Plant Physiol. (1987) 84, 862–867
0032-0889/87/84/0862/06/$01.00/0

Quantitation of the O_2-Dependent, CO_2-Reversible Component of the Postillumination CO_2 Exchange Transient in Tobacco and Maize Leaves

Received for publication January 12, 1987 and in revised form March 25, 1987

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ABSTRACT

The postillumination transient of CO_2 exchange and its relation to photorespiration has been examined in leaf discs from tobacco (*Nicotiana tabacum*) and maize (*Zea mays*). Studies of the transients observed by infrared gas analysis at 1, 21, and 43% O_2 in an open system were extended using the nonsteady state model described previously (Peterson and Ferrandino 1984 Plant Physiol 76: 976–978). Cumulative CO_2 exchange equivalents (*i.e.* nanomoles CO_2) *versus* time were derived from the analyzer responses of individual transients. In tobacco (C_3), subtraction of the time course of cumulative CO_2 exchange under photorespiratory conditions (21 or 43% O_2) from that obtained under nonphotorespiratory conditions (1% O_2) revealed the presence of an O_2-dependent and CO_2-reversible component within the first 60 seconds following darkening. This component was absent in maize (C_4) and at low external O_2:CO_2 ratios (*i.e.* <100) in tobacco. The size of the component in tobacco increased with net photosynthesis as irradiance was increased and was positively associated with inhibition of net photosynthesis by O_2. This relatively simple and rapid method of analysis of the transient is introduced to eliminate some uncertainties associated with estimation of photorespiration based on the maximal rate of postillumination CO_2 evolution. This method also provides a useful and complementary tool for detecting variation in photorespiration.

In spite of lingering controversy regarding the magnitude and detailed biochemical mechanism of photorespiration in C_3 leaves, two key properties of the process are strongly supported by all investigators. These are the O_2-dependent nature of photorespiration and the antagonistic effect of CO_2. Each of the various methods for measurement of photorespiration possesses biases that produce divergent quantitative estimates of this process. Nevertheless, all indicate that a higher gas phase concentration ratio of O_2:CO_2 results in a greater proportion of recently fixed carbon being partitioned to the photorespiratory pathway (1, 17). Such observations with intact leaf tissue have been strengthened by the discovery of the bifunctional nature of RuBP[1] carboxylase-oxygenase (8). *In vitro* studies clearly indicate that the enzyme will either carboxylate a RuBP to form 2 PGAs or oxygenate a RuBP to form P-glycolate plus PGA. The relative velocities of the two reactions depend on the relative availabilities of CO_2 and O_2 and upon temperature (1, 8). The accurate *in vivo* estimation of photorespiration is difficult because neither net depletion nor accumulation of any metabolite occurs, therefore no straightforward basis is provided for assay. Also, leaf stomatal and mesophyll diffusive resistances to CO_2 and O_2 exchange complicate interpretation of photorespiration assays using $^{14}CO_2$ or $^{18}O_2$ (17).

One method frequently employed to estimate photorespiration is the postillumination burst of CO_2 originally described by Decker (3). The phenomenon is commonly interpreted in terms of metabolism of a residual pool of light-generated photorespiratory substrate in a suddenly darkened leaf, and since CO_2-fixing capacity rapidly disappears upon darkening much of the photorespiratory CO_2 may diffuse out of the leaf and be detected (3, 7, 16, 17). The method has been criticized, however, since (a) the CO_2 evolved is being driven by a continuously depleting substrate pool, (b) it contains an unknown contribution from dark respiration, and (c) some CO_2 fixation capacity must persist after darkening so that an unknown portion of the photorespiratory CO_2 is refixed. The purpose of this study was to introduce a method of analysis of the CO_2 exchange transient that would circumvent these limitations.

In previous publications (4–6, 11, 12) a numerical simulator was used to estimate the time course of the leaf CO_2 exchange rate during the postillumination transient. Open flow-through systems employing an IRGA typically exhibit a nonsteady state response to the rapid changes in CO_2 exchange rate associated with the transient. This study uses the model to compare transients produced under nonphotorespiratory (1% O_2) and photorespiratory (21 and 43% O_2) conditions when all other conditions are constant. An initial O_2-dependent and totally CO_2-reversible component may be isolated and its size (expressed as CO_2 exchange equivalents) determined. The presence of this component is dependent upon photosynthesis and its magnitude is highly correlated with the inhibition of net photosynthesis by O_2. Estimation of this O_2-dependent component constitutes a new alternative to other means of analysis of the postillumination transient in the study of photorespiration.

MATERIALS AND METHODS

Plant Material. *Nicotiana tabacum* var. Havana Seed was grown in a greenhouse in pots containing a soil:perlite (3:1) medium. Leaf samples of *Zea mays* var. Sweet Sue were collected from field-grown plants just prior to the tasseling stage.

Sample Preparation. Excised leaves were washed and leaf discs prepared as described previously (11). A sample consisted of ten discs (20.1 cm^2, 400 mg fresh weight, 0.6 mg Chl).

[1] Abbreviations: RuBP, ribulose-1,5-bisphosphate; PGA, 3-phosphoglycerate; IRGA, infrared gas analyzer; NPS, net photosynthetic rate; F, flow rate; R_i, mean CO_2 exchange rate over time interval i; C_1, incoming [CO_2]; AP, assimilatory power; P, cumulative CO_2 exchange.

CO_2 Exchange Measurements. The IRGA open flow-through system described previously (11) was used with the following modifications.

Dry, CO_2-free flushing gas (1, 21, or 43% O_2 [balance N_2]) was blended with either 1% or 5% CO_2 (balance air) to provide the desired final [CO_2]. The gas stream was divided with a portion undergoing humidification by passage through a gas washing bottle containing distilled H_2O. A flowmeter and needle valve in the nonhumidified branch of the gas stream provided control of the RH of the flushing gas when the two streams subsequently reconverged. The dew point of the flushing gas was preset at 11°C as monitored by a Dew Point Hygrometer (EG and G Co. model 880-C1, Waltham, MA) situated downstream from the Beckman 865 IRGA. The [CO_2] in the flushing gas, C_1 (μl L^{-1}), was measured by injection of a sample into a second IRGA (13). The differential IRGA response (Δ[μl CO_2 L^{-1}]mV^{-1}) was calibrated by controlled injection of N_2 into the gas stream to generate a known Δ[CO_2] between the reference and sample cells. A system of valves permitted convenient bypassing of the assimilation chamber by the gas stream to permit zeroing of the IRGA.

In order to maximize the resolution of the IRGA to CO_2 exchange transients no desiccants or traps were used to remove excess water vapor arising from transpiration by the leaf discs or evaporation of the surrounding water. Thus a water vapor concentration differential (Δ[H_2O]) existed in the flushing gas leaving the chamber (chamber gas) relative to that entering (reference gas). The Δ[H_2O] results potentially in overestimation of the CO_2 assimilation rate due to dilution of CO_2 (15). The Δ[H_2O] was calculated (as $\Delta\mu$l H_2O L^{-1}), assuming ideal gas behavior, from dew point measurements of the reference and chamber gases and tabular data on dew point-relative humidity and weight per liter of saturated aqueous vapor. A correction, C_D (μl L^{-1}) = $C_1 \times \Delta[H_2O] \times 10^{-6}$, was calculated and added to the observed CO_2 concentration of the chamber gas (C_{obs}) to compensate for the effect of dilution by H_2O vapor.

Experiments with water only in the chamber revealed that $C_1 - C_{obs} \neq C_D$. This was judged to be caused by IR absorption by H_2O due to overlapping absorption by H_2O and CO_2 at wavelengths to which the IRGA is sensitive. In the absence of net CO_2 exchange a second algebraic correction, C_A (μl CO_2 L^{-1}), for IR absorption by H_2O was determined such that $C_A = C_1 - C_{obs} - C_D$. The IRGA response [$\Delta$($\mu$l H_2O L^{-1})mV^{-1}] to IR absorption by a water vapor concentration differential was nearly constant over a range of C_1 of zero to 2400 μl CO_2 L^{-1} when the reference gas dew point was 11° C. Since the IRGA response to Δ[CO_2] expressed as Δ(μl CO_2 L^{-1})mV^{-1} increases with C_1 so too does C_A. In the presence of net CO_2 exchange C_A is given by [Δ(μl CO_2 L^{-1})mV^{-1}] $\times$ Δ(μl H_2O L^{-1})/[Δ(μl H_2O L^{-1})mV^{-1}]. The IR absorption error opposes the dilution error so that the corrected chamber gas [CO_2] is $C_{corr} = C_{obs} + C_D - C_A$. Considering IR absorption alone the ratio of the individual concentration differentials of H_2O and CO_2 associated with equal IRGA response (*i.e.* ΔH_2O:ΔCO_2) ranged from 7300 at C_1 = 0 to 440 at C_1 = 2430 μl L^{-1}.

The IRGA output (5 V full scale) was recorded by a strip chart recorder and a Kaypro II microcomputer. The latter employed a model Q3024 analog-to-digital converter (Quansitronics Corp., Houston, PA). The system was capable of reading and storing a digitized IRGA response (mV) once every 0.146 s. A typical postillumination transient was initiated by recording the IRGA response for 5 s to provide an estimate of the steady state CO_2 assimilation rate in the light. Immediately following, the light source was extinguished on software command and the IRGA response recorded for 2 to 4 min. Transients were recorded in duplicate, each with at least 5 min of prior illumination under steady state conditions of photosynthesis.

All calculations and statistical analyses were performed with a Kaypro II or 2X microcomputer.

RESULTS

Test of the Nonsteady-State IRGA Response Model. The measuring system consists of a small leaf chamber connected by narrow inlet tubing to the IRGA measuring cell. The cell is represented as a series of eight interconnected compartments each of equal volume (11). The model simulates the IRGA response to leaf CO_2 exchange $R(t)$ over successive time intervals of Δt = 0.65 s (12). In the current study a sequential and unbiased approach for estimating $R(t)$ utilized the observed IRGA response and Equation 7 of Peterson (11). The mean CO_2 exchange rate R_i (μl s^{-1}) for a 0.65 s interval i was calculated using the integrated and rearranged Equations 7 and 10 of Peterson and Ferrandino (12). Cumulative CO_2 exchange (*i.e.* nmol CO_2) over n successive 0.65 s time intervals is

$$\sum_{i=1}^{n} 0.65\ R_i. \tag{1}$$

All time courses of cumulative CO_2 exchange described here have been corrected for the effect of the 1.42 s delay associated with the tubing connecting the leaf chamber and IRGA measuring cell.

An experiment was devised to test how well the model would predict known fluctuations in CO_2 exchange. A syringe pump injected CO_2 at a controlled rate into the empty leaf chamber on command. Figure 1 shows the IRGA response to three sequential pulses of CO_2 of 180, 60, and 30 s duration separated by 120 s intervals during which the pump was off. During the first and second pulses the system attained steady state as noted by the equilibrium response of the IRGA. The precise rate of CO_2 injection could be calculated using the steady state equation ($R = F\Delta[CO_2]$) and was found to be 18.3 μl CO_2 min^{-1}. When the observed IRGA response was analyzed using the IRGA response model, a linear increase in cumulative CO_2 exchange consistent with a constant injection rate was obtained for those intervals when the pump was activated (Fig. 2). The predicted injection of CO_2 halted abruptly when the pump was turned off even though the IRGA response (Fig. 1) required about 60 s to return fully to zero. Note also that the calculated total amount of CO_2 introduced during each pulse is proportional to the total time the pump was on and agrees satisfactorily with the amount

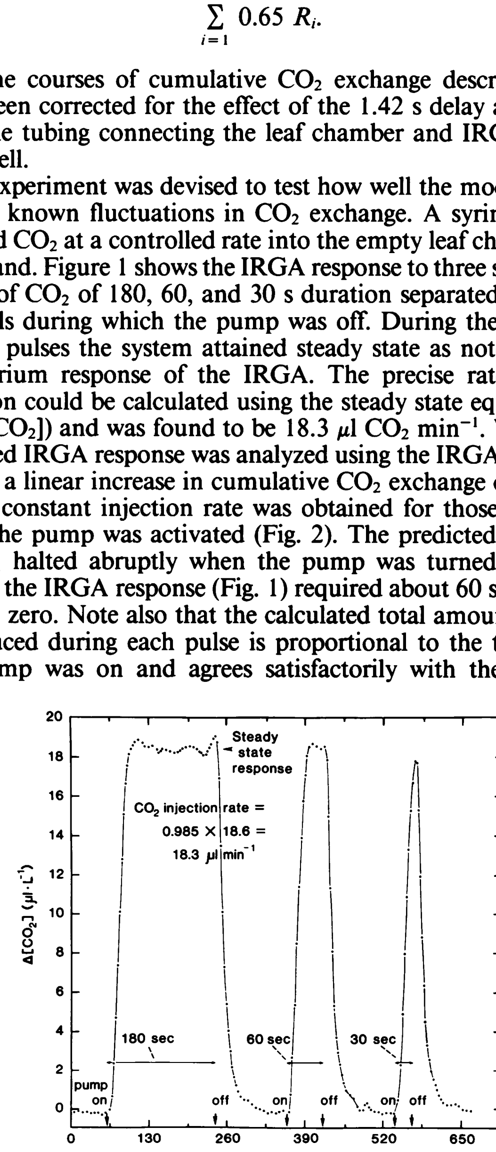

FIG. 1. IRGA response to repeated injections by a syringe pump. The open system described in "Materials and Methods" was equilibrated with air containing 407 μl CO_2 L^{-1} at a flow rate of 0.985 L min^{-1}. Arrows indicate when the pump was turned on and off. See text for additional details.

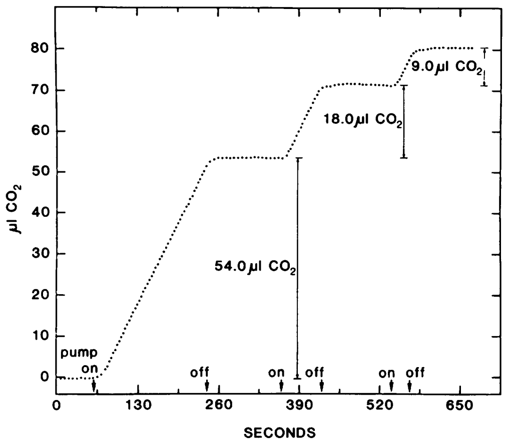

FIG. 2. Time course of cumulative CO_2 injection calculated from the response in Figure 1 using the IRGA response model.

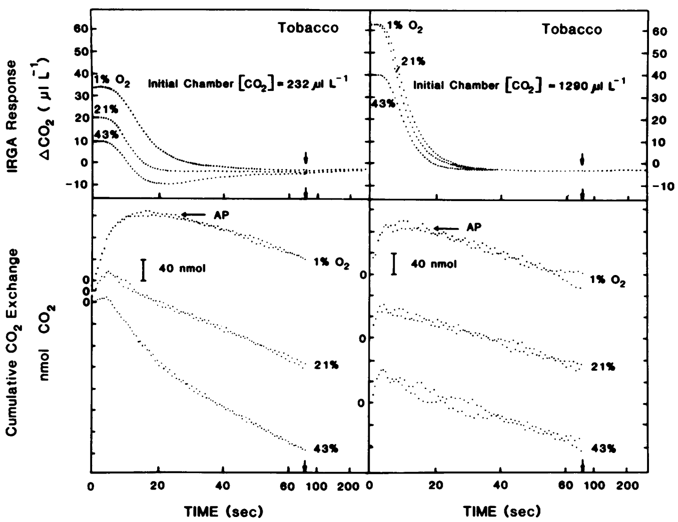

FIG. 3. Top panels: representative IRGA responses to postillumination transients recorded at 32°C and three levels of ambient O_2 and two levels of CO_2 with a single sample of tobacco leaf discs (20.1 cm^2). The flow rate was 1.0 L min^{-1} and the irradiance during preillumination was 484 μE m$^{-2}\cdot$s^{-1}. At (↓) the time scale has been divided by ten. Bottom panels: associated time courses of cumulative CO_2 exchange calculated from the first 65 s of the IRGA responses in the top panels according to Equation 1. Note that the curves have been displaced for clarity. Shown also (←) are minimal estimates of assimilatory power (AP) based on total net CO_2 uptake in the dark at 1% O_2 (see Laisk and Kiirats [9] and text). Values for AP were 124 and 88.5 nmol at 232 and 1290 μl CO_2 L^{-1}, respectively.

expected from the known rate of CO_2 injection.

Time Course of Cumulative CO_2 Exchange during the Postillumination Transient. Although the IRGA response model adequately predicts cumulative CO_2 exchange versus time (Equation 1), this is not true for the calculated time course of R_i itself. Due to even slight noise in the IRGA response the predicted time course of R_i quickly becomes extremely erratic and certainly does not reflect the true course of CO_2 exchange in the assimilation chamber. These seemingly contradictory observations are reconciled by the fact that since the noise contribution to the predicted time course of R_i is random in both magnitude and sign its cumulative effect becomes acceptably small after a few iterations of the model in comparison to the total true net CO_2 exchange (*i.e.* Fig. 2).

Figure 3 (lower panels) shows time courses of cumulative CO_2 exchange obtained with tobacco at varying levels of O_2 and CO_2. Some residual noise was evident in these curves due to the aforementioned oscillations in R_i, but the progress of cumulative CO_2 exchange may be readily discerned. The comparatively greater noise at 1290 μl CO_2 L^{-1} results from the reduced sensitivity of the IRGA at high [CO_2]. The cumulative CO_2 exchange always rose to a (+) peak 5 to 20 s following darkening and was associated with completion of net uptake of CO_2 by the leaf sample. Subsequently, net CO_2 release (−) occurred causing cumulative CO_2 exchange to decline. At low [CO_2] (232 μl L^{-1}) increasing [O_2] dramatically reduced the net quantity of CO_2 fixed in the dark and increased the rate of subsequent decline in cumulative CO_2 exchange. After about 1 min of darkness the rates of CO_2 evolution tended to converge across the various O_2 levels yet a residual O_2-dependence was still evident after 4 min of darkness. At high [CO_2] (1290 μl L^{-1}) the time course of cumulative CO_2 exchange was qualitatively similar to that observed at low [CO_2] yet the effect of [O_2] was not nearly as pronounced. Thus cumulative CO_2 exchange during the first minute of the postillumination transient shows a CO_2-reversible dependence upon O_2 consistent with the properties of photorespiration.

Perspective on Effect of O_2 on Postillumination Transient. The widely accepted explanation for the effect of O_2 on the transient (neglecting dark respiration for a moment) involves production of CO_2 during metabolism of residual photorespiratory substrate(s) immediately following darkening. Under such circumstances refixation of photorespiratory CO_2 declines and thus the CO_2 released may escape the leaf and be detected. This simple concept neglects, however, the finite pool of assimilatory power (9) composed primarily of RuBP which continues to consume CO_2 and O_2 in the dark until the AP is exhausted. For instance, the observation of a pronounced 'burst' of CO_2 release following darkening indicates that under such conditions the total quantity of photorespiratory CO_2 produced exceeds the amount of AP available for its refixation. When the quantity of photorespiratory CO_2 produced is similar to or less than the amount of AP available for refixation, a distinct 'burst' of CO_2 may not be observed and photorespiration would be seriously underestimated. Nevertheless, even a low rate of photorespiration will affect the time course of the transient insofar as it diminishes net uptake of atmospheric CO_2 by competing for the available RuBP through the processes of oxygenation and refixation of CO_2. Thus, at the moment of darkening, a leaf sample possesses (a) a pool of potentially 'CO_2-fixing' equivalents (*i.e.* AP) which overlaps partially with (b) a pool of 'photorespiratory' equivalents representing CO_2 to be released from the photorespiratory substrate(s) plus that proportion of the total RuBP pool which will be oxygenated by RuBP carboxylase-oxygenase. The observed total net quantity (nmol) of CO_2 exchanged (or cumulative CO_2 exchange, see above) at the end of the postillumination transient is the difference between the magnitudes of these two quantities. This concept assumes nothing about the time course of CO_2 exchange and does not distinguish photorespiratory CO_2 which escapes the leaf from photorespiratory CO_2 which is refixed by RuBP or from RuBP equivalents which are oxygenated.

The occurrence during the transient of mitochondrial dark respiration which is supplied by a distinct and essentially infinite substrate pool represents a third, and time-dependent, component of cumulative postillumination CO_2 exchange but does not alter significantly the overall concept described. The CO_2 released by dark respiration should merge with the photorespiratory CO_2 flux and similarly undergo refixation or escape from the leaf. If independent estimates of the contributions of dark respiration and AP to cumulative CO_2 exchange were available, direct measurement of the photorespiratory equivalent pool would be possible.

Estimation of the Photorespiratory Equivalent Pool. Two useful conjectures are advanced to construct a practical basis for performing the stated objective. These are, when all other conditions are constant, (a) dark respiration is unaffected by increasing the $[O_2]$ above 1%, and (b) any effect of increasing the $[O_2]$ above 1% on the steady state AP pool is mediated by RuBP carboxylase-oxygenase. Thus, the difference in cumulative CO_2 exchange between transients recorded at 1% O_2 (no photorespiration) and high $[O_2]$ would be associated with decarboxylation of photorespiratory substrate(s), oxygenation of RuBP, and any CO_2-reversible effect of O_2 on AP. The significance and validity of the above assumptions will be discussed later within the context of effects of varying environmental conditions and CO_2 fixation pathway (C_3 *versus* C_4) on estimates of O_2-dependent cumulative CO_2 exchange.

The CO_2 exchange transients were recorded at 1% O_2 and at 21 and 43% O_2 under otherwise identical conditions of irradiance, temperature, and ambient $[CO_2]$ with the same or replicate samples of leaf discs. The IRGA response model and Equation 1 were used to calculate the difference (nmol) in cumulative CO_2 exchange for the two transients using $P = \Sigma[R_i(1\%\ O_2) - R_i(k\%\ O_2)]\cdot\Delta t$ where $\Sigma R_i(1\%\ O_2)\cdot\Delta t$ and $\Sigma R_i(k\%\ O_2)\cdot\Delta t$ are estimates of cumulative CO_2 exchange at 1% O_2 and a specified higher O_2 concentration k, respectively, and $\Delta t = 0.65$ s.

Representative time courses of P at 21% O_2 and 43% O_2 for tobacco at four levels of $[CO_2]$ and for maize at these O_2 concentrations and 340 $\mu l\ CO_2\ L^{-1}$ are shown in Figure 4. The results with tobacco indicate the time course of P has an initial rapidly increasing O_2-dependent component which was usually complete within 30 s followed by a linear slow phase. The initial rapid phase became less evident as the $[CO_2]$ was increased with tobacco and was greatly suppressed in maize. The quantity of CO_2 contained in the rapid phase was estimated by linear extrapolation to time zero (P_f). The slope of the slow phase sometimes increased with $[O_2]$ in tobacco (Fig. 4). Also, there was occasionally a lag between completion of the rapid phase and onset of the slow phase (Fig. 4C). The characteristics of the lag were not associated with any particular set of environmental conditions but varied among leaf samples. A similar prominent lag was noted for maize at 21% O_2 (Fig. 4E). The initial rapid phase was consistently observed among samples of tobacco and its relative magnitude appeared to diminish with increasing $[CO_2]$. Thus particular attention was focused on P_f.

The results in Table I compare photosynthetic properties of tobacco at 21% and 43% O_2 over a wide range of $[CO_2]$. At both levels of O_2, NPS increased up to 1273 $\mu l\ CO_2\ L^{-1}$ but decreased somewhat at 2252 $\mu l\ L^{-1}$. Inhibition of NPS by O_2 profoundly decreased over this range of $[CO_2]$. The estimated P_f decreased markedly with increasing $[CO_2]$. Other results presented here (see next section) suggested that NPS exerts an independent effect on P_f. Thus, values of P_f were normalized to their corresponding NPS values before examining further the effects of O_2 and CO_2. When results were combined from several experiments and values of P_f/NPS were plotted against their associated O_2:CO_2 ratios, the points fell along an exponential function (Fig. 5, top). The line of best fit extrapolated to very near the origin indicating that any detectable effect of O_2 on the early phase of the transient is eliminated at infinite $[CO_2]$. Shown for comparison are the associated values of O_2 inhibition normalized to the NPS rate as described in the legend to Figure 5 (bottom).

Effect of Irradiance on P_f. Table II shows results obtained when irradiance was varied from just above light compensation (89 $\mu E\ m^{-2}\cdot s^{-1}$) to saturation (638 $\mu E\ m^{-2}\cdot s^{-1}$) and the $[CO_2]$

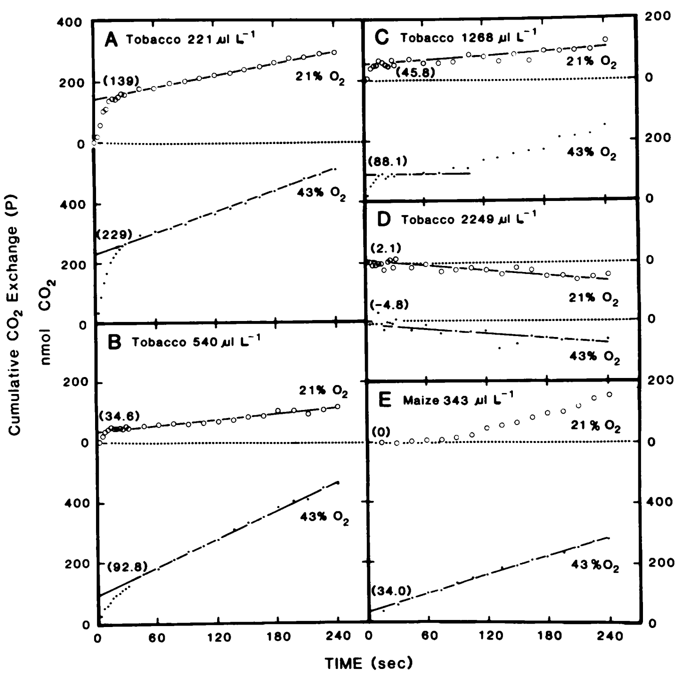

FIG. 4. Typical time courses obtained for P (see text) for two samples consisting of 10 leaf discs (20.1 cm^2) from tobacco (A–D) and for a single sample from maize (E). The results with tobacco were acquired from two leaf samples over two consecutive days; the data in panels (A) and (C) were from the first day and the data in panels (B) and (D) from the second day. The ambient CO_2 concentrations ($\mu l\ L^{-1}$) were (A) 221; (B) 540; (C) 1268; (D) 2249; and (E) 343. The irradiance was 484 $\mu E\ m^{-2}\cdot s^{-1}$ in the experiments with tobacco and 692 $\mu E\ m^{-2}\cdot s^{-1}$ for maize. The temperature was 32°C. The solid lines are linear regression fits of the slow phase and are employed to estimate the total CO_2 exchanged during the rapid phases (*i.e.* P_f= Y-intercept values [nmol] in parentheses).

Table I. *Interactive Effects of O_2 and CO_2 on Net Photosynthesis, O_2 Inhibition, and P_f in Leaf Discs from Tobacco and Maize*

The irradiance was 484 $\mu E\ m^{-2}\cdot s^{-1}$ in the experiments with tobacco and 692 $\mu E\ m^{-2}\cdot s^{-1}$ for maize. The temperature was 32°C. The O_2 inhibition was calculated as (NPS $[1\%\ O_2]$ − NPS $[k\%\ O_2]$)/NPS($1\%\ O_2$) where k is 21 or 43. Each value is a mean obtained from three leaf samples and includes the results of Figure 4 (±SD). Values of NPS (1% O_2) were 4.54 ± 0.65, 7.56 ± 1.14, 8.39 ± 1.36, and 5.86 ± 0.70 $\mu mol\ CO_2\ cm^{-2}\cdot h^{-1}$ for tobacco at 226, 544, 1273, and 2252 $\mu l\ CO_2\ L^{-1}$, respectively. The NPS (1% O_2) for maize was 8.34 ± 1.96 $\mu mol\ CO_2\ cm^{-2}\cdot h^{-1}$. Details of experimental procedures are in "Materials and Methods." See text for method of estimation of P_f.

Species	CO_2 Concentration		O_2 Concentration 21%	O_2 Concentration 43%
	µl L⁻¹			
Tobacco	226	NPS[a]	2.74 ± 0.42	1.30 ± 0.20
		O_2 inhib.[b]	39.9 ± 1.0	71.4 ± 11.5
		P_f[c]	8.13 ± 1.10	14.68 ± 2.95
	544	NPS	5.51 ± 0.97	3.39 ± 1.29
		O_2 inhib.	27.4 ± 2.3	56.0 ± 11.5
		P_f	2.26 ± 0.51	5.25 ± 0.61
	1273	NPS	7.86 ± 0.96	6.29 ± 1.60
		O_2 inhib.	6.0 ± 3.9	25.9 ± 8.7
		P_f	1.53 ± 0.86	3.65 ± 0.79
	2252	NPS	6.55 ± 0.64	5.65 ± 0.28
		O_2 inhib.	−12.1 ± 7.7	3.1 ± 6.4
		P_f	0.67 ± 1.04	1.10 ± 1.24
Maize	343	NPS	8.31 ± 1.78	7.9 ± 1.77
		O_2 inhib.	−0.2 ± 4.8	4.6 ± 2.2
		P_f	0.14 ± 0.14	0.21 ± 0.69

[a] $\mu mol\ CO_2\ cm^{-2}\ h^{-1}$. [b] %. [c] $nmol\ cm^{-2}$.

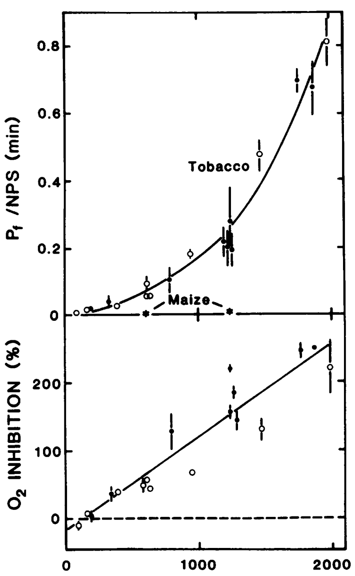

FIG. 5. (Top) Relationship of P_f/NPS with ambient O_2/CO_2 for tobacco and maize. The points shown represent means of triplicate determinations (±SD) and were collected from experiments described herein (Tables I and II). Results from several additional experiments not included in the Tables are presented also (100–340 μl CO_2 L^{-1}). All experiments with tobacco were performed at 490 to 640 μE $m^{-2}\cdot s^{-1}$ and 30° to 32°C. The experiments with tobacco performed at 21% O_2 (○) are distinguished from those performed at 43% (●). Shown also are results observed with leaf discs from maize (irradiance = 692 μE $m^{-2}\cdot s^{-1}$, 32°C) under 21% and 43% O_2 and 343 μl CO_2 L^{-1} (∗). The solid line is an exponential function relating the two variables for tobacco according to $y = me^{ax} + b$, where $b = -6.53 \times 10^{-2}$, $m = 5.65 \times 10^{-2}$, $a = 1.40 \times 10^{-3}$, and the correlation coefficient = 0.985 ($P < 0.001$). (Bottom) Associated O_2 inhibition values for tobacco calculated as (NPS [1% O_2] − NPS [k% O_2])/NPS(k% O_2), where k is 21 or 43, for comparison with the data in the upper half of the figure. Solid line is the linear regression fit to the data ($y = mx + b$) where $m = 0.138$, $b = -20.15$ and the correlation coefficient = 0.939 ($P < 0.001$).

Table II. *Effects of Irradiance on Net Photosynthesis, O_2 Inhibition, and P_f in Leaf Discs of Tobacco*

The external [CO_2] was 340 μl L^{-1} and the temperature was 32°C. Values of NPS (1% O_2) were 6.67 ± 0.21, 5.61 ± 0.22, 3.42 ± 0.07, and 1.86 ± 0.06 μmol CO_2 $cm^{-2}\cdot h^{-1}$ at 638, 307, 151, and 89 μE $m^{-2}\cdot s^{-1}$, respectively. Each value is a mean obtained from three leaf samples (± SD). The O_2 inhibition values are calculated as described in Table I.

Irradiance		O_2 Concentration 21%	O_2 Concentration 43%
638	NPS[a]	4.48 ± 0.56	2.61 ± 0.34
	O_2 inhib.[b]	32.9 ± 6.7	60.9 ± 4.1
	P_f[c]	3.86 ± 0.75	8.34 ± 2.11
307	NPS	3.56 ± 0.25	1.98 ± 0.25
	O_2 inhib.	36.6 ± 2.1	64.8 ± 3.1
	P_f	3.14 ± 0.26	6.35 ± 1.03
151	NPS	2.06 ± 0.10	1.07 ± 0.06
	O_2 inhib.	39.7 ± 1.8	68.8 ± 1.5
	P_f	1.92 ± 0.58	3.78 ± 0.59
89	NPS	1.08 ± 0.01	0.49 ± 0.05
	O_2 inhib.	41.8 ± 1.5	73.8 ± 1.7
	P_f	1.63 ± 0.39	3.23 ± 0.33

[a] μmol CO_2 cm^{-2} h^{-1}. [b] %. [c] nmol cm^{-2}.

was maintained at a constant 340 μl L^{-1}. The percent inhibition of NPS by either 21 or 43% O_2 increased somewhat as the irradiance decreased. Of special interest, the values of P_f increased with irradiance at constant external O_2:CO_2 suggesting that this quantity is associated with one or more products of photosynthesis.

DISCUSSION

The reproducible O_2-dependent, CO_2-reversible component of cumulative CO_2 exchange (P_f) observed during the early phase of the postillumination transient is assumed to be generated from a combination of (a) decarboxylation of residual photorespiratory substrate(s), (b) oxygenation of a portion of the remaining RuBP, and (c) any effect of photorespiration on the size of the AP pool during steady state photosynthesis. Together these three elements comprise the 'photorespiratory equivalent pool.'

Since the AP pool figures prominently in the estimation of the photorespiratory equivalent pool consideration will be given first to its characteristics and response to [O_2]. Laisk *et al.* (9) concluded that this capacity for CO_2 uptake in the dark was largely composed of RuBP based on similar absolute magnitudes and dependencies on [O_2] and [CO_2]. In the present study, minimal estimates of the AP pool in tobacco leaf discs could be obtained at 1% O_2 from the (+) maximum in postillumination cumulative CO_2 exchange (Fig. 3). At 32°C and 484 μE $m^{-2}\cdot s^{-1}$, mean values (±SD) of 6.5 ± 0.2, 4.0 ± 0.5, 4.7 ± 0.6, and 2.6 ± 1.2 nmol CO_2 cm^{-2} were observed at 220, 536, 1264, and 2249 μl CO_2 L^{-1}, respectively ($N = 3$). My values are comparable to the RuBP concentration of C_3 leaves as published elsewhere especially when expressed on a Chl basis (2, 9, 10) and also agree with these earlier results since increasing [CO_2] lowers the RuBP level. My results are consistent with the assumption that the capacity for postillumination CO_2 uptake and the RuBP pool are equivalent. Direct estimation of assimilatory power at elevated [O_2] was not possible with the apparatus described here.

Badger *et al.* (2) reported that 21% O_2 lowers the level of RuBP in bean and sunflower leaves by 50% relative to 2% O_2 (see also Ref. 9). Perchorowicz and Jensen (10) found no effect of O_2 on the RuBP level at 350 and 1400 μl CO_2 L^{-1} in wheat seedlings. At 100 μl CO_2 L^{-1}, however, 21% O_2 lowered the RuBP level by about 60% relative to 1.1% O_2. Lack of more extensive and systematic information regarding the mediating effects of [CO_2], irradiance, species, and sample history make generalization difficult regarding the effect of O_2 on the RuBP concentration. This subject is relevant to the present study because if elevated [O_2] lowered the RuBP content, this would likely result in a commensurate increase in the measured P_f. A probable cause for a lower RuBP level at high [O_2] is the depleting effect of the RuBP oxygenase activity on the RuBP pool (2). As all three elements that make up the P_f depend on the interaction of RuBP carboxylase-oxygenase with O_2, all should be reversible with CO_2. Figure 5 shows that the effect of O_2 on P_f (and, thus, including effects of O_2 on the RuBP level) disappears as CO_2 concentrations reach levels high enough to suppress RuBP oxygenase activity (8). The results with maize (Fig. 5, Table I) reinforce this interpretation overall since little or no P_f was detectable in this C_4 species which does not photorespire significantly (16).

The origin of the O_2-dependent CO_2 release during the 'slow' phase of the time course of P (Fig. 4) is less certain. This process may simply represent an O_2-dependent dark respiration above 1% O_2. Alternatively, it may be associated with utilization of photorespiratory intermediates, such as glycerate, by mitochondrial dark respiration. The linear extrapolation to time zero (Fig. 4) should remove, however, any contribution of this phase of the transient to P_f.

A possible source of error in estimation of P_f concerns the solubility of CO_2 in water ($\alpha_{CO_2} = 0.66$ at 30°C; see Umbreit

[14]). During measurement of the transient the chamber [CO_2] rises as NPS falls to zero and CO_2 evolution proceeds. Thus, some CO_2 will dissolve in the unbuffered H_2O present in the chamber as the aqueous phase equilibrates with the gas phase, thereby falsely resembling CO_2 uptake by the leaf tissue. A representative example employing conditions of, and NPS values interpolated from, Table I shows that this error has a minor effect on the estimate of P_f. With the chamber [CO_2] initially at 340 μl L^{-1} at high irradiance and $F = 1.0$ L min^{-1} the final [CO_2] rises to approximately 400 μl CO_2 L^{-1} at 1% O_2 and approximately 380 μl L^{-1} at 21% O_2. Thus, about 0.40 and 0.27 μl CO_2, respectively, will dissolve in the 10 ml aqueous phase subsequent to darkening of the chamber. The difference, 0.13 μl (0.25 nmol CO_2 cm^{-2} leaf area), could add to P_f to the extent of about 4%. Since this is a maximal estimate and assumes instantaneous equilibration of gaseous and aqueous phases, the influence of this effect on estimates of P_f may be safely neglected.

My previous studies of the postillumination transient have been concerned with estimation of the peak rate of net postillumination CO_2 evolution occurring 6 to 10 s after darkening as a measure of photorespiration (4–6, 11, 12). The method used a reiterative computerized fitting routine to maximize agreement between predicted and observed IRGA responses. The current analysis, while not capable of yielding an estimate of the absolute rate of photorespiration, provides complementary information and requires much less time for processing the experimental data. Since no arbitrarily chosen rate function is imposed upon $R(t)$, the opportunity for introduction of bias is reduced. Earlier reports (4, 5, 12) have also described a strong dependence of the peak rate of postillumination CO_2 evolution on the O_2:CO_2 ratio so that the latter and P_f are directly associated and reflect different aspects of the same process.

The size of the photorespiratory equivalent pool (P_f) is positively associated with inhibition of NPS by O_2 (Table I, Fig. 5). Interestingly, 21% O_2 was generally somewhat less inhibitory to NPS than was 43% when the external O_2:CO_2 ratio was constant (Fig. 5, bottom). This could reflect differences between the external O_2:CO_2 ratio and that which occurred at the site of CO_2 fixation in the chloroplast. The apparatus employed in these studies did not allow measurement of leaf internal [CO_2]. Nevertheless, the response of P_f/NPS to the O_2:CO_2 ratio was independent of the external [O_2] (Fig. 5, top). Studies of the kinetics of RuBP carboxylase-oxygenase *in vitro* indicate that the partitioning of carbon to P-glycolate is controlled by the O_2:CO_2 ratio at the enzyme active site (8). While not providing a comprehensive test of the validity of this enzyme mechanism in accounting for the regulation of photorespiration *in vivo*, the results of Figure 5 (top) are consistent with the aforementioned key property of RuBP carboxylase-oxygenase assuming that the chloroplast O_2:CO_2 ratio is proportional to the atmospheric ratio. Thus, the nonreciprocal effects of [O_2] and [CO_2] on O_2 inhibition of NPS may be associated with other direct or indirect effects of O_2 on photosynthesis besides promotion of photorespiration.

Release of CO_2 from photorespiratory intermediates, oxygenation of RuBP in the dark, and RuBP oxygenase-mediated regulation of the RuBP pool size in the light would all have additive effects on the measured value of P_f. Varieties which partition less carbon to the glycolate pathway because of reduced RuBP oxygenase activity and/or convert a smaller proportion of glycolate carbon to CO_2 (4–6) should show a lower P_f/NPS. The strong effect of the O_2:CO_2 ratio on this quantity (Fig. 5) tends to support this contention. One strategy in efforts to improve crop yields by manipulation of photosynthesis is to identify genetic differences in photorespiratory capacity among existing C_3 varieties or in mutants derived from plant cell culture (18). Estimation of the photorespiratory equivalent pool could prove useful in this search.

Acknowledgments—The author wishes to thank F. J. Ferrandino, K. R. Hanson, and I. Zelitch for helpful discussions and E. Hennessey for technical assistance.

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