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# Estimation of Photorespiration Based on the Initial Rate of Postillumination $CO_2$ Release

## II. EFFECTS OF $O_2$, $CO_2$, AND TEMPERATURE

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### ABSTRACT

**An open system associated with an infrared gas analyzer was employed to study transients in $CO_2$ exchange generated upon darkening preilluminated leaf discs of tobacco (*Nicotiana tabacum* vars John Williams Broadleaf and Havana Seed). An empirical formula presented previously enabled prediction of the analyzer response under nonsteady state conditions as a function of time and of the leaf $CO_2$ exchange rate. A computer was used to evaluate parameters of the leaf $CO_2$ release rate to provide an estimate of the initial rate of postillumination $CO_2$ evolution and to produce maximal agreement between predicted and observed analyzer responses. In 21% $O_2$, the decline in rate of $CO_2$ evolution upon darkening followed first order kinetics. Initial rates of $CO_2$ evolution following darkening were relatively independent of the prior ambient $CO_2$ concentrations. However, rates of photorespiration expressed as a fraction of net photosynthesis declined rapidly with increasing external $CO_2$ concentration at 21% $O_2$. Under normal atmospheric conditions, photorespiration was 45 to 50% of the net $CO_2$ fixation rate at 32°C and high irradiance. The rapid initial $CO_2$ evolution observed upon darkening at 21% $O_2$ was absent in 3% $O_2$. Rates of photorespiration under normal atmospheric concentrations of $CO_2$ and $O_2$ as measured by the postillumination burst were highly dependent upon temperature (observed activation energy = 30.1 kilocalories per mole). The results are discussed with respect to previously published estimates of photorespiration in $C_3$ leaf tissue.**

In spite of considerable effort aimed at elucidating the physiology, identity of metabolic intermediates, and regulation of photorespiration over the past 20 years, much uncertainty remains concerning the magnitude of this important process in $C_3$ leaves. Existing assays are indirect and those which are conducted in light certainly underestimate photorespiration due to refixation of the released $CO_2$ before it can escape the leaf and be detected (24). Although of comparative value, some methods such as $CO_2$ evolution into $CO_2$-free air are conducted under nonphysiological conditions. Other assays, such as the $CO_2$ compensation point as an indicator of photorespiration, are ambiguous or insensitive (8). Inhibition of net $CO_2$ uptake by 21% $O_2$ *versus* 3% $O_2$ has been used, and the results are largely due to increased photorespiration but also include other inhibitory effects of high $O_2$ concentrations (7).

Decker (3, 4) reported that preilluminated leaves of $C_3$ plants displayed vigorous $CO_2$ evolution, detectable by IR gas analysis in a closed system, immediately after darkening (the PIB[1]). This rate of $CO_2$ evolution declined rapidly with time. The principle of this assay is attractive because it minimizes the problem of refixation of photorespiratory $CO_2$ during photosynthesis, can be conducted over a wide range of $CO_2$ and $O_2$ concentrations, and is technically and conceptually straightforward. A serious limitation of closed systems for measuring the $CO_2$ released was that photorespiration was based on an estimate of the tangent to the initial increase in $CO_2$ concentration *versus* time in the recirculated gas (3, 4). Even under ideal conditions, this approach would somewhat underestimate the rate. Also, both open and closed systems operate under nonsteady state conditions (14, 16, 19). The IRGA response model presented in the previous paper (16) enables estimation of transient rates of $CO_2$ exchange under nonsteady state conditions characteristic of the early stage of the PIB.

Most studies of the effects of environmental conditions on photorespiration indicate that increasing $O_2$ concentrations or temperatures enhance this process (4, 7, 8, 21). Increasing $CO_2$ concentrations appear to diminish the importance of photorespiration when compared to net photosynthesis (1, 2, 4, 12, 13). In this paper, the IRGA response model presented previously (16) has been employed to reexamine these fundamental relationships and further verify the model. Observed responses of photosynthesis and photorespiration to $CO_2$, $O_2$, and temperature in tobacco leaf discs provided data that are consistent with many earlier reports. Hence, the IRGA response model produces reliable results. Also, the experiments suggest that photorespiration accounts for about 50% of net photosynthesis under normal atmospheric conditions and high temperature and irradiance.

## MATERIALS AND METHODS

These are described in the companion paper (16). Unless stated otherwise, all correlation coefficients reported here are significant at $P < 0.01$.

## RESULTS

Initial attempts to measure the PIB using the model described previously (16) indicated that net $CO_2$ uptake continues for a short period immediately after darkening the samples. This decline in the rate ($R(t)$) of $CO_2$ uptake must be included in the application of the model ([16]; equation 3) in order to obtain an accurate estimate of the subsequent PIB. Two parameters were defined to describe the decline in $CO_2$ uptake with time ($t$). The

[1] Abbreviations: PIB, postillumination burst; IRGA, infrared gas analyzer; RuBP, ribulose bisphosphate.

first, $S_1$ is the time (s) after darkening during which net $CO_2$ uptake continues at the previously determined steady state rate in the light ($R_o$). Subsequently, the rate of $CO_2$ uptake is assumed to decline linearly to zero at time $S_2$. Hence

$$R(t) = \frac{R_o (S_2 - t)}{(S_2 - S_1)} \quad (S_1 \leq t \leq S_2) \tag{1}$$

Figure 1 shows typical IRGA responses to darkening of leaf discs at atmospheric $CO_2$ levels and 21% and 3% $O_2$. At high concentrations of $O_2$, the first order equation

$$R(t) = K + Ae^{B(t-S_2)} \quad (K, A, B < 0) \tag{2}$$

describes the decline in the rate of $CO_2$ evolution with time (16). At 3% $O_2$, equation 2 does not apply. In this case, the initial $R(t)$ is given by $Q$ followed by a linear increase in $R(t)$ to a constant value ($K$) at $P$ seconds after the onset of $CO_2$ evolution. Hence

$$R(t) = Q + \frac{K - Q}{P} \cdot t \quad (S_2 \leq t \leq P) \tag{3}$$

For given values of the adjustable parameters $S_1$, $S_2$, and $A$, $B$ (or $P$, $Q$) the model produces a unique prediction of IRGA response. In these experiments, the predicted response was compared to the observed response at 1-s intervals by summing the squares of the differences ($d$) between respective values. In practice, it was convenient to test a large number of combinations of $S_1$ and $S_2$ for their ability to predict actual IRGA response during the first few seconds of an experiment. Thus, a few combinations of $S_1$ and $S_2$ were in turn employed to find corresponding optimal values of $A$ and $B$ using a computerized search routine until a minimal $\Sigma d^2$ was obtained for each experiment (23).

For example, given values of $S_1$ and $S_2$ at 21% $O_2$, $\Sigma d^2$ *versus* $A$ and $B$ describes a curved surface with a single minimum. Figure 2 shows how $\Sigma d^2$ varies with $A$ or $B$ for the three determinations of experiment 2 in Table I. Clearly, the model is quite sensitive to variations in $A$ and $B$ and, hence, variations in the magnitude of the PIB. Furthermore, two-way analyses of variance of predicted *versus* observed IRGA responses *versus* time for the individual experiments described here indicated that an average of only 0.06% of the total variance per experiment was unaccounted for after employing the specified parameters in the IRGA response model (Tables I and II; Figs. 3 and 4). Hence, although while making assumptions pertaining to the natures of the decline in net $CO_2$ uptake and subsequent $CO_2$ evolution, this approach is routinely capable of accounting for a very high degree of observed IRGA response following darkening.

The effect of $CO_2$ concentration on photorespiration by tobacco leaf discs (var John Williams Broadleaf) under 21% $O_2$ as estimated from the PIB is shown in Table I and Figure 3. Rates of photorespiration were relatively constant with varying $CO_2$ concentration within either of the two experiments shown. Net $CO_2$ uptake at 21% and 3% $O_2$ increased with $CO_2$ concentration. A decrease in net $CO_2$ uptake at 811 $\mu$l $CO_2$ $l^{-1}$ (Exp. 1) was probably a result of increased stomatal resistance caused by this high $CO_2$ level. Interestingly, although photorespiration was still active, the inhibition of net photosynthesis by 21% $O_2$ relative to 3% $O_2$ was virtually eliminated at the higher levels of $CO_2$ in each experiment. Figure 3 clearly shows that although the absolute rate of photorespiration changes little, it accounts for a progressively smaller fraction of the total turnover of carbon inside the leaf as the external $CO_2$ concentration is raised. This would explain, in part, the corresponding decrease in inhibition by $O_2$ observed here. Reversal of other mechanisms of inhibition

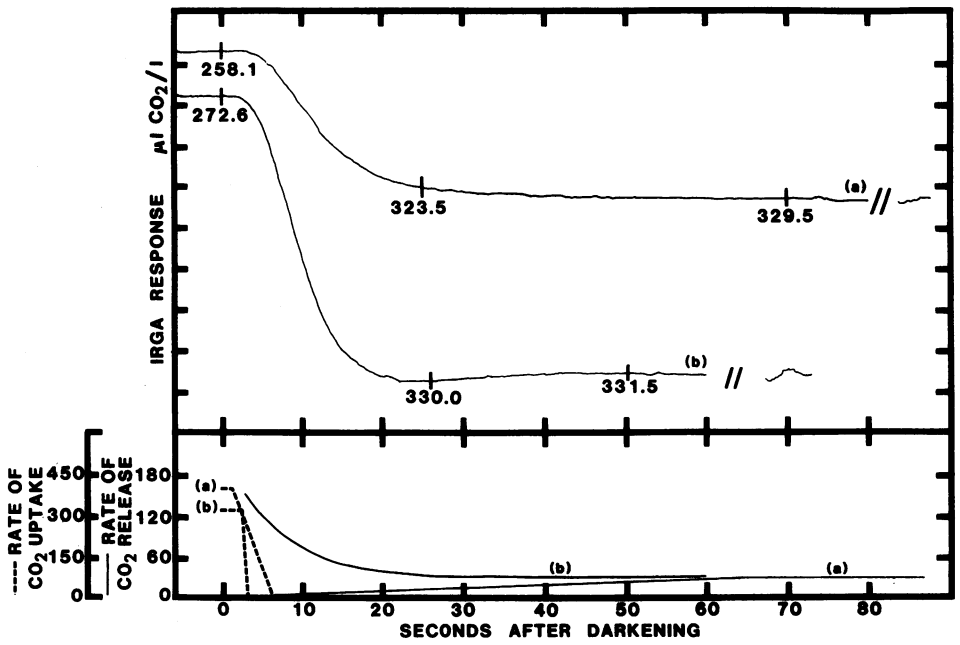


FIG. 1. Upper panel shows typical IRGA responses to darkening of 10 tobacco leaf discs under 3% $O_2$ (curve a) and 21% $O_2$ (curve b) in the system described in "Materials and Methods" of (16). One scale unit on the ordinate corresponds to 19.4 and 8.7 $\mu$l $CO_2$ $l^{-1}$, respectively, for curves a and b. The mean $CO_2$ concentration in the measuring cell was calculated from the IRGA response at selected points in time and these are shown on the curves for convenient reference. The irradiance was 850 $\mu$E $m^{-2}$ $s^{-1}$. In curve a, the inlet $CO_2$ concentration was 325 $\mu$l $l^{-1}$ and the rate of steady state $CO_2$ uptake in the light was 394.2 $\mu$mol $CO_2$ (g fresh weight)$^{-1}$ $h^{-1}$. In curve b, the inlet $CO_2$ concentration was 327 $\mu$l $l^{-1}$ and the corresponding rate was 318.0 $\mu$mol (g fresh weight)$^{-1}$ $h^{-1}$. Lower panel shows the predicted changes in leaf $CO_2$ exchange rate *versus* time for the two curves in the upper panel. Rates of $CO_2$ exchange are given by equations 1 to 3 in combination with the IRGA response model described in (16). Only 0.05% (curve a) and 0.006% (curve b) of total mean squares for predicted *versus* observed analyzer responses were associated with error after two-way analyses of variance (see text). The profound effect of $O_2$ concentration on the magnitude of the initial rate of $CO_2$ evolution is clearly demonstrated.

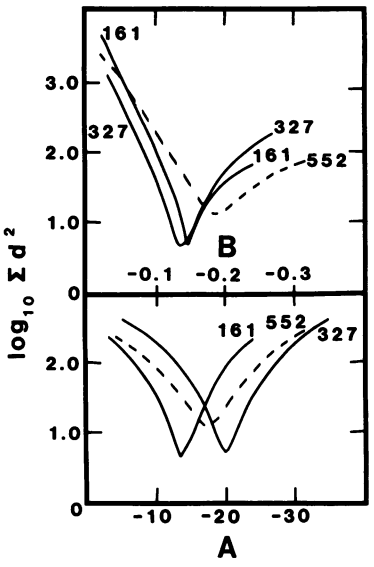


FIG. 2. Sensitivity of the regression of IRGA response with time to variation in $A$ and $B$ (equation 2) for the results in Table I, Exp. 2 (21% $O_2$). The difference ($d$) between predicted IRGA response and the observed response for given values of $A$ ($\mu$mol $CO_2$ (g fresh weight)$^{-1}$ $h^{-1}$) and $B$ ($s^{-1}$) at constant $K$ ($\mu$mol $CO_2$ (g fresh weight)$^{-1}$ $h^{-1}$) was measured at 1-s intervals. Curves were identified by associated values of $C_i$ (inlet $CO_2$ concentration) for the three experiments shown. The minima of the curves shown represent optimal values of $A$ and $B$ resulting in best agreement between predicted and observed responses. See text for further explanation.

by $O_2$, such as direct inhibition of carboxylation, by increasing $CO_2$ concentration is likely to contribute to these observations. Also, the high rates of net photosynthesis encountered at the higher $CO_2$ concentrations supplied suggest that ATP synthesis or regeneration of a Calvin cycle intermediate may have become limiting. Hence, lowering the $O_2$ concentration would do little to increase net photosynthesis under these conditions.

Rates of photorespiration at atmospheric $CO_2$ concentration and 21% $O_2$ were highly dependent on temperature as shown in Figure 4. The Arrhenius activation energy for photorespiration was calculated from the slope in Figure 4 and was found to be 30.1 kcal mol$^{-1}$, an unusually high value. Figure 5 shows the photorespiration/net photosynthesis ratio for the experiments in Figure 4. Photorespiration increases greatly, compared to net photosynthesis, with increasing temperature indicating a $Q_{10}$ of 3.8 for the ratio of these processes from 20 to 35°C.

## DISCUSSION

A number of investigators have shown that two successive peaks of $CO_2$ evolution occur when $C_3$ leaves are darkened (8, 9, 20, 21). The initial peak is considered most important with respect to photorespiration due to its early occurrence and response to $O_2$ concentration and irradiance (8, 17, 20, 21). Vines *et al.*, (22) reported that merely lowering the irradiance caused an immediate transient surge of $CO_2$ release for several species of $C_3$ leaves. I have also observed this response in leaf discs from tobacco (data not shown). Only one peak was evident in these experiments. No such transient was observed with leaves from $C_4$ plants which lack photorespiration (2, 22).

The first peak of $CO_2$ evolution which is observed at 21% $O_2$ is essentially absent at 3% $O_2$ (Fig. 1) regardless of $CO_2$ concentration (Tables I and II). The best agreement between predicted and observed IRGA responses at 3% $O_2$ was associated with initially low rates of $CO_2$ evolution upon darkening and after net $CO_2$ uptake had ceased. The regression method described (16) was not sufficiently sensitive to evaluate accurately these initial rates of $CO_2$ evolution, but they are certainly much less than the respective $CO_2$ evolution rates ($K$, Table II) estimated about 60 s after darkening. Although the magnitude of $CO_2$ evolution from mitochondrial respiration in the light is uncertain (7), it is probably independent of $O_2$ concentration in the range of 3% to 21% $O_2$. Hence, $CO_2$ evolution occurring within the first 60 to 80 s after darkening at normal levels of $O_2$ may be resolved into two components: first, a major component which declines with first order kinetics and which is sensitive to temperature and $O_2$

Table I. *Effect of $CO_2$ Concentration on Net Photosynthesis and Photorespiration by Tobacco Leaf Tissue*

Leaf discs floating topside down on water were preincubated at 850 to 900 $\mu$E $m^{-2}$ $s^{-1}$ and 32°C for 2 h before measurements began thus establishing steady state $CO_2$ uptake. The flow rate was 1.0 l min$^{-1}$. Net $CO_2$ uptake was given by equation 4 (16). The chamber was darkened at time zero and the IRGA response recorded for 60 to 80 s. The IRGA response model described previously (16) was used to determine the decline in rate of $CO_2$ evolution with time (see equation 2). The initial rate of $CO_2$ evolution ($K + A$) constitutes an estimate of steady state photorespiration. Parameters $S_1$ and $S_2$ describe the decline in rate of $CO_2$ uptake upon darkening (see text, equation 1). The constant $B$ describes the exponential decline in the rate of $CO_2$ evolution with time (equation 2). Steady state rates of $CO_2$ uptake were reestablished within 10 min upon illumination of the darkened leaf discs. Both experiments represent results obtained for 10 discs (1.6 cm diameter). See text and "Materials and Methods" (16) for further details.

| Exp. No. | Initial Chamber $CO_2$ Concn. | $K$ | $A$ | Photo-respiration | $B$ | Net $CO_2$ Uptake | | Inhibition by $O_2$ | $S_1$ | $S_2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 21% $O_2$ | 3% $O_2$ | | | |
| | $\mu l\ l^{-1}$ | $\mu mol\ CO_2\ g^{-1}\ h^{-1}$ | | | $s^{-1}$ | $\mu mol\ CO_2\ g^{-1}\ h^{-1}$ | | % | $s$ | |
| 1 | 164 | −29.5 | −67.8 | −97.3 | −0.053 | 86.6 | 152.1 | 43.1 | 0 | 4.5 |
| | 238 | −26.6 | −75.0 | −101.6 | −0.053 | 166.5 | 259.4 | 35.8 | 0 | 4.0 |
| | 425 | −21.4 | −78.0 | −99.4 | −0.092 | 273.1 | 356.9 | 23.4 | 0 | 2.7 |
| | 623 | −19.1 | −70.5 | −89.6 | −0.117 | 328.5 | 366.1 | 10.3 | 0 | 2.0 |
| | 753 | −16.7 | −82.5 | −99.2 | −0.157 | 380.7 | 398.3 | 4.4 | 0 | 2.2 |
| | 811 | −13.4 | −66.0 | −79.4 | −0.170 | 337.9 | 338.9 | 0.3 | 0 | 3.0 |
| 2 | 142 | −46.3 | −80.6 | −129.0 | −0.135 | 110.4 | 166.9 | 33.9 | 0 | 5.0 |
| | 273 | −26.3 | −120.9 | −147.2 | −0.146 | 318.0 | 394.2 | 19.3 | 2.3 | 3.0 |
| | 472 | −42.7 | −105.9 | −148.6 | −0.184 | 466.8 | 489.2 | 4.6 | 0 | 4.0 |

Table II. *Effect of Low $O_2$ Concentration (3% $O_2$) on the PIB*

Variables $K$, $Q$, and $P$ defined in equation 3. $S_1$ and $S_2$ describe the decline in $CO_2$ uptake rate upon darkening (equation 1). Net $CO_2$ uptake rates in the light ($PS$) are shown in Table I as are the corresponding rates at 21% $O_2$. All other details described in legend to Table I and in "Materials and Methods" of (16).

| $CO_2$ Concn. | Photorespiration | | | $\frac{Q}{PS}$ | $S_1$ | $S_2$ |
|---|---|---|---|---|---|---|
| | $K$ | $Q$ | $P$ | | | |
| $\mu l\ l^{-1}$ | $\mu mol\ CO_2\ g^{-1}\ h^{-1}$ | | $s$ | | $s$ | |
| 129 | −37.4 | −3.5 | 50 | 0.02 | 2 | 10 |
| 258 | −30.8 | −0.2 | 64 | <0.01 | 1 | 6 |
| 470 | −61.1 | −22.2 | 37 | 0.05 | 1 | 5 |

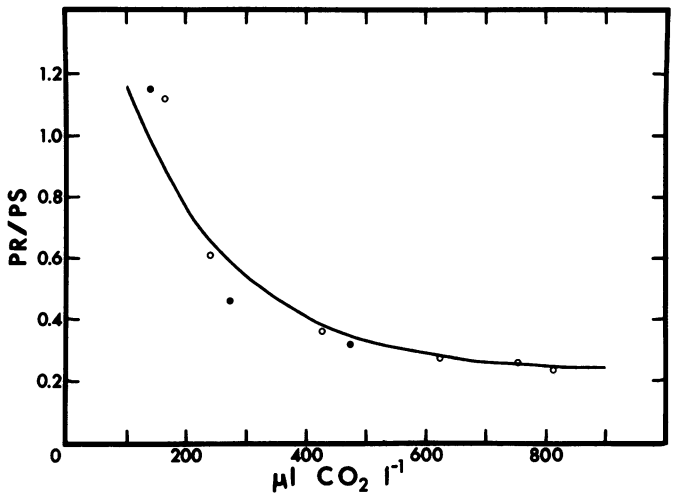


FIG. 3. Effect of $CO_2$ concentration on the ratio of photorespiration to net photosynthesis (PR/PS) in tobacco (var John Williams Broadleaf) as measured by the PIB. Results are from Table I. Irradiance was 850 $\mu E\ m^{-2}\ s^{-1}$ and the temperature was 32°C. (——), Exponential regression line for the ratio of (PR/PS) minus 0.23 *versus* $\mu l\ CO_2\ l^{-1}$ (correlation coefficient = 0.98). The data show that the ratio of photorespiration to net photosynthesis appears to become constant at high $CO_2$ concentrations.

levels (characteristics of glycolate biosynthesis and subsequent oxidation), and second, a slowly increasing minor component characteristic of dark respiration. The resultant rate equation for the PIB would be most adequately described by equation 1 when the first component is large compared to the second (*i.e.* when photorespiration is active).

As has been shown in both algal and higher plant systems (15, 17), net $CO_2$ uptake by the leaf discs occurred for up to 10 s after darkening, albeit at a declining rate (Fig. 1). The total length of the dark photosynthetic period was somewhat longer at low $CO_2$ concentrations and low temperatures. Lowering the $O_2$ concentration greatly increased dark $CO_2$ fixation (Table II). At 21% $O_2$, the area enclosed by the rate of dark $CO_2$ fixation *versus* time curve (data not shown) was linearly related to the logarithm of the $CO_2$ concentrations (correlation coefficient = 0.81, $P < 0.05$). Furthermore, the area was relatively independent of temperature at an average $CO_2$ concentration of 334 $\mu l\ l^{-1}$ (data from Fig. 4). Most likely, $CO_2$ and $O_2$ levels control the pool size of a critical photosynthetic substrate in the light which, in turn, subsequently limits dark $CO_2$ fixation.

If photorespiration were to decline during the initial period of dark net $CO_2$ uptake, the rates of photorespiration shown here would be underestimated. Thus, I conclude that the rates in Tables I and II and Figures 3 to 5 are minimal estimates of true steady state photorespiration.

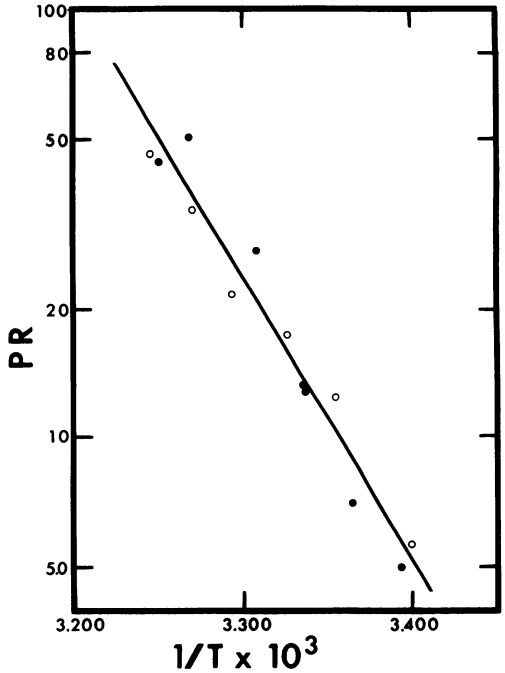


FIG. 4. Effect of leaf temperature ($T$, degrees Kelvin) on rates of photorespiration (PR in $\mu mol\ CO_2$ [g fresh weight]$^{-1}\ h^{-1}$) in tobacco (var Havana Seed) as determined by the PIB. The mean chamber $CO_2$ concentration in these experiments was 334 $\mu l\ CO_2\ l^{-1}$. Rates of net photosynthesis (not shown) were relatively constant above 25°C but somewhat less at the lower temperatures used. Values ranged from an average of 246 $\mu mol\ CO_2$ (g fresh weight)$^{-1}\ h^{-1}$ below 25°C to an average of 322 $\mu mol\ CO_2$ (g fresh weight)$^{-1}\ h^{-1}$ above 25°C. Irradiance was 850 $\mu E\ m^{-2}\ s^{-1}$. (——), Linear regression from the data shown (correlation coefficient = −0.98). The slope of the line was used to calculate an Arrhenius activation energy of 30.1 kcal $mol^{-1}$. Shown are the results of two experiments. Individual rates have been normalized to the grand mean rate for the two experiments.

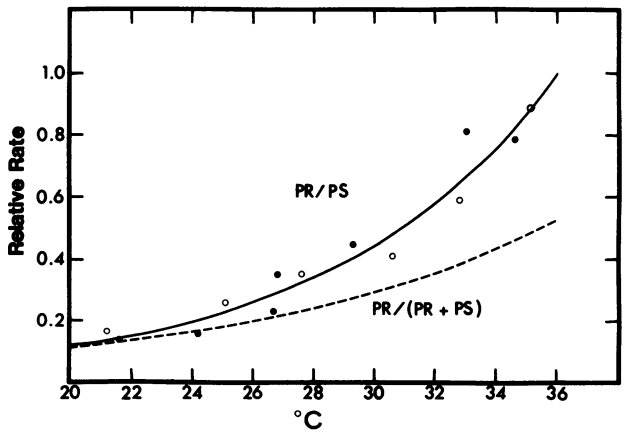


FIG. 5. Effect of leaf temperature on the ratio of photorespiration to net photosynthesis (PR/PS). Data are from the same experiments shown in Figure 4. (——), Exponential regression for the ratio of photorespiration to net photosynthesis (correlation coefficient = 0.97). (– – –), Exponential regression of the ratio of photorespiration to gross photosynthesis (PR/(PR + PS)) where gross photosynthesis equals net photosynthesis plus photorespiration. If X = PR/PS, then the ratio of photorespiration to gross photosynthesis is given by X/(1 + X). Thus, the PR/(PR + PS) values were calculated from the points shown but were not replotted for purposes of clarity.

Since photorespiration is entirely dependent upon photosynthetically generated substrate, a convenient means of comparing estimates of photorespiration from other laboratories is by use of the ratio of photorespiration to net photosynthesis. The ratios reported here for tobacco at 334 $\mu$l $CO_2$ $l^{-1}$ and 21% $O_2$ were 0.25 and 0.71 at 25.5 and 33.5°C, respectively (Fig. 5). At 300 $\mu$l $CO_2$ $l^{-1}$ and 21% $O_2$, Decker (4), using the PIB method in a closed system with whole leaves, reported ratios of 0.44 and 0.66 at the same two temperatures, respectively. Overall, the ratios presented here are comparable to those derived from (4).

Doehlert *et al.* (5) have employed an open system similar to the one used here to study the PIB in wheat leaves, and this warrants a comparison. The nonsteady state equation described by Marynick and Marynick (14) was used by them to calculate rates of postillumination $CO_2$ evolution directly from the slope of the IRGA response *versus* time curve. The equation presented in (14) describes only $CO_2$ concentration changes occurring within the leaf chamber, and it is invalid to apply it to a system consisting of a leaf chamber plus an IRGA. This error appears to have led to serious underestimation of photorespiratory rates in those experiments.

Great uncertainty exists concerning the influence of external $CO_2$ concentration on photorespiration because of conflicting results, often from the same assay of photorespiration. Early reports by Canvin and colleagues (12, 13) suggested that photorespiration by sunflower leaves at 25°C and 21% $O_2$ was relatively constant at atmospheric $CO_2$ levels and below but was inhibited at higher levels of $CO_2$. Fock and Przybylla (6) used the same assay based on the short-time measurement of differential uptake of $^{14}CO_2$-$^{12}CO_2$ with sunflower. Surprisingly, their rates of photorespiration were independent of temperature from 15 to 35°C but increased by over 50% on increasing the $CO_2$ concentration from 88 to 378 $\mu$l $l^{-1}$. Furthermore, a recent report from Canvin's laboratory (1), using a similar but improved assay, appears to suggest that the response to $CO_2$ concentration was modulated by temperature and/or irradiance. Sunflower leaves incubated at 25°C and at subsaturating irradiance exhibited increasing photorespiration with increasing $CO_2$ concentration. However, at 31°C and saturating irradiance, photorespiration was constant from the compensation point to 1200 $\mu$l $CO_2$ $l^{-1}$ (see also 2).

The $^{14}CO_2$-$^{12}CO_2$ method has gained acceptance because it is conducted in the light during steady state photosynthesis. Nevertheless, the method relies upon quantitation of a nonsteady state transient rate of $^{14}CO_2$ uptake shortly after introduction of the isotope to the gas stream flowing through the chamber. Finally, Bravdo and Canvin (1) acknowledge that the method underestimates photorespiration due to recycling of $CO_2$ within the leaf. The magnitude of this error under various conditions is unknown. The nonsteady state method of estimating the PIB could permit a more precise definition of the effect of $CO_2$ on photorespiration to be obtained.

The ratio of photorespiration to net photosynthesis declined from about 0.48 at 21% $O_2$, 340 $\mu$l $CO_2$ $l^{-1}$, and 32°C to 0.23 at the highest $CO_2$ levels studied here (Fig. 3). This observation is certainly consistent with the expected antagonistic effect of $CO_2$ on photorespiration. However, the RuBP carboxylase-oxygenase model does not easily accommodate the observation of a relatively constant photorespiratory rate with increasing $CO_2$ concentration at 21% $O_2$. According to the model (11), $CO_2$ competes directly with $O_2$ for the same enzyme site thus inhibiting oxygenation of RuBP and synthesis of P-glycolate, the proposed precursor of glycolate which is the substrate for photorespiration. The stoichiometry of $CO_2$ evolution (*i.e.* number of $CO_2$ molecules evolved per glycolate synthesized) may vary with $CO_2$ concentration and thereby confound this issue, although no firm evidence for this exists.

Estimates of photorespiration based on the PIB (Fig. 4) reported here were highly temperature dependent at normal atmospheric concentrations of $CO_2$ and $O_2$ ($Q_{10}$ = 5.2 at 25°C). The Arrhenius activation energy of 30.1 kcal $mol^{-1}$ is much higher than can be accounted for by the temperature dependence of the RuBP oxygenase reaction (11) and is, indeed, higher than the reported activation energies for most enzyme-catalyzed reactions (18). The observed temperature dependence of photorespiration (Fig. 4) certainly reflects the associated temperature dependencies of the several reactions involved in the metabolism of glycolate. Since glycolate synthesis is highly dependent upon photosynthesis (24), its temperature dependence will also contribute to that of photorespiration. Evidence is provided by the lowest rates of photorespiration observed in the experiments of Figure 4 which were associated with the lowest rates of net photosynthesis at temperatures less than 25°C.

To assess the temperature dependence of photorespiration alone, $CO_2$ release under steady state conditions in the light may be viewed as occurring stoichiometrically with total (gross) $CO_2$ fixation (*i.e.* a specific fraction of recently fixed carbon is released as $CO_2$). This stoichiometry will vary with the concentrations of $CO_2$ and $O_2$ present and possibly with other factors as well. The $CO_2$ released inside the leaf merges with, and becomes indistinguishable from, the incoming $CO_2$ from the atmosphere and is refixed by RuBP carboxylase. Hence, gross photosynthesis equals the sum of net photosynthesis plus photorespiration. Increasing temperature raises the fraction of recently fixed carbon which is released through photorespiration. This is shown in Figure 5 (– – –) where photorespiration (as measured by the PIB) per unit of gross photosynthesis increases with temperature. The activation energy for this process is 18.3 kcal $mol^{-1}$ ($Q_{10}$ = 2.7 for 20 to 35°C). This is still about twice as large as the activation energies reported for mitochondrial dark respiration in various plant species (18), and is consistent with a large body of evidence indicating that photorespiration is metabolically distinct from dark respiration (7, 24).

Photorespiration will be promoted with increasing temperature, in part, due to changing solubilities of $CO_2$ and $O_2$ (10). But without information on the $pCO_2$ and $pO_2$ in the chloroplast, together with the relative response of photorespiration to changes in the concentrations of these dissolved gases, precise corrections cannot be made. Furthermore, the results in Table I suggest that photorespiration varies only slightly with $CO_2$ concentration. Thus the effect of changing solubilities of $O_2$ and $CO_2$ on photorespiration is probably minimal.

Studies of photorespiration should be greatly facilitated by improved methods for its detection and quantitation. The nonsteady approach to measurement of the PIB described here offers an opportunity for achieving a more rigorous understanding of photorespiration in leaf tissue.

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## LITERATURE CITED

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