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Partitioning of the Leaf CO_2 Exchange into Components Using CO_2 Exchange and Fluorescence Measurements[1]

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Photorespiration was calculated from chlorophyll fluorescence and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) kinetics and compared with CO_2 evolution rate in the light, measured by three gas-exchange methods in mature sunflower (*Helianthus annuus* L.) leaves. The gas-exchange methods were (a) postillumination CO_2 burst at unchanged CO_2 concentration, (b) postillumination CO_2 burst with simultaneous transfer into CO_2-free air, and (c) extrapolation of the CO_2 uptake to zero CO_2 concentration at Rubisco active sites. The steady-state CO_2 compensation point was proportional to O_2 concentration, revealing the Rubisco specificity coefficient (K_{sp}) of 86. Electron transport rate (*ETR*) was calculated from fluorescence, and photorespiration rate was calculated from *ETR* using CO_2 and O_2 concentrations, K_{sp}, and diffusion resistances. The values of the best-fit mesophyll diffusion resistance for CO_2 ranged between 0.3 and 0.8 s cm^{-1}. Comparison of the gas-exchange and fluorescence data showed that only ribulose-1,5-bisphosphate (RuBP) carboxylation and photorespiratory CO_2 evolution were present at limiting CO_2 concentrations. Carboxylation of a substrate other than RuBP, in addition to RuBP carboxylation, was detected at high CO_2 concentrations. A simultaneous decarboxylation process not related to RuBP oxygenation was also detected at high CO_2 concentrations in the light. We propose that these processes reflect carboxylation of phospho*enol*pyruvate, formed from phosphoglyceric acid and the subsequent decarboxylation of malate.

Photorespiration of C_3 plants was discovered by Decker (1955) from measurements of a postillumination CO_2 burst. Photorespiration can also be observed as CO_2 evolution after rapid transfer of the leaf into a CO_2-free gas (Forrester et al., 1966). Since the CO_2 concentration at which photosynthesis and photorespiration equilibrate was found to be proportional to O_2 concentration and independent of light intensity, it was suggested that CO_2 and O_2 compete for the primary acceptor RuBP at the carboxylase/oxygenase sites (Laisk, 1970). This was proven with the partially purified enzyme (Ogren and Bowes, 1971) and at present it is generally accepted that photorespiration is a result of the functioning of the glycolate cycle (Hess and Tolbert, 1966; Kisaki and Tolbert, 1970; Lorimer, 1981).

Measurements of photorespiration are complicated because reassimilation of CO_2 obscures the true CO_2 evolution rate in the light. Reassimilation in leaf intercellular spaces can be accounted for by calculating the intercellular CO_2 concentration (Laisk and Oja, 1972). The postillumination photorespiratory CO_2 burst is also partially reassimilated by the assimilatory charge (Laisk et al., 1984; Laisk et al., 1987; Sharkey, 1988), which is closely equivalent to the pool of RuBP present in the leaf when illumination is interrupted. Calculations of reassimilation in the mesophyll cells are complicated, since the CO_2 transport resistance in the mesophyll cells is difficult to determine (Evans et al., 1986; von Caemmerer and Evans, 1991; Harley et al., 1992; Loreto et al., 1992). The mechanism based on the competition of CO_2 and O_2 at Rubisco has generally been found to fit the experimental data (Laisk, 1970; Laisk and Oja, 1972; Peterson, 1987, 1989; Cornic and Briantais, 1991). The range of CO_2 pressures applied in these measurements has usually been below 600 μbars, which has left out the interesting range of depression of photorespiration by CO_2. At saturating CO_2 concentrations the CO_2 evolution in the light proceeds faster than predicted from the theory, and this CO_2 is evolved from freshly assimilated carbon pools. This has led to suggestions that the CO_2/O_2 competition mechanism of photorespiration is not adequate at high CO_2 concentrations (Bravdo and Canvin, 1979; Keerberg et al., 1983; Pärnik, 1985).

In this work we have used a fast-response gas-exchange system for the measurements of photorespiration over a wide range of CO_2 concentrations. Simultaneously, electron transport rates were determined from fluorescence measurements. This allowed a comparison of the rates of photorespiration obtained from gas exchange with those calculated from the electron transport rate. As a result we can distinguish four components of gas exchange in the light. We confirm that at high CO_2 concentrations photorespiration is suppressed, as predicted by the Rubisco kinetics. Instead, a carboxylation and a decarboxylation, not directly related to the photosynthetic electron transport, dominate at these CO_2 concentrations.

MATERIALS AND METHODS

Sunflower (*Helianthus annuus* L.) plants were grown in a growth chamber at a *PFD* of 46 nmol cm^{-2} s^{-1}, 18/6 h day/

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Abbreviations: $B(CO_2)$, postillumination CO_2 burst in the presence of a stated concentration of CO_2; $B(0)$, postillumination CO_2 burst in the absence of CO_2; C_c, chloroplast CO_2 concentration; *ETR*, electron transport rate; Γ, steady-state CO_2 compensation point; Γ^*, CO_2 photocompensation point; K_{sp}, Rubisco specificity coefficient; $P(CO_2)$, net CO_2 assimilation rate; *PAD*, photon absorption density; *PFD*, photon flux density; PGA, 3-phosphoglyceric acid; R_L, total respiration in the light; r_{md}, CO_2 transport resistance in mesophyll cells; RuBP, ribulose-1,5-bisphosphate.

night cycle, air temperature 28/20°C day/night, RH 50 to 60% by day, on well-fertilized peat-soil mixture in 4-L pots. Attached upper, fully expanded leaves of 4-week-old plants were used.

The gas-exchange apparatus contained two open gas-flow systems (channels A and B) in which the gas composition could be adjusted separately (Oja, 1983; Laisk et al., 1984). An Infralyt 3 analyzer (Junkalor, Dresden, Germany) was used for the recording of CO_2 concentration at the end of channel A, and an LI-6262 analyzer (Li-Cor, Lincoln, NE) was used in channel B. Water vapor was recorded by psychrometers in each channel. Gas was dried to a constant humidity in small Peltier-cooled condensers before entering the CO_2 analyzers. During the measurements a part of the leaf was enclosed in a sandwich-type cuvette (4.4 × 4.4 × 0.3 cm^3, gas flow rate 20 cm^3 s^{-1}). For temperature stabilization the leaf blade was fixed with starch paste directly to the cuvette window, which was thermostated by water from the other side. Water temperature was 21.5°C, and leaf temperature was 22.2°C in the light. Gas exchange proceeded via the lower epidermis of the leaf only. As a result, the CO_2-limited gas-exchange rates were probably somewhat reduced. This did not influence the values of the CO_2 bursts nor the other results that were based on calculated chloroplast CO_2 concentration.

The leaf chamber could be connected with the gas flow of either channel A or channel B by a special stopcock, which made it possible to rapidly change the CO_2 concentration in the leaf chamber to the levels preadjusted in the channels. While the chamber was in channel A, channel B was circuited through an equivalent resistance so that the gas analyzer in channel B showed the reference line and that in channel A showed the leaf response and vice versa when the chamber was connected with channel B. The full response time of the system, determined mainly by gas flow: volume ratios, was 2 s for CO_2 with the LI-6262 in channel B. Channel A was used mainly for the preconditioning of the leaf to its steady state and channel B was used for the measurement of the fast leaf responses.

The CO_2 analyzers were calibrated by means of dynamic capillary gas mixers with 1% accuracy in the range from 0 to 2000 ppm CO_2 (Oja, 1983). C_c was calculated as outlined by Laisk (1977), taking into account the effect of water vapor counterflow for CO_2 diffusion in stomatal pores, the solubility of CO_2 in cell water, and CO_2 transport resistance in cells. Following the precedent laid down by Brown and Escombe (1900) and Laisk and Oja (1971, 1972), we express CO_2 as its concentration, not as partial pressure or mole fraction as suggested by Cowan (1977). Accordingly, the CO_2 and O_2 will be expressed in μM (nmol cm^{-3}), rates (including photon flux rate) in nmol cm^{-2} s^{-1}, and diffusion resistances in s cm^{-1}. These units are compatible with molar concentrations of metabolites and allow us to calculate the Rubisco specificity factor in vivo. The Bunsen solubility constants of 0.89 for CO_2 and 0.029 for O_2 at 22.2°C were applied. We still use mole fraction (ppm) to denote the CO_2 concentration at the inlet of the leaf chamber.

Three procedures for determining photorespiration were used: (a) postillumination CO_2 burst without changing the CO_2 concentration, (b) postillumination CO_2 burst with simultaneously changing to CO_2-free gas, and (c) initial CO_2 evolution into CO_2-free gas in the light, corrected for CO_2 reassimilation.

A special, six-branch fiber optic was designed for leaf illumination and optical measurements. Plastic fibers of 1 mm diameter (Toray polymer optical fiber, PF-series, from Laser Components, Gröbenzell/München, Germany) were arranged into a bundle of 45 × 45 mm^2, which was attached to the leaf cuvette. The free ends of the fibers were divided into branches; two were used for illumination by actinic light and saturation pulses and two for excitation and measurement of the Chl fluorescence. Chl fluorescence was measured by a PAM 101 fluorometer (H. Walz, Effeltrich, Germany) at two spots of 10 × 20 mm symmetrically placed over the leaf chamber, avoiding the midrib. A Schott KL 1500 light source was used for actinic illumination and a 1000-W DC xenon arc lamp, equipped with a cold mirror and a heat-reflecting filter, provided saturation flashes of 800 nmol cm^{-2} s^{-1}, of 1 s duration. The electron transport rate was calculated as proposed by Genty et al. (1989):

$$ETR = \frac{F'_m - F}{F'_m} \times PAD \times 0.5 \quad (1)$$

where PAD was measured by the LI-190SB quantum sensor (Li-Cor) and multiplied for the leaf absorbance measured in an integrating sphere with the same actinic light and sensor F is stedy-state fluorescence yield; and F'_m is fluorescence yield in saturation flashes. The assumption that 0.5 PAD was absorbed by PSII gave reasonably good coincidence between the fluorescence and gas-exchange data under nonphotorespiratory conditions.

RESULTS

Measuring Respiration in the Light by Transients of the CO_2 Exchange

Postillumination CO_2 burst is the simplest method for the measurement of photorespiration and is based on the consideration of the different decay kinetics of photosynthesis and photorespiration. Such a postillumination transient at normal atmospheric CO_2 and O_2 concentrations is shown in Figure 1 (curve 1). The CO_2 uptake approached zero after 5 s and the rate of CO_2 evolution peaked 13 s from darkening. From this recording it is evident that the maximum rate for the postillumination CO_2 burst $B(CO_2)$ (arrow) is a compromise between the postillumination CO_2 reassimilation in the first 10 s and a declining rate of photorespiration. In Figure 2 the same experiment was repeated with 2000 ppm CO_2, which would severely suppress photorespiration. There was no CO_2 burst and at 13 s from the transition to darkness the CO_2 evolution rate was still increasing.

Curve 2 in Figure 1 is the postillumination CO_2 burst after transition of the leaf into CO_2-free air of channel B simultaneously with darkening, $B(0)$. With this method we avoided the uptake of external CO_2 during the postillumination CO_2 burst (but not reassimilation of the photorespiratory CO_2). To bypass the big flux of dissolved CO_2, which evolved from the leaf during the first 2 s (Oja et al., 1986), the gas flow was not connected with the analyzer of channel B for 2 s

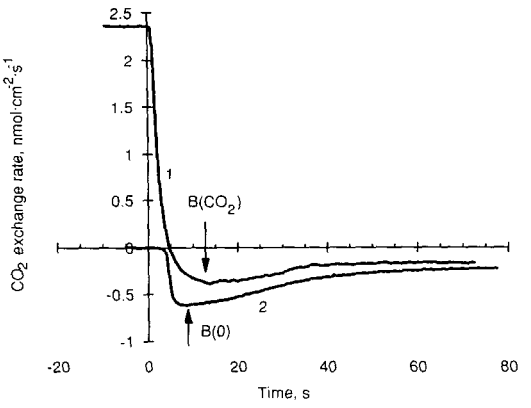

Figure 1. Postillumination transients in CO_2 exchange rates. Sunflower leaf was exposed at 300 ppm CO_2, 21% O_2, *PAD* of 120 nmol cm^{-2} s^{-1}, in steady state. At time = 0 light was switched off (curve 1). In another, similar experiment CO_2 concentration was changed to 0 simultaneously with darkening by switching the leaf chamber from channel A to channel B (curve 2). Values of the postillumination CO_2 burst in the presence, $B(CO_2)$, and absence, $B(O)$, of CO_2 were read at arrows.

from the moment of the transition. As a result, the analyzer of channel B continued to record its baseline for the first 2 s in the dark, as seen from curve 2 in Figure 1.

In CO_2-free air the postillumination CO_2 burst, $B(0)$, was greater and the maximum occurred earlier than in the presence of CO_2. After 60 s the CO_2 evolution declined to a minimum and then increased again (not shown in Figs. 1 and 2). The difference between the two curves for the CO_2 bursts in Figure 1 shows that there was a small postillumination CO_2 uptake component in gas exchange that shifted the $B(CO_2)$ curve upward. In similar experiments started after photosynthesis with 2000 ppm CO_2 (Fig. 2, curve 2), the fast initial burst (until 7 s) was probably desolubilization of residual CO_2. However, the subsequent time course of the CO_2 evolution did not represent photorespiration but reflected another metabolic CO_2 evolution process with a relaxation time of about 50 s. Because $B(CO_2)$ in the presence of 2000 ppm CO_2 was considerably higher than $B(0)$, the alternative carboxylation process was also present at this CO_2 concentration. Thus, the two postillumination CO_2 exchange curves, one recorded in the presence and the other in the absence of CO_2, reveal two simultaneous processes, a carboxylation and a decarboxylation, which have similar relaxation times of about 50 s.

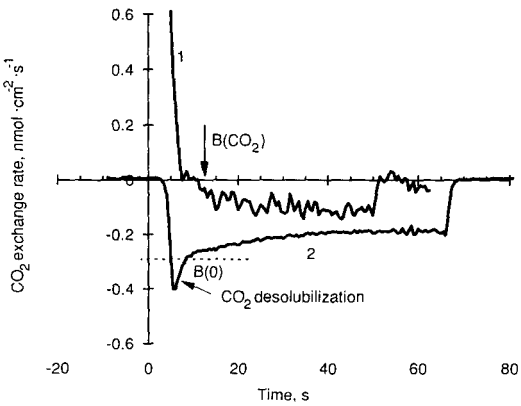

Figure 2. The same experiment as in Figure 1, except that the leaf was previously exposed in steady state to 2000 ppm CO_2. Recordings ended with switching the leaf chamber back to channel A to establish the reference line.

The third gas-exchange method to measure photorespiration was the extrapolation of the linear part of the P versus C_c response curve to $C_c = 0$. This method has been used primarily starting from limiting CO_2 concentrations. When used from saturating CO_2 concentrations, fast recording is necessary to see the initial photorespiration rate before it increases at low CO_2 levels in the light. Two recordings made starting from 2000 ppm CO_2, one to 100 and the other to 0 ppm, are shown in Figure 3. Gas was flushed out during 2 s after the transition, as in the case of curve 2 in Figure 2. The slow changes in the CO_2 exchange were extrapolated to the beginning of the recordings, disregarding the initial peak of CO_2 desolubilization. The values obtained (A and B) were assumed to correspond to the previous CO_2 evolution at 2000 ppm. In Figure 4 the points A and B are plotted against the C_c values, calculated for the same initial rates (curve 4). Extrapolation of this plot to $C_c = 0$ represents the sum of all CO_2 evolution processes in the light at 2000 ppm CO_2, R_L. Figure 4 also shows other, similar P versus C_c plots from transients starting from 0, 100, and 300 ppm CO_2 (curves 1–3). It must be emphasized that the CO_2 compensation points obtained by interpolation between the two transient measurements are apparent, representing a varying equilibrium between rapidly increasing photosynthesis and slowly increasing photorespiration. The transient from 0 to 100 ppm may also be influenced by the partial deactivation of Rubisco.

Γ was found from the plot of the steady-state CO_2 exchange rates (filled squares in Fig. 4). The values of Γ were found from similar experiments at 1.1, 10, and 50% O_2 and plotted against the dissolved O_2 concentration (Fig. 5). The

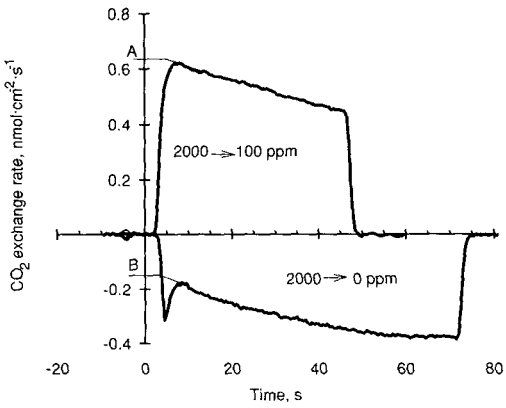

Figure 3. Transients in CO_2 exchange rate after changing CO_2 concentration in the light. The leaf was exposed at 2000 ppm CO_2, 21% O_2, in steady state. At time = 0 CO_2 concentration was changed to 100 ppm or to 0. The gas-exchange rate was extrapolated to the beginning of the transient disregarding the CO_2 desolubilization peak (A and B).

proportionality of Γ with O_2 concentration (Forrester et al., 1966; Laisk, 1977) was confirmed with great accuracy. The straight line in Figure 5 extrapolates very close to the origin of coordinates. This indicates that primarily photorespiratory CO_2 evolution was present in the light at Γ in mature sunflower leaves, with very little "dark" respiration. In a younger leaf this plot showed a small residual respiration, which did not disappear with photorespiration when O_2 approached zero. According to Laisk (1970) and Farquhar et al. (1980) the slope of this line is characteristic for the CO_2/O_2 specificity of Rubisco in vivo, $K_{sp} = 0.5[O_2]/\Gamma = 86$. In the younger leaf $K_{sp} = 92$. The value of $K_{sp} = 86$ was used in the analysis of the fluorescence data with the aim of finding the photorespiratory rate in an independent way.

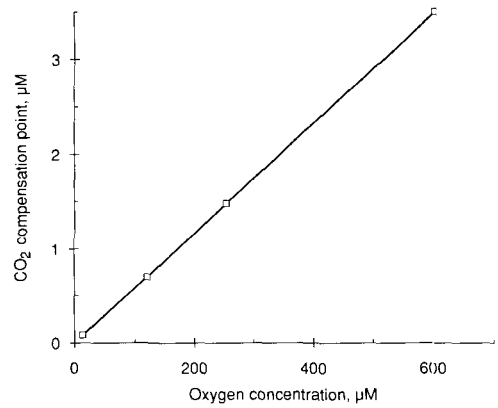

Figure 5. Dependence of Γ on the O_2 concentration in liquid phase of mesophyll cells.

The Influence of CO_2 Concentration on Respiration in the Light

Measurements similar to those in Figures 1 to 4 were carried out at different CO_2 and O_2 concentrations. Figure 6 shows the CO_2 dependencies of $P(CO_2)$, $B(CO_2)$, $B(0)$, and R_L, as revealed from the extrapolation of the P versus C_c plot. At 1.1% O_2, RuBP oxygenation and, correspondingly, photorespiration were very low. The difference between the two CO_2 burst measurements, $B(CO_2)$ and $B(0)$, remained, but $B(CO_2)$ showed slightly positive values (it was always measured 13 s after the darkening). This result indicates that a postillumination CO_2 uptake process and not suppression of respiration at higher CO_2 levels was the cause of the difference between $B(CO_2)$ and $B(0)$. The maximum of $B(CO_2)$ at low CO_2 concentrations may be an artifact caused by lasting RuBP carboxylation.

In 21% O_2, *ETR* was maximal at C_c values around 5 to 8 µM which are typical for leaves with open stomata under normal atmospheric conditions. It declines toward lower CO_2 concentrations because of the lack of sufficient electron acceptor and also toward higher CO_2 concentrations due to the inhibition of photorespiration by CO_2. At Γ (the situation that occurs with closed stomata when no net CO_2 fixation is possible) *ETR* was reduced by only half. It was maintained so high due to the simultaneous turnover of CO_2 by the carbon reduction and glycolate cycles. Similar measurements done at 10 and 50% O_2 with the same leaf showed that the maximum *ETR* occurred at 21% O_2.

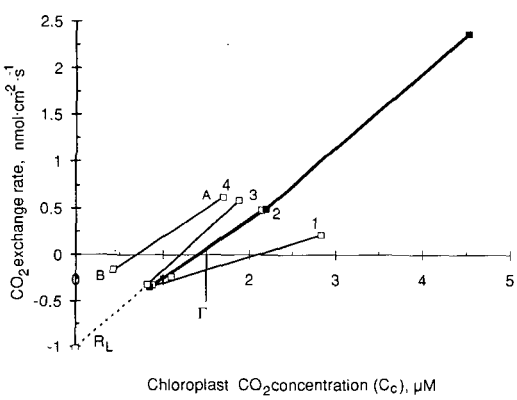

Figure 4. Determining CO_2 evolution in the light, R_L, by extrapolation of the P versus C_c curve to $C_c = 0$. Data points A and B from Figure 3 are plotted against calculated C_c (line 4). Other, similar transitions are from 0 ppm (steady state) to 100 ppm (line 1) from 100 ppm (steady state) to 0 ppm (line 2), and from 300 ppm (steady state) to 100 ppm and 0 ppm (line 3, extrapolation to $C_c = 0$ to find R_L is shown for this line). Filled squares represent the steady-state CO_2 curve used to find Γ at 21% O_2.

The theory of photorespiration predicts a certain ratio of oxygenation and carboxylation (V_o/V_c) at any given $[O_2]/[CO_2]$ ratio (Farquhar and von Caemmerer, 1982):

$$\frac{V_o}{V_c} = \frac{V_{omax}}{K_{mo}} \times \frac{K_{mc}}{V_{cmax}} \times \frac{[O_2]}{[CO_2]} = \frac{1}{K_{sp}} \times \frac{[O_2]}{[CO_2]} \quad (2)$$

where

$$\frac{V_{omax}}{K_{mo}} \times \frac{K_{mc}}{V_{cmax}} = \frac{1}{K_{sp}} \quad (3)$$

Denoting the true photosynthetic rate $F = V_c$ and the photorespiration rate $R_p = 0.5V_o$ we have

$$\frac{F}{R_p} = 2 \times K_{sp} \times \frac{[CO_2]}{[O_2]} \quad (4)$$

Replacing the CO_2 fluxes by the corresponding electron flow rates, denoted here as J, we have

$$J_F = 4 \times F \quad (5)$$

and

$$J_{Rp} = 8 \times R_p, \quad (6)$$

which yields

$$\frac{J_F}{J_{Rp}} = K_{sp} \times \frac{[CO_2]}{[O_2]}. \quad (7)$$

On the other hand,

$$J_F + J_{Rp} = J \quad (8)$$

Equations 7 and 8 form a system for the two unknowns J_F

and J_{Rp} from which

$$J_F = J \times \frac{K_{sp} \times C_c/[O_2]}{1 + K_{sp} \times C_c/[O_2]} \quad (9)$$

and

$$J_{Rp} = J \times \frac{1}{1 + K_{sp} \times C_c/[O_2]} \quad (10)$$

where C_c is substituted for $[CO_2]$ at the carboxylation sites. The corresponding CO_2 fluxes are

$$F = \frac{J}{4} \times \frac{K_{sp} \times C_c/[O_2]}{1 + K_{sp} \times C_c/[O_2]} \quad (11)$$

$$R_p = \frac{J}{8} \times \frac{1}{1 + K_{sp} \times C_c/[O_2]} \quad (12)$$

and the CO_2 exchange rate is

$$P = F - R_p = \frac{J}{4} \times \frac{K_{sp} \times C_c/[O_2] - 0.5}{1 + K_{sp} \times C_c/[O_2]}. \quad (13)$$

On the other hand,

$$C_c = B \times C_a - P \times (B \times r_g + r_{md}) \quad (14)$$

Substituting C_c from Equation 14 into Equation 13 yields a quadratic equation

$$P = \frac{-b + \sqrt{(b^2 - 4ac)}}{2a} \quad (15)$$

where

$$a = -4 \times K_{sp} \times r/[O_2]$$

$$b = 4 \times (1 + K_{sp} \times B \times C_a/[O_2]) + J \times K_{sp} \times r/[O_2]$$

$$c = J \times (0.5 - K_{sp} \times B \times C_a/[O_2])$$

$$r = B \times r_g + r_{md}.$$

In these equations B is the CO_2 solubility in water, C_a is ambient CO_2 concentration, r_g is diffusion resistance in the gas phase, and r_{md} is the CO_2 transport resistance in the liquid phase of mesophyll cells.

Equation 15 makes it possible to calculate the CO_2 exchange rate P from fluorescence data when diffusion resistances are known. The value of r_{md} was varied to find the best fit between the calculated and measured gas-exchange curves, as suggested by Harley et al. (1992). Once P was calculated, C_c was found from Equation 14 and substituted

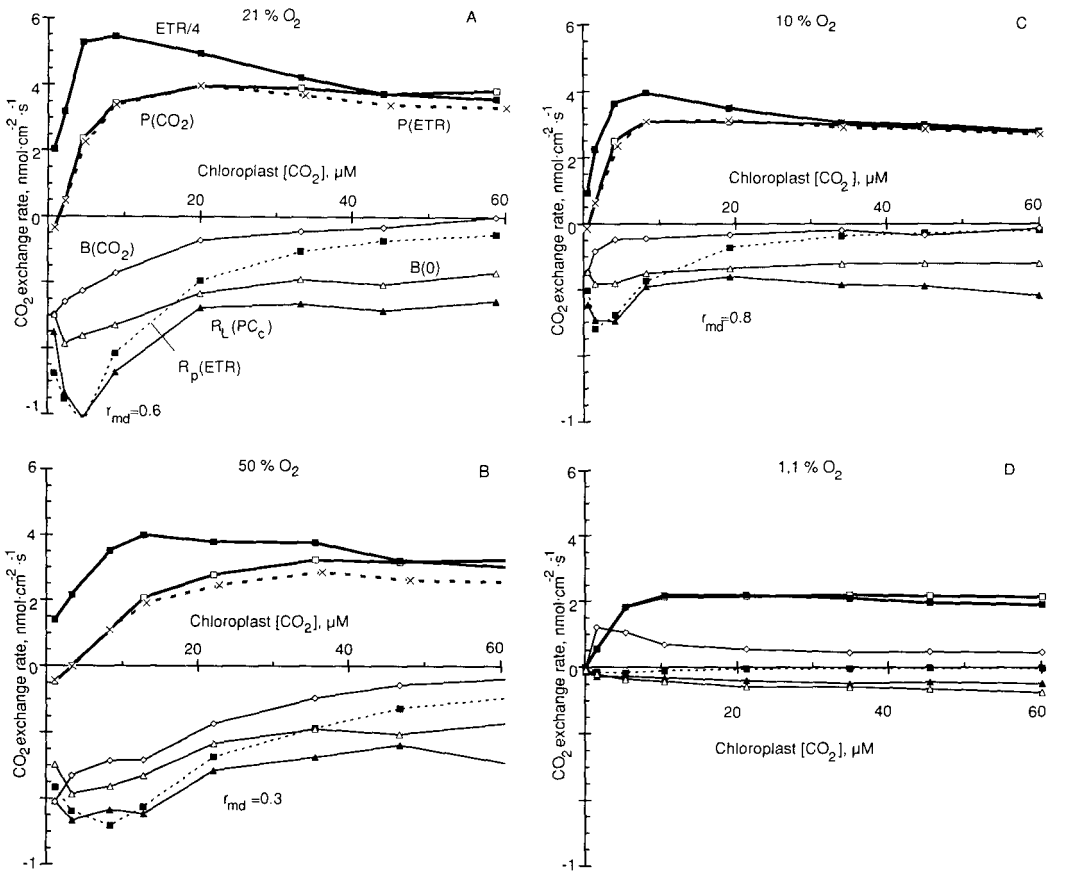

Figure 6. CO_2 dependencies of the measured $P(CO_2)$ (empty squares and thick line), $ETR/4$ (filled squares and thick line), $B(CO_2)$ (empty diamonds), $B(0)$ (empty triangles), and CO_2 evolution in the mesophyll cells in the light, $R_L(PC_c)$ (filled triangles). Dotted lines represent the rates calculated from ETR, the CO_2 exchange rate $P(ETR)$ (crosses), and photorespiration rate $R_p(ETR)$ (filled squares). Notice that the scales are stretched ×6 for all CO_2 burst and respiration curves, but not for P below zero. A, 21% O_2; B, 50% O_2; C, 10% O_2; D, 1.1% O_2.

into Equations 11 and 12 to find the carboxylation and photorespiratory rates separately.

The thick dotted line and crosses in Figure 6 were calculated from Equation 15. Coincidence between the measured and calculated values for CO_2 net exchange is good at low CO_2 concentrations. At high CO_2 concentrations the measured gas-exchange rate tends to be greater than predicted. This shift is greater than experimental error and is present at all O_2 concentrations, pointing to a carboxylation that is not coupled to electron transport. The dotted line and filled squares in Figure 6 were calculated from Equation 12. The value of r_{md} was varied until the best fit between the fluorescence and gas-exchange measurements was obtained at the maximum of photorespiration. The values of the best-fit r_{md} ranged between 0.3 and 0.8 s cm^{-1} for the same leaf at different O_2 concentrations.

DISCUSSION

In this work we measured CO_2 evolution in the light and electron transport rate in a wider range of CO_2 concentrations than previously reported (Peterson, 1989; Cornic and Briantais, 1991; Loreto et al., 1992). The fastest electron transport occurs around normal atmospheric CO_2 and O_2 levels, when both carboxylation and oxygenation of RuBP contribute the most. In the range of CO_2 saturation, *ETR* declines due to the outcompetition of O_2 by CO_2 at the Rubisco sites. The extent of this decline depends on the capacity of the leaf to activate starch synthesis (Sharkey and Vassey, 1989; Eichelmann and Laisk, 1994). Higher resistance to electron transport at saturating CO_2 has also been observed from the measurements of P700 redox state (Weis and Lechtenberg, 1989; Lechtenberg et al., 1990; Laisk et al., 1992). The decline of the net CO_2 assimilation rate with increasing CO_2 has been reported before (Woo and Wong, 1983), but the reasons for this phenomenon are obscure. One possibility is that Pi is being trapped in hexosephosphates and is no longer available for phosphorylation (Sharkey and Vassey, 1989; Eichelmann and Laisk, 1994).

This work shows that the best $^{12}CO_2$ gas-exchange method to estimate photorespiration rate is the extrapolation of the $P(CO_2)$ versus C_c plot to $C_c = 0$, but it gives satisfactory results only at limiting CO_2 concentrations and requires knowledge of r_{md}. The values of the best-fit r_{md} ranged between 0.3 and 0.8 s cm^{-1}, which corresponds to the conductance of 0.52 to 1.04 μmol m^{-2} s^{-1} bar^{-1}. These values of CO_2 transport conductance in the mesophyll of sunflower-leaves extend the range of conductances obtained by Loreto et al. (1992), which ranged from 0.113 to 0.638 μmol m^{-2} s^{-1} bar^{-1} for a number of species. However, such variability of r_{md} in one leaf as obtained in our experiments raises doubts about how adequately the simple resistance network model describes the CO_2 reassimilation in the mesophyll cells. Nevertheless, the result should be considered as a demonstration of the considerable reassimilation of the photorespiratory CO_2 in the mesophyll cells. Reassimilation almost as efficient as that occurring in steady state occurs during the postillumination burst into CO_2-free air, despite the fact that the RuBP level should rapidly decrease during the burst.

The application of the Rubisco kinetics for the calculation of carboxylation and oxygenation rates is based on K_{sp}. The value of K_{sp} obtained in this work is in the range of the K_{sp} values of 77 to 88 determined in vitro (Jordan and Ogren, 1981, 1984) but is lower than about 100, the value frequently obtained with leaves (Laisk, 1977; Brooks and Farquhar, 1985; Peterson, 1989). It may be that the method of determining the Γ^* in the presence of dark respiration (Laisk, 1977; Brooks and Farquhar, 1985) leads to underestimation of Γ^* or that the variability of K_{sp} in vivo is real.

None of the lines from the gas-exchange measurements follow the pattern of the CO_2 dependence of photorespiration, $R_p(ETR)$, calculated from fluorescence and Rubisco kinetics in the range of high CO_2 concentrations. $B(CO_2)$ shows the closest trend but is shifted toward more CO_2 uptake. Both $B(0)$ and R_L from the $P(CO_2)$ versus C_c plot show a continuing CO_2 evolution at high CO_2 concentrations, which cannot be explained by RuBP oxygenation. This CO_2 evolution is not present at low CO_2 concentrations limiting photosynthesis, but appears when photosynthesis becomes CO_2 saturated. It is inhibited neither by high CO_2 nor by low O_2, from which we can conclude that it is not related to the RuBP oxygenase reaction. In parallel with the CO_2 evolution, a CO_2 uptake component is present, which can be seen from the shift of the $B(CO_2)$ curve toward positive values. This CO_2 uptake is not present during photosynthesis at low CO_2 but appears rapidly after light is switched off. It is continuously present during photosynthesis at saturating CO_2. This component of CO_2 uptake explains the difference between *ETR*/4 and $P(CO_2)$ at high CO_2 levels, where *ETR* declines with increasing CO_2 faster than $P(CO_2)$. Similar extra CO_2 uptake, concomitant with enhanced CO_2 evolution, has been observed at the final phase of the postillumination CO_2 uptake, when PGA concentration had reached its maximum level (Laisk, 1985). It is known that under conditions at which PGA levels are high, considerable export of the PGA to the cytosol and its further metabolism occur (Keerberg et al., 1971). We suggest that carboxylation of PEP and the subsequent decarboxylation of malate and pyruvate (Keerberg et al., 1983) are the dominating non-RuBP carboxylation and decarboxylation during the postillumination period and at high CO_2 levels in the light. The corresponding anaplerotic CO_2 uptake should not be classified as photosynthesis, nor should the corresponding CO_2 evolution be classified as photorespiration.

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