C_a was calculated to be 16.2 mL L^{-1} (Table II). To calculate O_2 effects, C_{BS} was assumed to decrease by 50% (0.085 mL L^{-1}) when $[O_2]$ was reduced from 210 to 20 mL L^{-1}. This is approximately the percentage change in C_{BS} due to reduced $[O_2]$ predicted by a model of C_4 photosynthesis when C_a was 0.01 mL L^{-1} (Berry and Farquhar, 1978). Since C_a and g_{BSL} were assumed to stay the same when $[O_2]$ was changed, enhancement of **A** at 20 mL L^{-1} O_2 was calculated as follows:

$$\mathbf{A}_{20} - \mathbf{A}_{210} = (C_{BS210} - C_{BS20})\, g_{BSL} \quad (2)$$

where $\mathbf{A}_{20}$, $\mathbf{A}_{210}$, and C_{BS20}, C_{BS210} are assimilation and $[CO_2]$ in BSC at 20 and 210 mL L^{-1} O_2, respectively. When $[O_2]$ was reduced to 20 mL L^{-1}, **A** was calculated to rise by only 0.11 μmol m^{-2} s^{-1} or 0.5% (Table II). In other calculations increasing $C_{BS210} - C_{BS20}$ from 0.085 to 0.120 mL L^{-1} (C_{BS210} assumed to be 0.240 mL L^{-1}), increasing **A** from 20 to 35 μmol m^{-2} s^{-1}, or changing g_{BSL} from 1.25 to 2.35 mmol m^{-2} s^{-1} (Table II) had only small effects on the estimated enhancement of **A**. Maximum enhancement of **A** was only 1.4%. Even if extreme changes in C_{BS} occurred, say from 0.6 mL L^{-1} at 210 mL L^{-1} O_2 to 0.1 at 20 mL L^{-1} O_2, enhancement is still only 1.17 μmol m^{-2} s^{-1} or 3.3% (Table II). Such changes are hardly measurable by gas-exchange techniques.

Thus, the low g_{BS} greatly buffers any change in **A** due to changes in $[O_2]$. A somewhat analogous buffering was reported from a comparison of O_2 effects on two C_3 species, where **A** in *Quercus rubra* was less sensitive to O_2 than in *Xanthium strumarium* because of the lower mesophyll conductance of *Quercus* (Loreto et al., 1992). In *Quercus*, mesophyll conductance was approximately 100 times greater than g_{BSL} of *P. maximum*, so dampening of the O_2 effect was much less than in DCDP-treated C_4 leaves, where C_{BS} is likely to be low enough to cause considerable oxygenation of RuBP.

The low g_{BS} may also largely prevent O_2 effects on **A** in control leaves of C_4 plants. Results predicted by Berry and Farquhar (1978) from a model of C_4 photosynthesis indicated that **A** at a C_a of 0.01 mL L^{-1} would increase only from 3.83 to 3.97 μmol m^{-2} s^{-1} if $[O_2]$ was reduced from 210 to 20 mL L^{-1}, an increase of less than 4%. Conductance of the bundle sheath was assumed to be 1.0 mmol m^{-2} s^{-1} and reduction of $[O_2]$ caused a predicted drop in C_{BS} from 0.312 to 0.171 mL L^{-1}. Thus, even though reduced $[O_2]$ may cause an increase in carboxylation by Rubisco in BSC, O_2 effects on CO_2 exchange are minimal even under conditions likely to result in low C_{BS}.

The low BSC conductance of C_4 plants may explain the apparently contradictory reports of O_2 stimulation of ^{14}C labeling of glycolate pathway intermediates on the one hand and a lack of O_2 effect on CO_2 exchange on the other (Osmond and Björkman, 1972; Mahon et al., 1974; Morot-Gaudry et al., 1980). Even though O_2 may stimulate synthesis and oxidation of glycolate in BSC of C_4 species, especially at low $[CO_2]$, g_{BS} is too low to allow a measurable effect on CO_2 exchange by leaves. Although emphasis has been placed on refixation of CO_2 leaked from BSC by PEPcase in the surrounding mesophyll cells as an explanation for lack of demonstrable photorespiration in C_4 leaves at low C_a (Troughton, 1971; Morot-Gaudry et al., 1980), it is unlikely that appreciable leakage of CO_2 from BSC occurs, especially at low $[CO_2]$. If C_{BS} is assumed to be 0.5 mL L^{-1} at Γ, which is near 0 for C_4 leaves, then using a g_{BSL} value of 2.35 mmol m^{-2} s^{-1} and substituting Γ for C_a in Equation 1, **A** is calculated to be -1.17 μmol m^{-2} s^{-1}. Refixation by PEPcase of this rate of CO_2 release is likely, but a C_{BS} of 0.5 mL L^{-1} appears to be much too high for reassimilation alone and at such a low rate. Thus, at Γ, leakage from the BSC is probably much lower than 1.17 μmol m^{-2} s^{-1}. In summary, previously estimated conductances of BSC have been confirmed and the low values are likely to greatly restrict CO_2 leakage and to limit the influence of $[O_2]$ on **A**, especially at low $[CO_2]$.

Table II. *Calculated values for enhancement of **A** by 20 mL L^{-1} compared with 210 mL L^{-1} of O_2 for DCDP-treated leaves of P. maximum*

g_{BSL}	A_{210}	C_a[a]	$C_{BS210} - C_{BS20}$[b]	A Enhancement by 20 mL L^{-1} O_2 ($A_{20} - A_{210}$)	
mmol m^{-2} s^{-1}	*μmol m^{-2} s^{-1}*		*mL L^{-1}*	*μmol m^{-2} s^{-1}*	*%*
1.25	20	16.2	0.085	0.11	0.5
1.25	20	16.2	0.120	0.15	0.7
1.25	35	28.2	0.085	0.11	0.3
2.35	20	8.7	0.085	0.20	1.0
2.35	20	8.7	0.120	0.28	1.4
2.35	35	15.1	0.085	0.20	0.6
2.35	35	15.5	0.500	1.17	3.3

[a] C_a was first calculated from assumed values of **A**, g_{BSL}, and C_{BS210} using the equation $C_a = (\mathbf{A}/g_{BSL}) + C_{BS}$. Then enhancement of **A** by reduced $[O_2]$ was calculated as $\mathbf{A}_{20} - \mathbf{A}_{210} = (C_{BS210} - C_{BS20})\, g_{BSL}$. [b] C_{BS210} was twice the values given here, except 0.6 mL L^{-1} in the case of $C_{BS210} - C_{BS20} = 0.5$.

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