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# Solubility of Gases and the Temperature Dependency of Whole Leaf Affinities for Carbon Dioxide and Oxygen

## AN ALTERNATIVE PERSPECTIVE[1]

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JOHN D. TENHUNEN, JAMES A. WEBER, CONRAD S. YOCUM, AND DAVID M. GATES
*The Biological Station and Matthaei Botanical Gardens, Division of Biological Sciences, The University of Michigan, Ann Arbor, Michigan 48109*

### ABSTRACT

**An analysis of the kinetics of simultaneous photosynthesis and photorespiration at the end of a diffusion path is applied to observed net photosynthetic rate as a function of $O_2$ and $CO_2$ concentrations. The data of Ku and Edwards (Plant Physiol. 59: 991-999, 1977) from wheat (*Triticum aestivum* L.) are analyzed in detail. Ku and Edwards, using an analysis that ignored diffusion resistance between the intercellular air space and fixation site, the competitive effect of $CO_2$ on photorespiration, and the actual concentrations of gases at the fixation site, concluded that: (*a*) the affinity coefficient of the leaf for $CO_2$ was approximately 3.5 to 5 micromolar; (*b*) this affinity coefficient is independent of temperature between 25 and 35 C; (*c*) the effect of $O_2$ was independent of temperature over this range; and (*d*) competition between $CO_2$ and $O_2$ is responsible for the major share of $CO_2$ loss from photosynthesis due to photorespiration. They suggest that using gas concentrations calculated as equilibium values in the liquid phase is very important in reaching these conclusions. By applying a more complete analysis to their data which includes diffusion in the cell, it is concluded that: (*a*) the affinity coefficient of the leaf for $CO_2$ is 0.1 to 1.1 micromolar; (*b*) the temperature dependence of this affinity coefficient cannot be determined from existing data, but there is no evidence to refute independent temperature effect on the two functions of ribulose-1,5-bisphosphate carboxylase-oxygenase being important in the regulation of whole leaf net photosynthesis; and (*c*) the competitive interplay of $CO_2$ and $O_2$ at ribulose-1,5-bisphosphate carboxylase may under certain conditions lead to a stimulation of fixation by the Calvin cycle because of photorespiration. These conclusions are reached whether $CO_2$ and $O_2$ are expressed as dissolved concentrations or as gas concentrations in the intercellular air space. The relative merits of these two expressions of concentration are discussed.**

Ku and Edwards (12, 13) recently obtained data from wheat comparing the $O_2$ and $CO_2$ dependencies of net photosynthesis at 25, 30, and 35 C. The following questions were addressed in the analysis and interpretation of these data: (*a*) what role does solubility of gases play in the increased percentage inhibition of photosynthesis by $O_2$ with increasing temperature; (*b*) what is the temperature dependence of the whole leaf affinity coefficient for $CO_2$; (*c*) is the increased percentage inhibition of photosynthesis by $O_2$ at high temperature due to different temperature effects on the whole leaf affinity coefficients for $CO_2$ and $O_2$ regulating total photosynthesis and the photorespiratory process according to the Bowes-Ogren hypothesis (2)? The objective of this paper is to reevaluate the conclusions of Ku and Edwards (12, 13) using an alternative analysis. This second interpretation explains the original data but may lead to very different conclusions, which are fundamental to understanding the photosynthetic response of whole plant leaves to environmental factors.

In our analysis (31-33), a stepwise procedure is used to elaborate the environmental regulation of net photosynthesis in terms of physiologically meaningful parameters that characterize: (*a*) the production of photoproducts in the light reactions of photosynthesis; (*b*) transport of $CO_2$ from the intercellular air space to the site of fixation; (*c*) enzymic fixation of $CO_2$; and (*d*) reutilization of photosynthate products in the simultaneous processes of photorespiration and dark respiration. Stomatal resistance is treated as a separate effect superimposed on these processes. This general model has many characteristics in common with models of Peisker (25), Charles-Edwards and Ludwig (3), Chartier (4), Laisk (15), Hall (8), and Lake (16, 17) but differs in that it is developed specifically to separate the effects of light, temperature, $CO_2$, and $O_2$ on the individual metabolic subprocesses mentioned above.

At a specific light intensity and leaf temperature, the simultaneous processes of photosynthesis and photorespiration have been successfully described based on the assumption that $CO_2$ and $O_2$ compete for the same active site on RuBP[2] carboxylase. Various criteria, important in evaluating the performance of such a submodel have been considered (29, 31). The single most important criterion, an accurate description of the net photosynthetic response as a function of $O_2$ and $CO_2$ concentration, is well satisfied.

The determination of values for the whole leaf affinity coefficients for $CO_2$ is dependent on the photosynthetic model used in the analysis. The most meaningful definition of the kinetic constants of whole leaf photosynthesis is one based on estimations of $CO_2$ concentrations at the enzyme active site. This concentration is dramatically affected by respiration rates and the geometric distribution of $CO_2$ sources and sinks within the leaf (30), making a careful formulation of the regulation of photosynthesis and photorespiration based on the Bowes-Ogren hypothesis of paramount importance. It has likewise become apparent that suggested simplifications of these kinetic equations may heavily bias results. Although Ku and Edwards (12, 13) considered extensive data, their conclusions may be misleading due to inadequacies of their model. Reinterpretation of their results, using our net photosynthesis model, produces quite different results with significant implications for regulation of whole leaf photosynthesis.

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[2] Abbreviation: RuBP: ribulose 1,5-bisphosphate.

## MATERIALS AND METHODS

The analysis summarized here describes net photosynthetic rate (P) as a function of $CO_2$ and $O_2$ concentrations in the air space with leaf temperature and light intensity held constant. The equations and their derivations have been discussed in detail elsewhere (19, 31). The following modifications from the equations of Lommen *et al.* (19) were made. First, stomatal resistance is removed and viewed as a separate process, *i.e.* description of photosynthesis is elaborated as seen inside the leaf. Second, the *apparent* affinity coefficient for $CO_2$ (K in the equation of Lommen *et al.*) is a function of the affinity coefficient ($K_C$) of the photosynthetic process for $CO_2$ and of $O_2$ as a competitive inhibitor, *i.e.* $K_{Lommen} = K_C(1 + [O_2]/K_{O_2})$. When the $O_2$ concentration is low, the kinetics of the photosynthesis process reflects the properties of $K_C$. This affinity coefficient is not the concentration of air space $CO_2$ at which net photosynthesis proceeds at half-maximal rate even with low $O_2$ concentration. It is the concentration of $CO_2$ at the enzyme fixation site which results in half-maximal photosynthesis (for simplicity we refer to this concentration as chloroplast $CO_2$ concentration). If significant diffusion resistances are assumed to exist between the air space and chloroplast, respiration sites and chloroplast, and respiration sites and air space, then $K_C$ values different from the air space concentration producing half-maximal net photosynthesis are determined from observed data.

Photorespiration (see equation 1 below) is described in a manner similar to that of photosynthesis and is assumed to be competitively inhibited by chloroplast $CO_2$ concentration. The resistance network of Lommen *et al.* describing the geometric distribution of $CO_2$ sources and sinks within the leaf is collapsed by considering the site of photorespiration to be close to the site of photosynthesis. As we have discussed extensively elsewhere (30), this obviously affects the predicted values for affinity coefficients. At present these can be based only on net photosynthesis data and only measurements on responses of other dependent variables (photorespiration, dark respiration) can lead to a more correct analysis. When fitting the photosynthesis equation to observed data, the calculated photosynthetic rate is obtained as a function of the chloroplast $CO_2$ concentration by an iterative solution of the equations describing simultaneous photosynthesis and photorespiration and diffusion (Fick's Law). This iterative procedure is discussed stepwise in the Appendix.

In summary, the rate of net photosynthesis (P) in nmol $cm^{-2}$ $s^{-1}$ as a function of $[CO_2]$ and $[O_2]$ with light and leaf temperature constant is given by:

$$P = \frac{AA - \{(AA)^2 - BB\}^{1/2}}{2 R_M} \quad (1)$$

where:

$$AA = C_w + K_C\beta + R_M(P_M - W_P) - W_P\left(\frac{R_M}{1 + M}\right)$$

$$BB = 4R_M\left[\left(C_w - W_P\frac{R_M}{1 + M}\right)(P_M - W_P) - W_PK_C\beta\right]$$

$$\beta = \left(1 + \frac{[O_2]}{K_{O_2}}\right)$$

$$W_P = \frac{W_M[O_2]}{[O_2] + K_{O_2}\left(1 + \frac{C_c}{K_C}\right)}$$

$P_M$ is the rate of photosynthesis at a specific light intensity, saturating $CO_2$, and a specific leaf temperature (nmol $cm^{-2}$ $s^{-1}$);

$K_C$ is a coefficient equal to the chloroplast $CO_2$ concentration (photorespiration $\simeq$ 0) at which $P = P_M/2$ (nmol $cm^{-2}$; see also alternative units below);

$C_w$ is the $CO_2$ concentration in the intercellular air space (nmol $cm^{-3}$ or alternatives discussed below);

$C_c$ is the $CO_2$ concentration at the site of fixation in the chloroplast (nmol $cm^{-3}$ or alternatives discussed below);

$R_M$ is the mesophyll diffusion resistance between $C_w$ and $C_c$ for $CO_2$ (s $cm^{-1}$)

M is some large number, arbitrarily 100; this reflects the ratio of the diffusion resistance between $C_w$ and $C_c$ to the diffusion resistance between the photorespiratory site and the fixation site; as M approaches infinity, the photorespiratory site is moved to and becomes identical with the fixation site.

$W_M$ is the rate of photorespiration (*$CO_2$ evolution*) at saturating $O_2$, a specific leaf temperature, and a specific light intensity (nmol $cm^{-2}$ $s^{-1}$);

$W_P$ is the rate of photorespiration (*$CO_2$ evolution*) (nmol $cm^{-2}$ $s^{-1}$);

$K_{O_2}$ is a constant equal to the $O_2$ concentration in the atmosphere with $C_c \simeq 0$ at which $W_P = W_M/2$ (v/v expressed as a decimal fraction or alternatives discussed below);

$[O_2]$ is the concentration of $O_2$ in the air (v/v expressed as a decimal fraction, *i.e.* 1% = 0.01, or alternatives discussed below).

To describe a particular set of data, it is necessary to determine the values of five parameters: $R_M$, the diffusion resistance from the intercellular air space to the fixation site; $K_C$, the leaf affinity coefficient for $CO_2$ in $CO_2$ fixation; $K_{O_2}$, the leaf affinity coefficient for $O_2$ in oxygenation (photorespiration based on the rate of $CO_2$ evolution); $P_M$, the maximum rate of $CO_2$ fixation at saturating $CO_2$; and $W_M$, the maximum rate of oxygenation at saturating $O_2$. It may be possible in the future to relate the leaf affinity coefficient for $CO_2$ more closely to biochemical kinetics (*cf.* ref. 25), but the definition used above will provide a focus for new ideas and promote progress in describing the interactive regulatory effects of environmental variables on leaf net photosynthesis (31–33).

Observed photosynthetic rates at different $O_2$ concentrations are expressed as a function of the air space $CO_2$ concentration ($C_w$). $O_2$ concentration is measured outside of the leaf (*cf.* ref. 26). This simplifiation may also lead to difficulties, (*cf.* ref. 28), but we have not considered it further at the present time. For raw data, $C_w$ is calculated as described by Gaastra (7) from:

$$P = \frac{C_A - C_w}{1.56\ R_1} \quad (2)$$

where:

$C_A$ is the concentration of $CO_2$ in air outside the boundary layer (nmol $cm^{-2}$ $s^{-1}$);

1.56 is the ratio of the diffusion coefficients for water vapor and $CO_2$;

$R_1$ is the total resistance to water flux out of the leaf determined from transpiration measurements (9).

In the present study, analysis proceeded in the following manner. Net photosynthetic rates as a function of dissolved $[CO_2]$ at 25, 30, and 35 C and 1 and 21% $O_2$ were obtained for wheat (*Triticum aestivum* L.) from Ku and Edwards (Fig 9 in ref. 13). Other data of Ku and Edwards could not be analyzed by our method because rates in the curvature region of the P *versus* $C_w$ response for $[O_2] = 0.01$ are required to obtain estimates of $K_C$, and rates at $CO_2$ saturation for $[O_2] = 0.01$ are required to obtain estimates of $P_M$. The data were transformed to express photosynthetic rates in nmol $cm^{-2}$ $s^{-1}$ and to express $CO_2$ concentrations in nmol $cm^{-3}$ in the intercellular air space corresponding to the $\mu$M concentrations in solution reported by Ku and Edwards (13). This transformation was done according to the method indicated by

Ku and Edwards using the temperature dependency of $CO_2$ solubility in water (34) and the gas law (PV = nRT) to consider the effect of temperature in the conversion from partial pressure to actual gas concentration.

Equation 1 describes photosynthesis as a process which at zero $CO_2$ and $O_2$ concentrations has zero net $CO_2$ exchange. In reality, photorespiration may continue at low rates, mitochondrial and/or glycolytic respiration may occur, and photosynthesis may continue to recycle the released $CO_2$. Initially we have not considered these aspects of recycling and have ascribed any negative net photosynthesis occurring at low $O_2$ concentration to residual mitochondrial respiration (32). We further assume that the residual respiration rate of $CO_2$ exchange can be estimated by extrapolating the initial slopes of the P *versus* $C_w$ response curves where $[O_2] = 0.01$ to zero $CO_2$ concentration. This rate is then added to each data set before analysis. The added correction was 0.0, 0.05, and 0.32 nmol $cm^{-2}$ $s^{-1}$ at 25, 30, and 35 C, respectively. These corrections are included in the data shown in Figure 1. Other treatments of mitochondrial respiration occurring in the light (or respiration resulting in a constant flux of decarboxylation from glycolate produced from other pathways) have been studied by modifying the expression $W_P$ in equation 1 of the analysis (30). Such modifications may change the estimated value for $K_C$ as much as an order of magnitude. We have no criterion for establishing the rates of such fluxes and therefore choose here to remain with the simpler description.

The corrected data were studied using nonlinear least squares analysis (6, 32), supplemented by three specific subroutines (31). These subroutines solve for the net photosynthesis rate at particular values of $CO_2$ concentration and $O_2$ concentration, and solve for partial derivatives of the function used for net photosynthesis rate in each case with respect to the parameters to be estimated. The subroutines are available from the authors.

Initial estimates of the parameters $K_C$, $P_M$, and $R_M$ are obtained from analysis of the P *versus* $C_w$ curve at 1% oxygen ($[O_2] = 0.01$). Photorespiration is essentially zero and a quadratic equation must be fit to the observed data (equation 12 in ref. 33). The solution is more or less straightforward since the only independent variable of equation 1 in this case ($C_w$) is known, and the only dependent variable (P) is observed. The partial derivatives of the quadratic photosynthesis equation with respect to $K_C$, $R_M$, and $P_M$ are required in the solution and are calculated in subroutine A.

In the subsequent analysis step, $K_{O_2}$ and $W_M$ are drived from the best fit solution of equation 1 to data where photosynthesis has been measured as a function of both $CO_2$ and $O_2$. As yet we have no procedure for determining $K_{O_2}$ and $W_M$ independently of each other. A practical solution to this problem is to eliminate one parameter by setting $W_M$ equal to a fixed proportionality or multiple of $P_M$. Whether this simplification is valid and what proportion to assume must still be determined. For the present study, we have set $W_M = P_M$. Essential to the determination of $K_{O_2}$ is an iterative determination of the chloroplast $CO_2$ concentration ($C_c$) which competitively interacts with $O_2$ in the chloroplast to determine the relative velocities of photosynthesis and photorespiration. Nonlinear least squares analysis with subroutine B results in estimates of the $O_2$-dependent parameters $K_{O_2}$ and $W_M$ at constant values of $K_C$, $R_M$, and $P_M$ as determined with subroutine A.

A final solution for parameter values on the basis of least squares is obtained with subroutine C, which is a generalized version of subroutine B. Iteration is performed with respect to all five parameters: $K_C$, $R_M$, $P_M$, $K_{O_2}$, and $W_M$. Very little change in $K_C$, $R_M$, and $P_M$ is observed in this final analysis step because these values are very strongly determined by the observations at low $O_2$ concentration. This three-step determination of $K_C$, $R_M$, $P_M$, $K_{O_2}$, and $W_M$ was carried out on the respiration corrected data of Figure 1. With experience and with prior knowledge of reasonable estimates for the five parameters, only the last step of the analysis need be performed.

Th data of Figure 1 were analyzed: (*a*) where $CO_2$ and $O_2$ concentrations were both expressed in $\mu$M dissolved gas; (*b*) where $CO_2$ concentration was expressed as $\mu$M dissolved $CO_2$ and $O_2$ concentration was expressed only as per cent composition of the gas in the intercellular air space; and (*c*) where $CO_2$ concentration was expressed in nmol $cm^{-3}$ and $O_2$ concentration was expressed as per cent composition of the gas in the intercellular air space. As pointed out by Ku and Edwards (12) per cent composition for $O_2$ concentration is technically incorrect as a measure of concentration. Nevertheless, it is desirable to determine what shift in parameter values might occur by analysis on these different bases especially since the third combination is very commonly used for expression of net photosynthesis data from whole leaves.

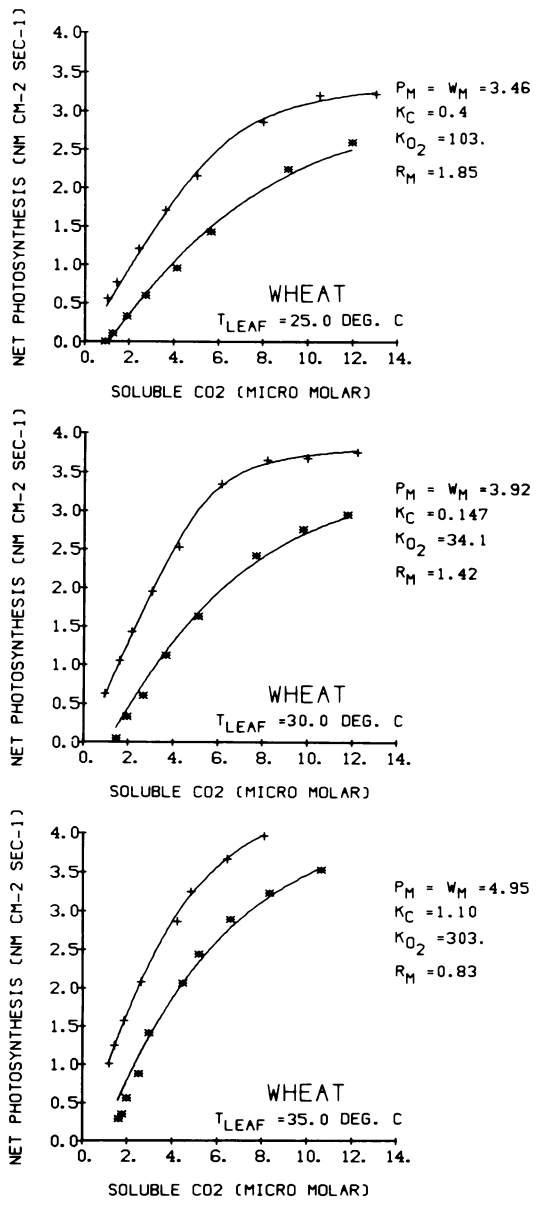


FIG. 1. Analysis of the wheat data of Ku and Edwards (13) using dissolved gas concentrations in equilibrium with measured gas concentrations in the intercellular air space and according to equation 1. Symbols are Ku and Edwards' observations at 1% (+) and 21% (*) $O_2$. Solid lines are predicted according to equation 1 with the parameter values shown. $P_M$ and $W_M$ are in nmol $cm^{-2}$ $s^{-1}$, $K_C$ and $K_{O_2}$ in $\mu$M, and $R_M$ in ($\mu$M/nmol $cm^{-2}$ $s^{-1}$). $W_M$ is based on the rate of $CO_2$ evolution in photorespiration. Light intensity = 0.15 $\mu$E $cm^{-2}$ $s^{-1}$ PAR. NM = nmol.

## RESULTS

The parameter values determined for the most correct case, *i.e.* where both $CO_2$ and $O_2$ concentrations are expressed in $\mu M$ dissolved $CO_2$ and $O_2$, are indicated in Figure 1 for the data at three temperatures. The solid lines in this figure were determined according to equation 1 with the parameters set at the values indicated. In each case, the model accurately describes the net photosynthesis rate as a function of $CO_2$ and $O_2$ concentration.

The solutions shown are not the best possible solutions with these equations but instead are those obtained under the restriction that $W_M = P_M$. This restriction was imposed due to a clear shortcoming in applying the least squares methodology to this system in which five parameters are determined from observations of net photosynthesis as a function of only two independent variables. The best solution will at times produce parameter estimates that seem very unlikely in order to minimize least squares. Such a situation was confronted in analysis of these data at 30 C and 35 C. The best solution produced estimates of $W_M$ of 8+ and 20+ nmol $cm^{-2}$ $s^{-1}$, producing response curves that vary almost imperceptibly from those shown in Figure 1.

There are several possible reasons why one might obtain such solutions with the data shown in Figure 1. As temperature increases, less information is available on photosynthesis under saturating conditions, thus decreasing the accuracy of determining $P_M$. Estimations of $CO_2$ and $O_2$ coefficients become more difficult with increasing temperature because of decreased data in the curvature region of the response curves, especially at 21% $O_2$. Superimposed on these problems is the possibility of experimental error and the possibility that a constant correction for residual respiration across the entire $CO_2$ concentration range may be invalid. Values for $W_M$ of the order of $P_M$ have been obtained with our analysis without restrictions for the sunflower data of Ludwig (21), for the data of Ku and Edwards (12) at 25 C, for the data of Lommen *et al.* (20), and for certain cases of the families of response curves presented by Joliffe and Tregunna (10). As further data are obtained and examined, predicted values of $W_M$ must be reevaluated. Further critical discussion and experimentation are needed to clarify this problem.

For many purposes, the solutions shown in Figure 1 are adequate. They emphasize the need to obtain very accurate data and to measure to very high $CO_2$ concentrations. Only then can we separate problems introduced by shortcomings of the model from those introduced by insufficient and/or inaccurate data. These estimates of $K_C$ and $K_{O_2}$ obtained with an analysis that attempts to account for the actual concentration of $CO_2$ at the reaction site and for photorespiration competitively inhibited by $CO_2$ are in our opinion the most reasonable estimates for the affinities of the whole leaf for $CO_2$ and $O_2$ from the data of Ku and Edwards. One observes that the estimates of $K_C$ and $K_{O_2}$ differ considerably from temperature to temperature and differ from those obtained by Ku and Edwards who considered the diffusion process to be negligible. While a solution based on dissolved gas concentration is advantageous in comparing whole leaf photosynthesis K values to those obtained for RuBP carboxylase *in vitro*, a disadvantage is found in determining a numerical value for mesophyll resistance which cannot be immediately compared to stomatal and boundary layer resistances. This problem arises from the fact that there is a change in the concentration (molar) of $CO_2$ when going from gas to liquid phase. This change is expressed as the solubility coefficient ($[CO_2]_{liquid}/[CO_2]_{gas}$) which for $CO_2$ in water at 25 C and 1 atmosphere is about 0.8. The solubility coefficient will be affected by solutes in the cell; for simplicity we are assuming that this effect is small. In order to evaluate the diffusion resistance over the path from the external atmosphere to the fixation site, it is necessary to take into account the solubility characteristics of $CO_2$ at the cell wall by converting the concentration of $CO_2$ at the cell wall and at the fixation site to their equivalent gas phase concentrations. This conversion leads to an increased concentration difference between the cell wall and fixation site thereby affecting the estimate of $R_M$. $R_M$ estimated from equivalent gas phase concentrations can be converted to the $R_M$ estimated from dissolved $CO_2$ concentrations by multiplying the former by the solubility coefficient. This was done for the estimates of $R_M$ (gas) (Table I) using the following solubility coefficients: 0.804 at 25 C, 0.709 at 30 C, and 0.633 at 35 C. Comparison with the estimates of $R_M$ based on dissolved $CO_2$ shows a good correspondence. One might ask whether the expression of whole leaf photosynthesis affinities for $CO_2$ and $O_2$ can be based, as has been customary, on concentrations in the gas phase, thus allowing present modeling definitions and units to remain unchanged, and whether considering a simple gas/liquid equilibrium conversion to dissolved concentrations would allow comparison of whole leaf affinities to enzyme affinities. In other words, if $CO_2$ concentration is in nmol $cm^{-3}$ in the intercellular air space and $O_2$ concentration is in per cent $O_2$ (v/v or partial pressure) in the air, are the predicted values of whole leaf affinities different? The data were expressed in these units and reanalyzed. The results are presented in Table I. When the parameters $K_C$ and $K_{O_2}$ are thus determined and converted to dissolved concentrations, the results are identical. The solution for the 25 C data based on the units most likely to effect different results, nmol $cm^{-3}$ for $[CO_2]$ and per cent for $[O_2]$, is shown in Figure 2 for comparison. Except for the scale on the abscissa, no difference can be seen between results presented in Figures 1 and 2. Results obtained for all cases summarized in Table I and at all three temperatures compare similarly. This suggests that present definitions and units of expressions are adequate.

## DISCUSSION

We will first comment on the effect of $O_2/CO_2$ solubility ratio on the $O_2$ inhibition of photosynthesis in light of our analysis and then consider the problem of whole leaf affinities for $CO_2$ and $O_2$. Ku and Edwards (12) have cited the experiments of Bowes *et al.* (2) and conclude that "$O_2$ competitively inhibits carboxylase activity with respect to $CO_2$ and $CO_2$ competitively inhibits oxygenase activity with respect to $O_2$." They then demonstrated that the per cent $O_2$ inhibition of photosynthesis is correlated with the solubility ratio of $O_2/CO_2$ calculated from concentrations in the intercellular air space (Fig 2 in ref. 12). They stated that the expression of $O_2$ and $CO_2$ concentrations as dissolved values eliminates differences in comparing certain photosynthetic parameters. The implication in conjunction with their second paper (13) is that expressing $O_2$ and $CO_2$ as dissolved concentrations eliminates differences in the calculated affinities of the whole leaf for $CO_2$ and $O_2$ at different temperatures and differences in the ratio of these affinities. The dissolved gas concentrations and their ratios, when calculated according to Ku and Edwards, assume gas-liquid equilibrium which does not necessarily reflect conditions at the site of fixation. This ratio to which RuBP carboxylase is responding can only be determined when the diffusion limitations and actual $CO_2$ fluxes are considered.

Net photosynthetic flux is seen to vary considerably in Ku and Edwards' tabulated data. The significance of diffusion resistance is obvious from observations that a $CO_2$ compensation point exists and that net photosynthesis in $CO_2$-free air reaches fairly large negative values, *i.e.* the concentration within the cells is considerably greater than zero to drive these rather large fluxes out of the leaf. While Ku and Edwards sought to describe "factors controlling photosynthesis at the cellular level such as affinity for $CO_2$," the $K_m$ for $CO_2$ that is derived is only an apparent affinity coefficient for $CO_2$ that also includes respiratory and diffusion effects. The expression of $CO_2$ and $O_2$ as dissolved concentrations may only coincidentally remove some variation in the data and parameters describing those data. As seen in Table I, the parameters derived from our analysis incorporating respiratory fluxes

Table I. Parameter values determined from the kinetic analysis outlined in the text applied to the data of Ku and Edwards (13) from wheat (symbols in Figure 1). Analyses are for three different expressions of these data with respect to concentration as explained in Materials and Methods.

| Case Considered | Leaf Temperature 25.0 | 30.0 | 35.0 | Units of Expression |
|---|---|---|---|---|
| µMolar dissolved $CO_2$ and $O_2$ | | | | |
| $W_M = P_M$* | 3.46 | 3.92 | 4.95 | nmol $cm^{-2}$ $sec^{-1}$ |
| $K_C$ | 0.400 | 0.147 | 1.10 | µMolar $CO_2$ |
| $K_{O_2}$ | 103. | 34.1 | 303. | µMolar $O_2$ |
| $R_M$ | 1.85 | 1.42 | 0.829 | $\frac{\text{µMolar}^{**}}{\text{nmol cm}^{-2}\ \text{sec}^{-1}}$ |
| µMolar dissolved $CO_2$; percent $O_2$ in air | | | | |
| $K_C$ | 0.402 | 0.144 | 1.07 | µMolar $CO_2$ |
| $K_{O_2}$ | 8.23 | 2.90 | 27.5 | percent $O_2$ |
| —— | 103. | 33.6 | 296. | µMolar $O_2$*** |
| $R_M$ | 1.85 | 1.42 | 0.841 | $\frac{\text{µMolar}^{**}}{\text{nmol cm}^{-2}\ \text{sec}^{-1}}$ |
| nmol $cm^{-3}$ $CO_2$ and percent $O_2$ in air space gas | | | | |
| $K_C$ | 0.493 | 0.201 | 1.61 | nmol $cm^{-3}$ |
| —— | 0.402 | 0.147 | 1.06 | µMolar $CO_2$*** |
| $K_{O_2}$ | 8.23 | 2.94 | 27.3 | percent $O_2$ |
| —— | 103. | 34.1 | 294. | µMolar $O_2$*** |
| $R_M$ | 2.27 | 1.94 | 1.28 | sec $cm^{-1}$ |
| —— | 1.83 | 1.38 | 0.811 | $\frac{\text{µMolar}^{**}}{\text{nmol cm}^{-2}\ \text{sec}^{-1}}$ |

*The values of $W_M = P_M$ are the same for all three analyses.
**This unit is technically reducible to sec $cm^{-1}$ but has been retained here to emphasize that a resistance based on dissolved concentration is not equal to the same numerical resistance based on gas concentrations.
***These values are simple conversions of the numbers tabulated immediately above to give equilibrium dissolved gas concentrations.

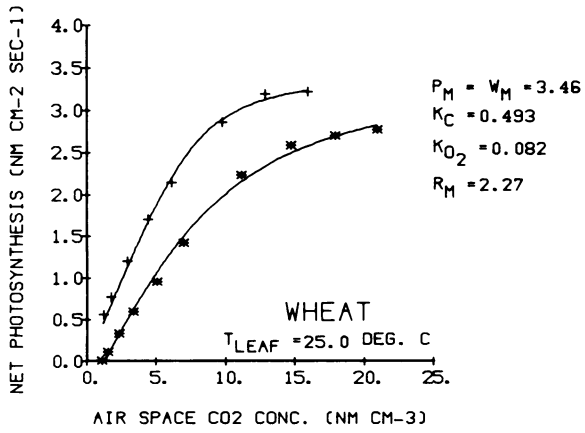


FIG. 2. Analysis is according to equation 1 of the wheat data of Ku and Edwards (13) obtained at 25 C with $CO_2$ concentration expressed in nmol $cm^{-3}$ in the intercellular air space and $O_2$ expressed as per cent. Symbols are Ku and Edwards' observations at 1% (+) and 21% (*) $O_2$. Solid lines are predicted according to equation 1 with the parameter values shown. $P_M$ and $W_M$ in nmol $cm^{-2}$ $s^{-1}$, $K_C$ in nmol $cm^{-3}$, $K_{O_2}$ as a partial pressure (decimal fraction) = 0.082 = 8.2% $O_2$, and $R_M$ in s $cm^{-1}$. Light intensity = 0.15 µE $cm^{-2}$ $s^{-1}$. NM = nmol.

and diffusion resistances are the same regardless of the way in which gas concentrations are expressed.

Due to simplifications in the analysis of Ku and Edwards, we do not agree with their conclusion that differential temperature effects on the carboxylase and oxygenase functions do not play a major role in temperature regulation of whole leaf net photosynthesis; we regard the possibility that temperature may play a role, proposed by Badger and Andrews (1) and Laing *et al.* (14), as theoretically sound and consider it still an open question.

Before proceeding, a few general comments are in order. The importance of RuBP carboxylase-oxygenase in the over-all regulation of carbon fixation emphasizes that an analysis based on the properties of RuBP carboxylase-oxygenase provides an effective means of describing whole leaf net photosynthesis. Although the actual values of parameters determined in that analysis may in fact reflect properties of that enzyme determined *in vitro*, they are only effective values of the whole leaf system (5, 18). We do make comparisons between the $K_C$ or $K_{O_2}$ of our analysis and the respective carboxylase or oxygenase affinities determined *in vitro*, but we do not simply equate the two.

On the basis of the present analysis, which is consistent with a system such as that proposed by Bowes *et al.* (2), it is possible to obtain values for the leaf affinity constants for $CO_2$ and $O_2$ as used in equation 1. In this analysis, the effect of diffusion on the

concentration of $CO_2$ at the carboxylation site is included at least in the simplest fashion, net photosynthetic rate is a function of this calculated value, and the nonlinear and saturation portions of response curves are included. The first of these with the iterative solution for net photosynthesis overcomes a major difficulty (estimation of $C_c$) that has plagued all other considerations of the problem to date. Including the nonlinear and saturation portions of the response is essential because it provides the only means of solving for the values of the five parameters required to describe both $O_2$ and $CO_2$ dependencies. Including the saturation portion also allows linking of this analysis with the $P_M$ surface described previously (32).

From Figure 1 it is apparent that the $K_C$ predicted by our method is an order of magnitude lower than that obtained by Ku and Edwards (13). In each case the magnitude of the affinity constant is simply a direct result of the model imposed. Ku and Edwards derived their affinity constants for $CO_2$ by fitting a transformation of the Michaelis-Menten equation to the observed data at 1% $O_2$. The photosynthesis model in that case was the Michaelis-Menten equation and the value obtained varies between 1 and 10 $\mu$M. If a model is used which includes a diffusion resistance, the affinity coefficient decreases by an order of magnitude.

Using a model which includes a diffusion resistance, Jones and Slatyer (11) reported values for the affinity coefficient for whole leaves of cotton as low as 0.23 $\mu$M. Shown in Figure 3 are determinations of $K_C$ from a Jones and Slatyer analysis (11) of $CO_2$ response curves at 1% $O_2$ for kidney bean measured by the authors in a manner similar (32) to the response curves of Ku and Edwards. The results of a similar analysis of the wheat data of Ku and Edwards and of Joliffe and Tregunna (10) are included. Note that with one exception the values for $K_C$ vary between 0.1 and 1.0 nmol $cm^{-3}$. Joliffe and Tregunna's data (connected by the solid line in Fig. 3), which are by far the best from the population response standpoint (mean of 80 seedlings in each case), suggest a temperature dependency in $K_C$ similar to the temperature dependency for the enzyme $K_m(CO_2)$ for RuBP carboxylase-oxygenase observed by Laing *et al.* (14). The final data point at 40 C is estimated to be around 3 nmol $cm^{-3}$. These data were not obtained at steady-state which may distort the response curves. The scatter of data points (Fig. 3) reemphasizes a point already made by Ku and Edwards that "the degree of temperature-dependent variation in factors controlling photosynthesis in $C_3$ plants at the cellular level such as affinity for $CO_2$, nature of $O_2$ inhibition of photosynthesis, maximum velocity of photosynthesis based on enzyme potential, and solubility of $O_2$ and $CO_2$ is uncertain." To this list we add values for our parameters $K_{O_2}$, $R_M$, and $W_M$. The variability observed in Figure 3 underscores the need for extreme care in future determinations of $CO_2$ concentrations and corresponding photosynthesis rates.

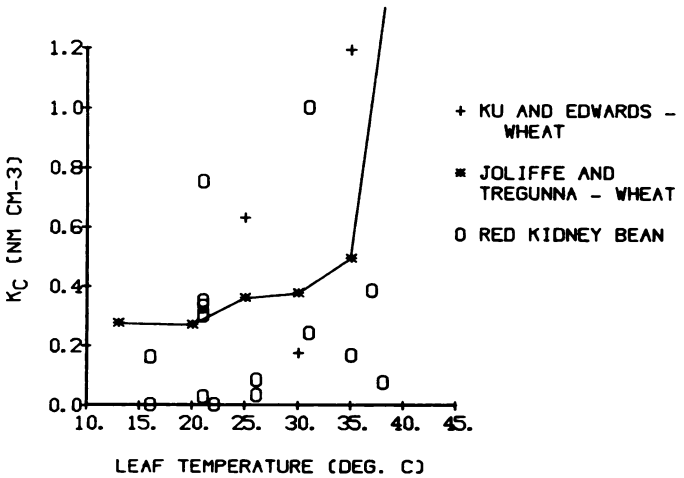


FIG. 3. Determinations of whole leaf affinity for $CO_2$ ($K_C$) at various leaf temperatures from the net photosynthesis *versus* air space $CO_2$ concentration response curves at 1% $O_2$ in red kidney bean (symbol = 0). Parameter estimates obtained from a Jones and Slatyer (11) type analysis modified for a nonlinear least squares approach (29, 31, 32). Similar values from the data of Ku and Edwards (13) are presented for wheat considering only the 1% response curve (+). Values obtained from analysis of Joliffe and Tregunna's data (10) at 1.8, 60.9, 78.6, and 99% $O_2$ according to the methods of this paper are connected by the solid line. Joliffe and Tregunna's data are mean values for 80 seedlings (final data point at 40 C ≃ 3 nmol $cm^{-3}$). Light intensity was high. NM = nmol.

Based on the above considerations, we feel that it is inappropriate to conclude as did Ku and Edwards that the temperature dependence of RuBP carboxylase-oxygenase activity is unimportant in the regulation of whole leaf net photosynthesis. Further application of the analysis presented above may help clarify the role of RuBP carboxylase-oxygenase in temperature regulation of whole leaf net photosynthesis but even this is uncertain. Different estimates for the affinity constants of the leaf for $CO_2$ and $O_2$ are obtained if the sources of photorespired $CO_2$ are assumed to be located further from the chloroplast, if dark respiration fluxes are included in the analysis, or if the function assumed to describe photorespiration is modified (30). These changes in the estimates of affinity coefficients are understandable because each modification of the model alters the relationship of net photosynthesis to $CO_2$ concentration at the fixation site. Final conclusions about affinity coefficients must await further information on respiratory fluxes determined independently of photosynthesis and on diffusion relationships between cellular organelles.

As with the model of Ku and Edwards, it is possible with our model to calculate net photosynthetic flux, photorespiratory flux, and total photosynthetic flux for any combination of external $CO_2$ and $O_2$ concentrations and to determine the contributions to net photosynthetic response. Several characteristics are thus illustrated that apply to a model of photosynthesis based on the Bowes-Ogren hypothesis These characteristics are strongly determined by the competitvie terms in equation 1 and are inconsistent with models that have assumed that competition between $CO_2$ and $O_2$ at low $CO_2$ concentration is negligible (23, 27) or that the rate of photorespiration is unaffected by $CO_2$ concentration below 300 $\mu$l/l $CO_2$ as observed by Ludwig and Canvin with the $^{14}CO_2$ technique (22).

In equation 1, the predicted photorespiratory rate always decreases as air space $CO_2$ concentration is increased above zero. The predicted photorespiration at 25 C based on nmol $cm^{-3}$ for $C_w$ and per cent for $[O_2]$ is shown in Figure 4. Contributions to

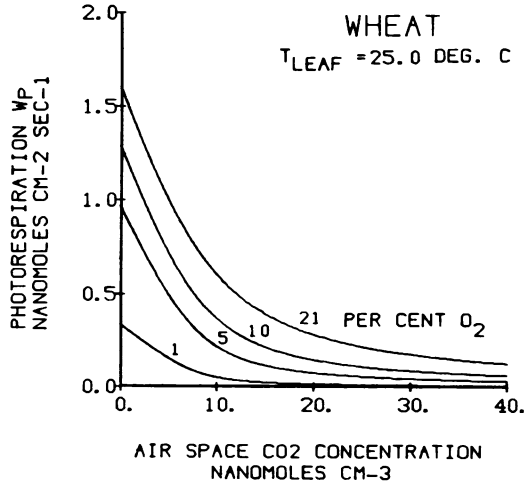


FIG. 4. Predicted relationship of photorespiration to air space $CO_2$ concentration at 1, 5, 10, and 21% $O_2$. This case corresponds to the solution shown in Figure 2 where $CO_2$ concentration is expressed in nmol $cm^{-3}$ and $O_2$ concentration is expressed in per cent. See Figure 2 for parameter values and units.

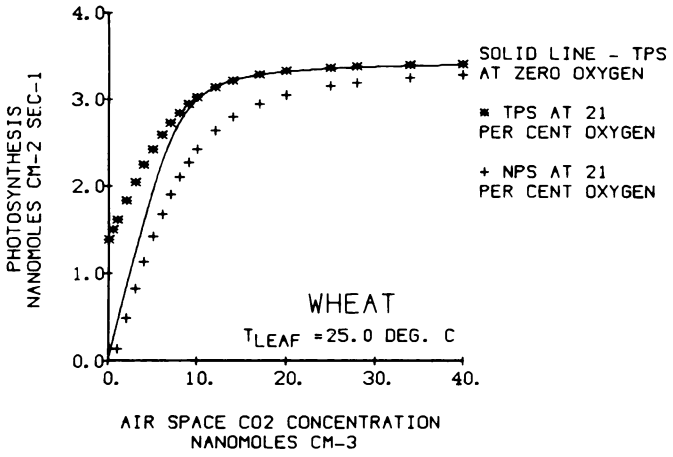


FIG. 5. Simulated contributions to the net photosynthesis response of wheat according to the solution presented in Figures 2 and 4 at 25 C as a function of air space $CO_2$ concentration. Solid line is the true or total photosynthetic rate (TPS) at 0% $O_2$ or with no photorespiration occurring. *Symbols indicate the true photosynthetic rate at 21% $O_2$ (net photosynthesis plus photorespiration) and demonstrate the stimulation at low $CO_2$ concentration effected by photorespiration. + symbols indicate the predicted net photosynthesis (NPS) due to loss of photorespiratory $CO_2$ from the leaf.

the net photosynthetic response at 21% $O_2$ due to competition for RuBP carboxylase-oxygenase and due to photorespiration are shown in Figure 5. With the photorespiratory site close to the fixation site, net photosythetic rate at zero air space $CO_2$ concentration and low $O_2$ is essentially zero rather than negative as suggested by Figure 4 because of recycling of $CO_2$. The analysis of Ku and Edwards does not account for recycling $CO_2$ and total photosynthesis at 21% $O_2$ can never be higher than the total (or net) photosynthesis at low $O_2$. With the present analysis, the chloroplast $CO_2$ concentration remains high due to respiratory $CO_2$ production and diffusion resistance to the escape of $CO_2$. According to our model, it would only be possible to achieve the photosynthetic $CO_2$ response indicated by the solid line in Figure 5 if photorespiration did not occur. When $O_2$ concentration is increased to 21%, net photosynthesis decreases at all $CO_2$ concentrations (+ symbols). At low air space $CO_2$ concentration, photorespiratory $CO_2$ is available for recycling and results in a stimulation of true photosynthesis (* symbols).

Although it is clearly speculative to extrapolate on the basis of this analysis in light of its shortcomings (30), the model results are nevertheless interesting. Since a leaf behaving according to this model uses photoproducts more effectively even at low $CO_2$ concentration in the intercellular air space, photorespiration of such a leaf might protect the leaf reaction centers from possibly damaging effects of excess photoproducts during periods of water stress and high irradiance as suggested by Osmond and Björkman (24). The calculated rate of total photosynthesis (rate of carboxylation or Calvin cycle cycling) at zero $CO_2$ concentration in the intercellular air space might be considered a measure of this possible protective ability of photorespiration and is obviously a function of $O_2$ concentration. One determines from equation 1, little protective ability at low $O_2$ concentrations and maximal protective ability at higher concentrations near normal ambient concentration.

Our analysis, which includes both kinetics and diffusion, provides more realistic estimates of whole leaf affinities for $CO_2$ and $O_2$, estimates of mesophyll resistance, and maximum rates of photosynthesis and photorespiration at one temperature than have been previously obtained. The analysis provides a mathetical description of the net photosynthetic response which separates the simultaneous effects of $O_2$ and $CO_2$ on the rates of the two processes, photosynthesis and photorespiration. The complexity of determining the temperature dependencies is illustrated by the scatter of estimated $K_C$ (Fig. 3). From our studies it appears inappropriate to draw conclusions on the effect of temperature of the leaf affinity coefficients at this time. We provide evidence that expression of $CO_2$ concentrations as nmol $cm^{-3}$ and $O_2$ as per cent is adequate for determination of kinetic constants and that the results are readily convertible to dissolved gas concentrations of interest in the study of enzyme data.

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## APPENDIX

To calculate net photosynthetic and photorespiration rates at steady-state, three equations must be balanced. At a constant $O_2$ concentration, the following iterative estimation of $CO_2$ concentration ($C_c$) at the chloroplast fixation site balances these equations and provides the desired rates in the final step. Consider the parameters $P_M$, $W_M$, $K_C$, $K_{O_2}$, and $R_M$ to be known from the analysis by nonlinear least squares as described in the text. (See equation 1 of text for definitions.) An initial value of $C_c$ is chosen and used in equation A.1 to calculate the rate of photorespiration ($W_P$).

$$W_P = \frac{W_M[O_2]}{[O_2] + K_{O_2}\left(1 + \frac{C_c}{K_C}\right)} \qquad \text{A.1}$$

Then $W_P$ is used in the net photosynthesis equation A.2.

$$P = \frac{AA - \{(AA)^2 - BB\}^{1/2}}{2R_M} \qquad \text{A.2}$$

where:

$$AA = C_w + K_C\beta + R_M(P_M - W_P) - W_P\left(\frac{R_M}{1 + M}\right)$$

$$BB = 4R_M\left[\left(C_w - W_P\frac{R_M}{1 + M}\right)(P_M - W_P) - W_PK_C\beta\right]$$

$$\beta = \left(1 + \frac{[O_2]}{K_{O_2}}\right)$$

$$M = 100$$

The values obtained for $W_P$ and P are then used in Fick's Law equation to calculate a new $C_c$ compatible with these fluxes (equation A.3).

$$C_c = C_w - P\,R_M - W_P\left(\frac{R_M}{1 + M}\right) \qquad \text{A.3}$$

The second estimate of $C_c$ is compared to the first and used again in equation A.1 until the change in $C_c$ between iteration steps is acceptably small. Calculations of this sort are possible by hand but are extremely tedious. In the above fashion, the predicted responses of net photosynthesis *versus* $C_w$ at different $O_2$ concentrations shown in Figure 1 are obtained.