

Development of a Photosynthesis Model with an Emphasis on Ecological Applications

II. Analysis of a Data Set Describing the P_M Surface

J. D. Tenhunen, J. A. Weber, C. S. Yocum, and D. M. Gates

Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan 48104, USA

Summary. Photosynthesis was measured in leaves of *Phaseolus vulgaris* and analyzed according to the set of equations outlined previously by Tenhunen et al. (1976).

Introduction

In a previous publication (Tenhunen et al., 1976), a theoretical model was elaborated for photosynthetic and respiratory processes in a leaf of a C_4 plant or a C_3 plant under special conditions. The model was designed to minimize problems confronted in experimentation. With further development, the model will function as an ecological tool allowing description of the photosynthetic system of particular leaves from data obtained in field studies of limited duration. The photosynthetic system is described so that sub-processes of metabolism are detailed. This should prove useful in investigating complex ecological problems such as acclimation responses to light or temperature changes.

The initial steps in model development call for the description of a three dimensional surface (termed the P_M surface) where total estimated photosynthesis is a function of incident light and leaf temperature. This is accomplished by measuring light response curves of net photosynthesis over a range of leaf temperatures, from well below the absolute photosynthetic maximum to well above it, while the carbon dioxide concentration is maintained at a saturating level and oxygen is maintained at 1.5% so that photorespiration is essentially turned off (see Tenhunen et al., 1976). Measurements of net photosynthesis to provide such a data set are reported here. These data are analyzed according to the formulation suggested previously.

Methods

1. Plant Growth

Due to the length of time involved in measurement of photosynthesis responses, the data necessary for description of the P_M surface must be derived from many leaves. Photosynthetic and respiratory

responses of leaves are strongly influenced by the stage of leaf development, genetic constitution, and environmental factors experienced during growth. We attempted to produce a population of leaves in which the complications introduced by these factors were minimized (for further discussion and another approach to this problem of variation see Chmora and Oya, 1967a).

A selected strain of red kidney beans (*Phaseolus vulgaris* var. Charlevoix) was used because of its low genetic variability and its rapid growth to usable size. Plants to be studied were further selected for uniformity by using only bean seeds 15 to 17 mm long. Seed size has been shown to be correlated with seedling photosynthetic and respiratory responses (Burriss et al., 1973). Selected seeds were germinated between moist paper towels in the dark at 20° C for 4 days and then placed on aluminum screens on top of 21 battery jars containing complete Hoagland's medium (made according to the method of Wagner and Cumming, 1970). These plants were then placed in the plant conditioning chamber. Water levels were maintained in the battery jars with distilled water and the Hoagland solution was changed every 2-3 days. The roots were constantly aerated. No deficiency symptoms were ever noticed.

A growth chamber was constructed so that plants would experience relatively constant conditions with respect to incident radiation, air temperature, relative humidity, and concentration of carbon dioxide in the air during their development prior to measurement of photosynthesis (a period of approximately 14 days). A chest type freezer with a 1/4 h.p. motor provided the actual chamber box and most of the cooling. The temperature within the chamber cycled between 21 and 24° C day and night. To raise the humidity to acceptable levels and maintain constant carbon dioxide concentration, a flow of air (from an outside source) was constantly passed into the chamber and escaped from the chamber through any available passageways. This air was first saturated with water vapor at room temperature (25° C) and then passed into the chamber, some of it being channeled by a network of tubing and sparging tubes into the battery jars containing plants. The relative humidity fluctuated between 65 and 75 % as the temperature fluctuated within the chamber. The carbon dioxide concentration remained between 300 and 330 ppm.

Plants were grown under cycles of 12 h light and 12 h dark. Lighting was provided with a bank of ten cool white fluorescent lights (General Electric-F48/T 12/CW/1500) and four Sylvania 75 W tubular incandescent bulbs. Incident photosynthetically active radiation was maintained between 0.029 and 0.035 $\mu\text{E cm}^{-2} \text{s}^{-1}$.

2. Photosynthesis Measurements

Overview. A plant and its battery jar assembly was supported so that the middle leaflet of the first compound leaf could be inserted into a plexiglass measurement chamber. The roots were aerated constantly, initial leaflet area was recorded, and the leaflet was sealed into the chamber with Mortite putty. Leaf temperature, light intensity, and gas concentrations were controlled while water vapor and carbon dioxide concentration changes due to transpiration and photosynthesis were measured in a gas stream flowing through the chamber. Final leaflet area was recorded at the end of each experiment.

Lighting. Light was provided by two 500 Watt PAR 56 Q NSP quartz iodide lamps (Sylvania). Voltage to the lamps was maintained at 150 Volts with a Superior 236B 240V variable transformer. A large plexiglass box was placed two feet below the lamps and filled with two inches of circulating water to remove heat. A Bausch and Lomb 90-32 polished glass heat filter was laid immediately on top of the leaf chamber during measurement. Light intensity was changed by inserting blackened screens in the light path above the Bausch and Lomb filter. Photosynthetically active radiation (PhAR) within the chamber incident on the leaf was measured constantly during experimentation with a silicon cell placed next to the leaf and wired to a multipoint recorder. This cell was calibrated often with a PhAR quantum sensor (Lambda Inst., Lincoln, Nebraska) placed in the leaf chamber.

Gas Analyzer Calibration. Two infra-red gas analyzers were used in the measuring system to determine carbon dioxide concentration changes. A Beckman Model 864 with 0-500 and 0-1,500 ppm ranges was used in the absolute mode to determine the absolute concentration of carbon dioxide in the gas stream. The analyzer was calibrated by passing known gas mixtures of 0, 59, 238, 330, 530, 605, 996, 1,603, and 2,025 ppm carbon dioxide in nitrogen through it at 780 mm Hg pressure. A sealed nitrogen

cell was used as reference. A family of calibration curves for a Beckman 315 differential carbon dioxide analyzer was generated by passing through the analyzer a series of known differentials (generated by mixing reference gas with nitrogen on the "low" side and holding the reference gas constant on the "high" side) for each of the standard calibration gases listed above. It was thus possible to determine for any reference gas, the concentration difference causing an observed recorder deflection. From this concentration difference (caused during experimentation by passing gas on the sample side first to the leaf chamber), photosynthesis rates were calculated.

All gas was dried with magnesium perchlorate before entering the gas analyzers. Water manometers were inserted in the two flow paths immediately after the differential analyzer and maintained at 780 mm Hg pressure (ambient pressure was always less than 780 mm Hg) during all measurements with needle valve adjustments. This proved to be the most effective way of controlling pressure effects in the gas analyzers. This pressure was also maintained during calibration. Flow rates were maintained between 500 and 700 cc/min. In this range, no effects of flow rates on any calibrations of the apparatus were observed.

Water Vapor Concentration. Humidification of the gas stream was accomplished by passing it through a gas washing bottle half full of distilled water at room temperature. The dew point of gas flowing into the chamber was adjusted by passing the humidified gas through a condenser in a temperature bath controlled by a Honeywell Elektronik One-Eleven controller. The dew point of gas exiting the chamber (also assumed equal to the dew point of gas external to the leaf in the chamber) was measured immediately with a dew point hygrometer (Model 880, Cambridge Systems Inc.). The gas was then dried with magnesium perchlorate and entered the gas analyzers as described above.

Leaf Chamber and Temperature Measurements. During measurement the leaf was placed in a five inch diameter plexiglass chamber one inch deep surrounded by a plexiglass water jacket. The air was well stirred by a fan located under a nylon network supporting the leaf. Calculated boundary layer resistance was very low at all times and is of little importance in these experiments as long as internal carbon dioxide concentration remained at saturating levels. Leaf temperature was sensed by several thermocouples (copper-constantan-36 gauge) on the undersurface. Leaf temperature was held constant with a Honeywell Elektronik One-Eleven controller that controlled the temperature of water in the plexiglass jacket surrounding the leaf chamber. All other temperatures in the system were measured with copper-constantan thermocouples and recorded on a West dual range multipoint recorder.

For each steady-state observation time, the rate of transpiration, the diffusion resistance for water, the concentration of carbon dioxide in the air external to the leaf, the rate of photosynthesis and the concentration of carbon dioxide at the cell walls of internal leaf cells were calculated. The observed raw data and methods of calculation are reported in detail by Tenhunen (1976).

Results

1. Plant Growth

The partitioning of biomass at all developmental stages from seeds to very large plants was observed. Total plant dry weight, dry weight of leaf, stem and support, and root tissue, and total leaf area were determined. The resulting measurements are shown graphically in Figure 1. Dry weight initially decreased due to respiration before photosynthetically active tissue was present. Then dry weight of stems, roots, and leaves increased linearly up to plant total dry weight of 4.0 g (28 days after imbibition). Total leaf area (total photosynthetically active surface) also appeared to increase linearly. A second subset of plants was selected and daily measurement of the area of each leaf was made by tracing the leaves onto paper, cutting out the paper, and weighing it. The increase in leaf area of individual primary leaves (undivided in bean plants) and first, second, and third trifoliate

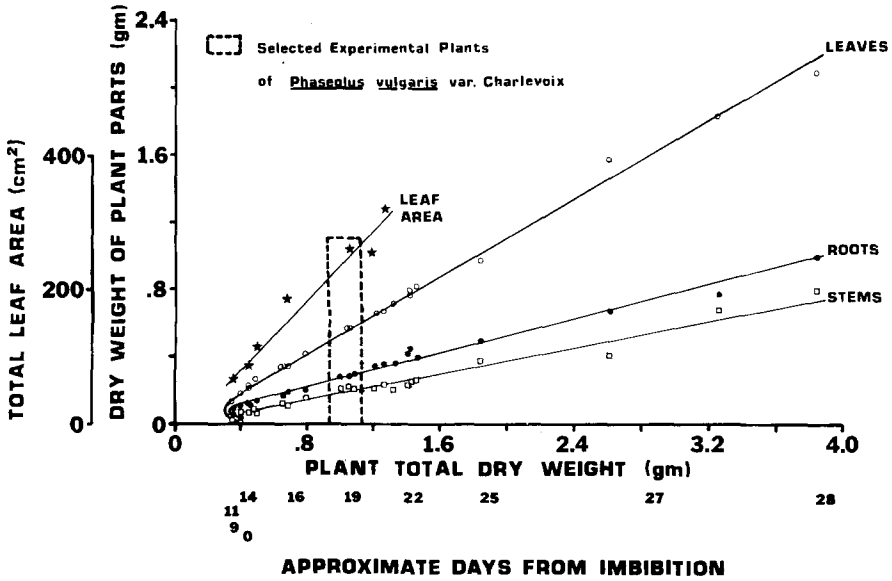


Fig. 1. Dry weight of plant parts and total leaf area of plants developing in the growth chamber as a function of time after imbibition and plant total dry weight

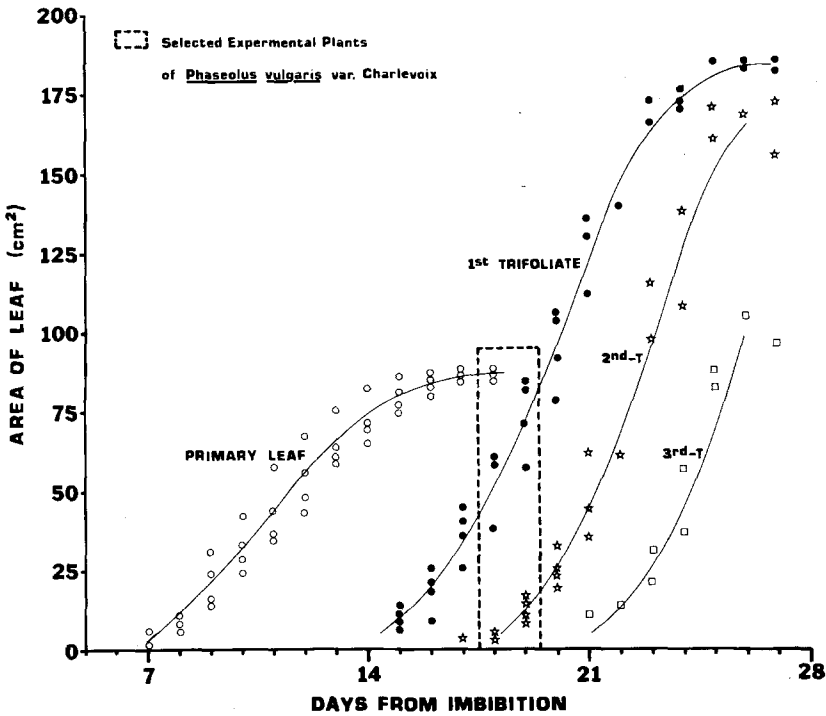


Fig. 2. Total area of the primary leaf and first, second, and third trifoliolate leaves of plants developing in the growth chamber as a function of time after imbibition

leaves as a function of time from germination (imbibition) is essentially sigmoid (Fig. 2). Plants from the boxed area in Figures 1 and 2 were selected as suitable material for the measurement of photosynthesis.

Seedling development was extremely regular. All plants measured developed in a similar manner with respect to leaf area. Slight differences with respect to time after imbibition may be due to small differences in time of day of planting, amount of water available during the 4-day dark germination, or actual seedling differences. These small differences presented no problem. The middle leaflet of the first trifoliolate leaf was selected for experimentation. A leaflet between 15 and 25 cm² was appropriate for the measurement chamber corresponding to a plant whose first trifoliolate total leaf area was between 35 and 70 cm². During experimentation, the leaf may expand as much as 10%. The measured photosynthesis rates were corrected for leaf area changes by assuming a linear change in leaf area during measurement and calculating on this basis the leaf area at the time individual measurements were taken.

2. *Photosynthesis Rates*

Carbon dioxide concentration was maintained at a saturating level (> 1,200 ppm) and oxygen concentration at 1.5%. Twenty-one light response curves at different temperatures were obtained according to the methods outlined. The incident radiant fluxes in $\mu\text{E cm}^{-2} \text{s}^{-1}$ and net photosynthesis rates in $\text{nM cm}^{-2} \text{s}^{-1}$ along with the temperature of observation are given in Table 1.

The light response curves are typical of light responses at high carbon dioxide concentration with a linear portion, curved portion, and plateau. It is difficult to reach the plateau at high temperature under these conditions and in certain instances it was not reached even though light intensity approached a value approximately equal to full sun light and almost 10 times as high as the light intensity in the growth chamber.

Analysis of the Photosynthesis Data

1. *Residual Respiration*

The initial portion of light response curves is linear due to limitation by the photoreactions (see Tenhunen et al., 1976). The initial portion of carbon dioxide response curves is linear due to mesophyll resistance to carbon dioxide transfer (Jones and Slatyer, 1972). Because of these linearities, estimates of the magnitude of residual respiration were made by linear extrapolation of the initial parts of the light response curves and also of several carbon dioxide response curves to a point below zero net photosynthesis. These estimates correspond to respiration occurring when incident light or carbon dioxide concentration is zero and are presented in Table 2.

If the extrapolated residual respiration is independent of light intensity and carbon dioxide concentration, as is assumed in our model, it is constant over the

Table 1. Light response curves of net photosynthesis (P_N) in *Phaseolus vulgaris* at saturating carbon dioxide concentration, 1.5% oxygen, and various leaf temperatures (T_L). P_N in nanomoles $\text{cm}^{-2} \text{s}^{-1}$; L in $\mu\text{Einsteins cm}^{-2} \text{s}^{-1}$

$T_L = 15.6^\circ \text{C}$		$T_L = 15.8^\circ \text{C}$		$T_L = 20.6^\circ \text{C}$	
L	P_N	L	P_N	L	P_N
0.177	0.976	0.169	1.08	0.177	1.90
0.108	0.897	0.104	1.08	0.111	1.87
0.068	0.912	0.063	0.988	0.067	1.79
0.043	0.868	0.036	0.927	0.041	1.68
0.030	0.846	0.022	0.819	0.027	1.57
0.017	0.715	0.012	0.588	0.017	1.22
0.010	0.464			0.012	0.811
0.007	0.320			0.010	0.683
$T_L = 20.9^\circ \text{C}$		$T_L = 25.6^\circ \text{C}$		$T_L = 25.9^\circ \text{C}$	
L	P_N	L	P_N	L	P_N
0.171	1.62	0.179	1.98	0.170	2.22
0.101	1.58	0.112	1.94	0.102	2.13
0.060	1.46	0.070	1.90	0.059	1.87
0.036	1.26	0.044	1.78	0.060	1.86
0.022	0.934	0.026	1.30	0.035	1.42
0.012	0.596	0.018	0.834	0.021	0.888
		0.011	0.506	0.012	0.512
$T_L = 30.4^\circ \text{C}$		$T_L = 30.6^\circ \text{C}$		$T_L = 31.0^\circ \text{C}$	
L	P_N	L	P_N	L	P_N
0.178	2.66	0.203	2.62	0.175	2.93
0.113	2.53	0.125	2.50	0.105	2.68
0.072	2.40	0.076	2.36	0.063	2.14
0.043	1.94	0.047	1.76	0.036	1.49
0.026	1.25	0.028	1.16	0.021	0.912
0.015	0.753	0.017	0.718	0.012	0.497
0.010	0.404	0.011	0.430		
		0.007	0.258		
$T_L = 35.4^\circ \text{C}$		$T_L = 35.5^\circ \text{C}$		$T_L = 36.0^\circ \text{C}$	
L	P_N	L	P_N	L	P_N
0.179	3.20	0.204	3.19	0.198	3.16
0.112	3.08	0.125	3.04	0.172	3.34
0.070	2.72	0.076	2.66	0.105	2.88
0.044	1.92	0.047	1.96	0.065	2.28
0.026	1.17	0.029	1.32	0.040	1.52
0.016	0.637	0.017	0.816	0.022	0.876
0.010	0.323	0.010	0.482	0.013	0.491
		0.007	0.299		

Table 1 (continued)

$T_L = 38.2^\circ \text{C}$		$T_L = 40.1^\circ \text{C}$		$T_L = 40.7^\circ \text{C}$	
L	P_N	L	P_N	L	P_N
0.204	3.44	0.195	3.46	0.205	3.45
0.123	3.24	0.167	3.34	0.125	3.25
0.078	2.70	0.101	2.84	0.076	2.66
0.046	1.89	0.063	2.21	0.046	1.85
0.029	1.18	0.038	1.45	0.029	1.20
0.017	0.709	0.022	0.853	0.017	0.728
0.010	0.410	0.012	0.477	0.010	0.428
0.007	0.257				
$T_L = 40.7^\circ \text{C}$		$T_L = 42.5^\circ \text{C}$		$T_L = 44.1^\circ \text{C}$	
L	P_N	L	P_N	L	P_N
0.173	3.47	0.199	3.37	0.174	2.28
0.108	3.27	0.122	3.42	0.106	2.29
0.066	2.51	0.078	2.94	0.066	2.10
0.041	1.69	0.056	2.32	0.041	1.60
0.026	1.05	0.049	2.21	0.025	1.07
0.017	0.634	0.034	1.54	0.017	0.700
0.009	0.323	0.022	1.01	0.011	0.436
		0.016	0.689		
$T_L = 44.2^\circ \text{C}$		$T_L = 44.6^\circ \text{C}$		$T_L = 44.7^\circ \text{C}$	
L	P_N	L	P_N	L	P_N
0.194	3.13	0.219	2.90	0.204	2.36
0.173	2.95	0.138	2.68	0.125	2.27
0.106	2.54	0.088	2.43	0.074	1.99
0.064	1.95	0.060	2.00	0.044	1.41
0.038	1.29	0.039	1.26	0.028	0.926
0.021	0.743	0.024	0.835	0.017	0.566
0.012	0.417	0.019	0.588	0.009	0.311

entire response curve. In such a case, the rate of total photosynthesis is obtained by adding this extrapolated estimate of respiration to measured net photosynthesis rates. This procedure will lead to incorrect estimates of total photosynthesis if carbon dioxide is being recycled within the leaf. On the other hand, even at the highest temperatures of measurement, the observed (extrapolated) rate of respiration is only 1/10 to 1/20 of maximum net photosynthesis rates. Hence any error in estimating residual respiration will have only a very small effect in the over-all determination. It was observed that the initial slopes of the carbon dioxide response curves at different light intensities coincide (Tenhunen, 1976) as carbon dioxide concentration is decreased to zero, suggesting a common extrapolated rate for respiration at all light intensities. Estimating respiration

Table 2. Residual respiration estimates for *Phaseolus vulgaris* leaves at saturating CO₂ for light response and constant light for CO₂ response estimates. Oxygen concentration = 1.5%

From light response curves		From CO ₂ response curves	
T _L (°C)	Respiration rate (nanomoles cm ⁻² s ⁻¹)	T _L (°C)	Respiration rate (nanomoles cm ⁻² s ⁻¹)
15.6	0.0	15.9	0.0
15.8	0.0	16.1	0.0
20.6	0.0	20.4	0.0
20.9	0.0	20.5	0.0
25.6	0.0847	20.9	0.0
25.9	0.0317	21.3	0.0
30.4	0.0876	21.7	0.0
30.6	0.0	21.8	0.0
31.0	0.0979	25.7	0.0
35.4	0.100	25.8	0.0
35.5	0.166	30.8	0.0300
36.0	0.180	31.0	0.00912
38.2	0.238	31.4	0.0
40.1	0.135	35.6	0.279
40.7	0.182	36.1	0.0
42.5	0.462	37.8	0.299
44.1	0.341	37.8	0.343
44.2	0.277		
44.6	0.588		
44.7	0.197		

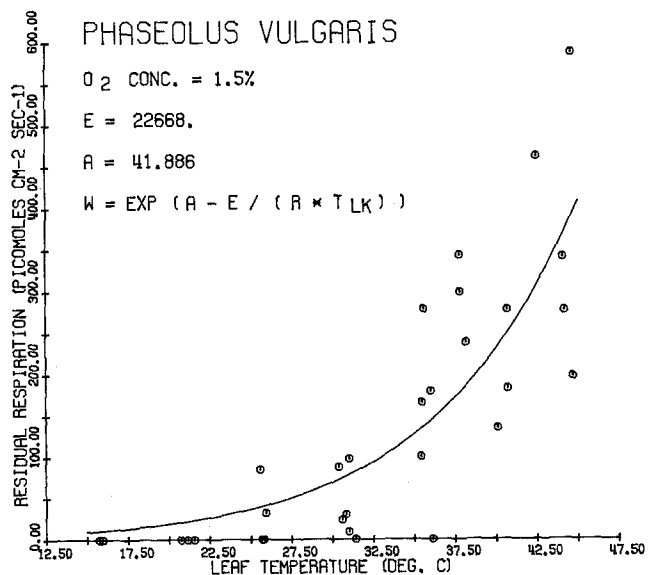


Fig. 3. Residual respiration as a function of leaf temperature. Solid line is the best fit function obtained from non-linear regression analysis according to the Arrhenius equation, $E=22,668$ calories/mole; $A=41.886$. Symbols are observed values. Rate must be multiplied by $J=1$ picomole $\text{cm}^{-2} \text{s}^{-1}$ to obtain proper units

by means other than extrapolation (biochemical methods) is much too time consuming to be practical for ecological modeling purposes and this method must suffice.

Residual respiration is described by the Arrhenius equation (shown in Fig. 3) in our model. Best values for the parameters E and A [see Tenhunen et al., 1976, Eq. (5); E is the apparent activation energy of residual respiration and A is an empirically determined constant] were obtained by non-linear regression analysis with a statistical program provided by The University of Michigan Statistical Laboratory [BMDX85; written by Paul Sampson of the Health Sciences Computing Facility at U.C.L.A. and described by Dixon (1969); see also Hartley, 1961; Jennrich and Sampson, 1968]. Initial estimates of E and A (obtained from an Arrhenius plot of transformed variables for those cases where extrapolated respiration is not equal to zero) and partial derivatives of the function to be fit with respect to the parameters to be estimated (here E and A) must be provided in a subroutine. All 38 values in Table 2 were considered in the solution. The predicted function for respiration as a function of leaf temperature is shown in Figure 3 superimposed on the scattered observations. This result is discussed further below.

2. Corrected Light Response Curves

Extrapolated residual respiration for each response curve was added to the observed net photosynthesis rates. *Total estimated photosynthesis* as a function of photosynthetically active radiation (PhAR) was thus determined for each of twenty-one light response curves. The unique aspect of this data set is that it provides related data that allow study of light and temperature dependencies simultaneously and that it is obtained at saturating carbon dioxide concentration with negligible photorespiration occurring.

3. Smith Equation: An Adequate Description of the Light Response

In the late 1930's there was considerable interest in analytical description of light response curves of photosynthesis. Emil Smith (1937, 1938) observed that light response curves in general approached the maximum more rapidly than expected, i.e. curvature occurs very rapidly. Interestingly enough, the data that Smith was studying were obtained at very high concentrations of carbon dioxide. The empirical expression that Smith derived to describe the light response in the presence of high carbon dioxide concentration is the following:

$$P_M = \frac{C \cdot P_{ML} \cdot L}{(1 + C^2 L^2)^{1/2}} \quad (1)$$

where: P_M is the rate of photosynthesis at a *specific* L , saturating carbon dioxide and a specific leaf temperature,

P_{ML} is the rate of photosynthesis at *saturating* L , saturating carbon dioxide and a specific leaf temperature,

C is an empirically determined constant,

L is the photosynthetically active radiant flux ($\mu\text{Einstein cm}^{-2} \text{ s}^{-1}$).

This expression is shown to adequately describe the light responses observed here.

As stated above, C is an empirically determined constant of obscure meaning. An alternative and more useful form of the above expression is obtained in the following manner. The first derivative of the Smith equation is:

$$\frac{dP_M}{dL} = \frac{C \cdot P_{ML}}{(1 + C^2 L^2)^{1/2}} - \frac{C^3 L^2 P_{ML}}{(1 + C^2 L^2)^{3/2}}. \quad (2)$$

Taking the limit as $L \rightarrow 0$, we find the initial slope α :

$$\lim_{L \rightarrow 0} \frac{dP_M}{dL} = \alpha = C \cdot P_{ML} \quad (3)$$

then,

$$C = \frac{\alpha}{P_{ML}} \quad (4)$$

substituting into Equation (1):

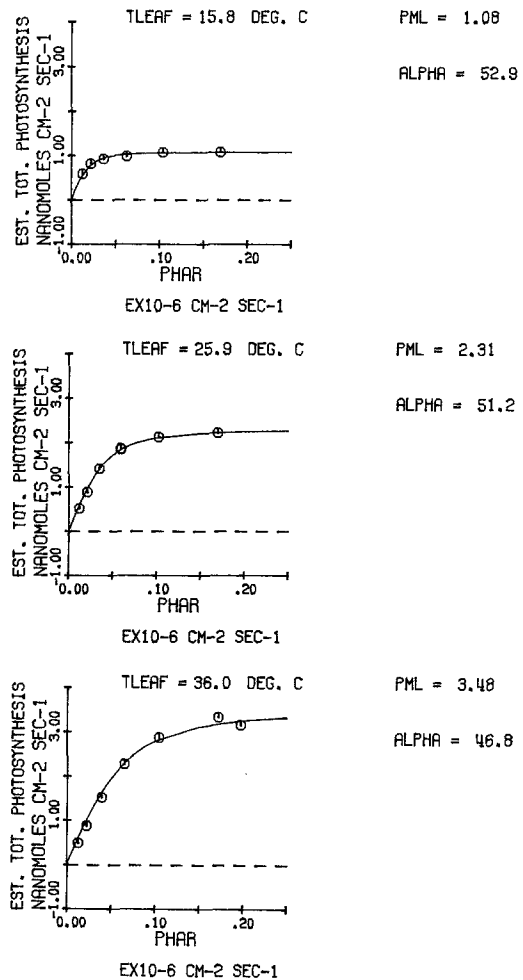
$$P_M = \frac{\alpha L}{\left(1 + \frac{\alpha^2 L^2}{P_{ML}^2}\right)^{1/2}} \quad (5)$$

where: α is the maximum efficiency of light energy conversion (Chartier, 1970).

Table 3. Best non-linear least squares determinations of parameters of the modified Smith equation as described in the text

T_L (°C)	α and P_{ML} free to take any value $\alpha = 50$ nM/ μ E		
	α (nM/ μ E)	P_{ML} (nM cm ⁻² s ⁻¹)	P_{ML}
15.6	56.0	0.950	0.957
15.8	52.9	1.08	1.09
20.6	87.9	1.91	2.00
20.9	52.9	1.65	1.66
25.6	66.4	2.01	2.08
25.9	51.2	2.31	2.33
30.4	60.3	2.75	2.84
30.6	50.2	2.73	2.74
31.0	48.1	3.13	3.10
35.4	54.1	3.39	3.54
35.5	54.2	3.33	3.41
36.0	46.8	3.48	3.45
38.2	49.2	3.68	3.70
40.1	42.4	3.79	3.64
40.7	48.7	3.79	3.86
40.7	49.9	3.66	3.70
42.5	56.0	3.61	3.77
44.1	54.1	2.40	2.44
44.2	36.0	3.44	3.15
44.6	40.7	3.07	2.95
44.7	41.3	2.47	2.41

Fig. 4. Corrected photosynthetic light responses. Symbols are observed rates for data corrected for residual respiration. Solid lines are the best fit functions obtained from non-linear regression analysis according to the modified Smith equation where P_{ML} and α are set at the values indicated



Equation (5) is useful for the purpose of description of the P_M surface. The unknowns are the initial slope or maximum light utilization efficiency and the maximum capacity for carboxylation.

A test to see whether the equation could be used to describe light responses was undertaken. Best parameter values for α and P_{ML} for the twenty-one corrected light responses were derived with the BMDX85 non-linear least squares program described. These values are given in Table 3. Typical examples of the predicted fits to the corrected photosynthesis data for selected response curves are shown graphically in Figure 4. The agreement between observed and predicted values is extremely good.

It is known from very careful experiments of Emerson and Lewis (1941) (also found here in preliminary analysis) that the initial slope (quantum efficiency) of light response curves (when photosynthesis is plotted versus *absorbed* radiation) is independent of temperature. Therefore, α was restricted to a constant value and

the data were reanalyzed. With $\alpha = 50$ nM/ μ E (a value close to the mean value determined for the 21 response curves), an excellent description of the responses was still obtained even though the error mean square values increased slightly as expected. P_{ML} values predicted for $\alpha = 50$ nM/ μ E are also presented in Table 3. P_{ML} values considered as a function of leaf temperature conform generally to the expected pattern. This relationship is similar to that of any enzymatically governed reaction with respect to temperature.

4. The Temperature Dependency of the Light Maxima

The temperature dependency of the light maxima (see Tenhunen et al., 1976, Eq. (8) for further details) $I(T_K)$ can be studied in detail once the values of P_{ML} have been obtained from the analysis of light response curves. $I(T_K)$ was studied for P_{ML} values determined from the Smith equation fits with $\alpha = 50$ nM/ μ E. P_{ML} values were analyzed according to the methods outlined by Johnson et al. (1954) and described for this function $I(T_K)$ in our previous publication. Values were divided by a best guess estimate of P_{MLT} (the maximum point on the curve) and then multiplied by 100. $\Delta H \ddagger$ (the energy of activation for the photosynthesis reaction) can be estimated from the slope of a regression obtained for $\ln(P_{ML}/P_{MLT} \times 100)$ as a function of inverse absolute leaf temperature, where leaf temperature is well below the optimum. From the data of Table 3, $\Delta H \ddagger$ is estimated $\approx 11,000$ calories per mole.

ΔH_1 (the energy of activation for denaturation of the photosynthesis reaction) is estimated from the slope of a regression for those data of $\ln(P_{ML}/P_{MLT} \times 100)$ where rate has been reduced to 20 to 40% of maximum due to denaturation. However, problems were encountered in operation of the measuring apparatus at temperatures above 40°C and the data do not provide this estimate even though the temperature optimum (maximum rate) was clearly surpassed. As an alternative approach, ΔH_1 was assumed equal to each of four widely differing values (200,000; 100,000; 50,000; and 25,000 calories per mole). ΔS (the entropy of the denaturation equilibrium) and C_2 (an empirical constant; see Johnson et al., 1954) were then calculated for $\Delta H \ddagger = 11,000$ calories/mole and $\Delta H_1 =$ four different values. At the optimal temperature (maximum):

$$\frac{\Delta H \ddagger + RT_K}{\Delta H_1 - \Delta H \ddagger - RT_K} = e^{-\Delta H_1/RT_K} e^{\Delta S/R} \quad (6)$$

where ΔS is the only unknown. Further:

$$\text{rate} = 100 = \frac{C_2 T_{K(\text{max rate})} e^{-\Delta H \ddagger/RT_{K(\text{max})}}}{1 + e^{-\Delta H_1/RT_{K(\text{max})}} e^{\Delta S/R}} \quad (7)$$

where C_2 is the only unknown.

As ΔH_1 is changed from 200,000 calories/mole to 25,000 calories/mole, there is an improvement in the fit of the predicted function to the observed data. It was assumed that ΔH_1 is between 25,000 and 50,000 calories/mole. A subroutine was written for the non-linear least squares program previously described that allowed

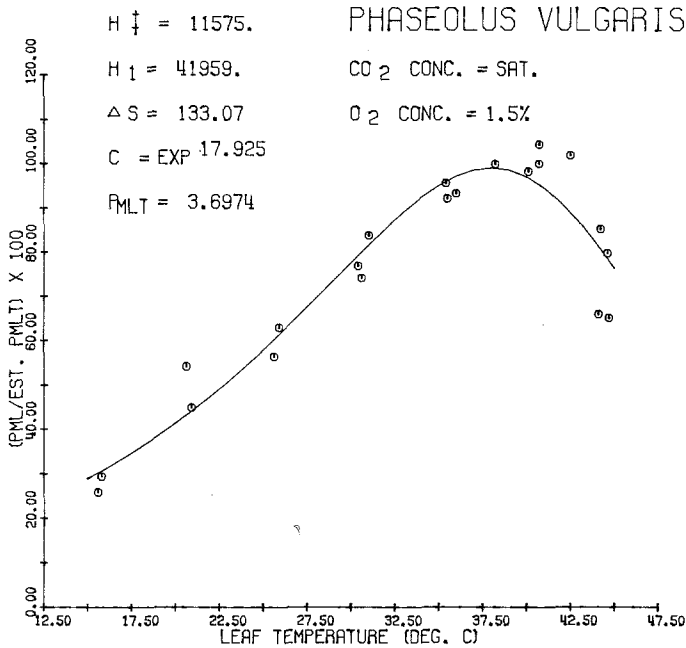


Fig. 5. Best fit of $I(T_K)$ (temperature dependency of P_{ML}) from non-linear regression analysis of the derived P_{ML} values presented in Table 3 where $\alpha = 50 \text{ nM}/\mu\text{E}$ (modified Smith equation). Parameter values determined as indicated

iteration with respect to $\Delta H \ddagger$, ΔH_1 , ΔS , and C_2 . Initial estimates determined in preliminary analysis of $\Delta H \ddagger = 11,000$ calories/mole, $\Delta H_1 = 39,000$ calories/mole, $\Delta S = 124$ entropy units, and $C_2 = e^{17.0}$ were used.

Best values for the parameters returned from the program and the response predicted when these values are substituted into the $I(T_K)$ equation described previously are shown in Figure 5 superimposed on the P_{ML} data values. From Figure 5, the conformity of the data to the $I(T_K)$ formulation is seen to be very good and the formulation is applicable. There is however scatter in the data above the maximum which can cause a problem in determination of P_{MLT} . Both reversible and irreversible denaturation processes have been shown for over-all photosynthesis and may contribute to this scatter depending on the duration of the experiments. The $I(T_K)$ equation used here does not apply to irreversible denaturation and it may be necessary in the future to use more rapid methods to measure the rate of photosynthesis.

Discussion and Conclusions

The following system of equations corresponding to those presented in our previous publication (Tenhunen et al., 1976) adequately describe photosynthesis (gross and net photosynthesis) under the restricted environmental conditions imposed in this research. Under conditions of saturating carbon dioxide and

1.5% oxygen, the rate of photosynthesis (I) at saturating light (expressed as a decimal fraction of the absolute maximum rate times 100) is given by:

$$I = \frac{T_K e^{17.925 - (11575/1.987 \times T_K)}}{1 + e^{(133.07/1.987) - (41959/1.987 \times T_K)}} \quad (8)$$

where: I is dimensionless,

T_K is the absolute leaf temperature ($^{\circ}$ Kelvin).

In nanomoles $\text{cm}^{-2} \text{s}^{-1}$, the rate of photosynthesis (P_{ML}) at saturating light and a specific temperature is given by:

$$P_{ML} = 3.6974 \text{ nM cm}^{-2} \text{ s}^{-1} \times \frac{I}{100} \quad (9)$$

where: I is determined in Equation (8).

The activation energy of the forward over-all photosynthetic reaction has been estimated previously by many researchers from Arrhenius plots (see Rabinowitch, 1951, pp. 1236-1237). Estimates have clustered around values of 10,000 to 12,000 calories per mole which is in good agreement with the value estimated here of 11,575 calories/mole. We are unaware of other estimates of the parameters in Equation (8) for a photosynthetic system except for P_{MLT} . $P_{MLT} = 3.6974 \text{ nM cm}^{-2} \text{ s}^{-1}$ agrees well with a value for tobacco of approximately $4.35 \text{ nM cm}^{-2} \text{ s}^{-1}$ (Hesketh, 1963) and with estimates made by others (see Rabinowitch, 1951, pp. 990-991).

The form of the Smith function used to describe the light response allows one to easily set the value of the initial slope α . With α equal to a constant, the error mean square (EMS) increases slightly over EMS values obtained when both α and P_{ML} are free to take on any value. EMS values (considering the set of 21) tend to pass through a minimum with $\alpha \approx 50 \text{ nM}/\mu\text{E}$. This value for α agrees well with a value of approximately $60 \text{ nM}/\mu\text{E}$ determined by Hall (1970) for *Beta vulgaris*.

The rate of photosynthesis at less than saturating light (P_M) and at a specific leaf temperature is given by:

$$P_M = \frac{50 \times L}{\left(1 + \frac{50^2 L^2}{P_{ML}^2}\right)^{1/2}} \quad (10)$$

where: P_{ML} depends on leaf temperature and is determined by Equation (9),

L is the incident photosynthetically active radiant flux ($\mu\text{E cm}^{-2} \text{ s}^{-1}$)

The response of photosynthesis to light has often been described by an equation analogous to the Michaelis-Menten equation. The Michaelis-Menten equation in various forms has been suggested as part of the structure of various models (Waggoner, 1969; Horn, 1971; Lommen et al., 1971) and has been used successfully to describe the light response of many different species of plants (Hesketh and Moss, 1963; Monteith, 1963; Monsi and Saeki, 1953; Chartier et al., 1970; Tamiya, 1951) [equations of the form $P = 1/(a + b/L)$ or $P = \alpha L P_M / (P_M + \alpha L)$ are different forms of the Michaelis-Menten equation]. While the Michaelis-

Menten equation may in fact adequately describe a light response under certain conditions, it is not satisfactory when considering the present data collected at 1.5% oxygen and saturating carbon dioxide. The light responses observed consist of two portions that are very linear over an extensive light intensity range, connected by a curving portion. If one considers the first derivative of the Michaelis-Menten equation, it is apparent that it is unsuitable for such a response (see also Bannister, 1974).

Equations (8)–(10) describe a three dimensional surface that has been termed the P_M surface. It is a fixed surface reference when considering data at lower carbon dioxide concentration and higher oxygen concentration if those two variables interact competitively. The surface is shown in two dimensions in Figure 6. Total estimated photosynthesis as a function of leaf temperature is shown for several constant light intensity values. The maximum for each curve (photosynthetic optimum) occurs as it must in this system of equations at the temperature of P_{MLT} .

The quantum efficiency (moles carbon fixed/Einstein absorbed radiation) corresponding to the light utilization efficiency $\alpha = 50 \text{ nM}/\mu\text{E}$ was approximated. The average % transmitted light was approximately 7%. The ratio of transmitted PhAR to reflected PhAR is fairly constant for plant leaves (Moss and Loomis, 1952; Rabideau et al., 1946; Yocum et al., 1964). This ratio (reflected PhAR/transmitted PhAR) from Yocum et al. (1964) was found to equal approximately 2.65. Using 2.65 for the bean leaves studied here, reflected PhAR must approximately equal 18% of incident. In total 25% is reflected or transmitted and 75% is absorbed.

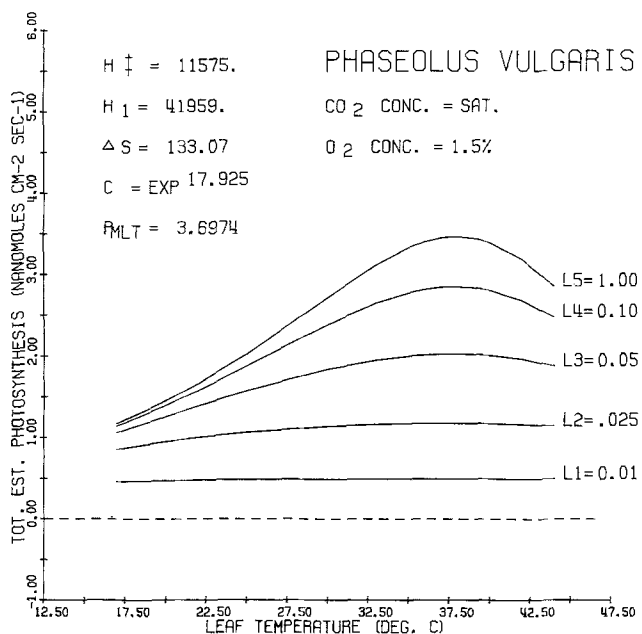


Fig. 6. The P_M surface projected onto the total photosynthesis and leaf temperature plane. Solid lines are equal photosynthetically active radiant flux lines ($PhAR$) as indicated ($L1, L2 \dots$) in $\mu\text{Einstein cm}^{-2} \text{ s}^{-1}$. Surface is based on analysis of light curves according to the Smith equation with $\alpha = 50 \text{ nM}/\mu\text{E}$

The quantum efficiency is then $50 \text{ nM}/0.75 \text{ } \mu\text{E}$ or $1/15$ moles per Einstein. This value falls in the middle of a range of values given by Rabinowitch (1951, p. 1096) for various horticultural plants.

Residual respiration is determined according to the Arrhenius equation:

$$W = J \cdot e^{(41.886 - (22,668/1.987 \times T_K))} \quad (11)$$

where: W is the residual respiration rate in picomoles $\text{cm}^{-2} \text{ s}^{-1}$,

J provides the proper units = $1 \text{ picomole } \text{cm}^{-2} \text{ s}^{-1}$.

This extrapolated respiration is believed to reflect active mitochondrial respiration. However, a contribution of the photorespiratory process to this residual can not be ruled out. One can only say that residual respiration is not strongly dependent on light intensity. There is adequate evidence that mitochondrial respiration occurs in the light (Raven, 1972; Marsh et al., 1965). Estimates of that activity from biochemical studies of turnover of intermediates (Raven, 1972) indicate its magnitude to be similar to the residual respiration rates found by extrapolation. The apparent energy of activation (E) found here, on the other hand, is higher than the activation for dark respiration in *Rhizobium* (13,000 calories/mole) found by Koffler et al. (1947). The value determined here (22,668 calories/mole) is at the high end of a range of values for dark respiration given by Forward (1960). The Q_{10} for residual respiration is likewise higher (approximately 3+) than most Q_{10} values that have been observed for dark respiration in algae and higher plant leaves (usually around 2) (Van der Paauw, 1934; Stålfelt, 1939; Altman and Ditmer, 1966). Since the true meaning of residual respiration is impossible to elaborate at this time, the high activation energy or Q_{10} is not understood. Regulation of mitochondrial respiration in the light is a very complex process (Reid, 1970; Raven, 1972) and it is difficult to even speculate on the effect of temperature on that regulation.

Since carbon dioxide has not yet been considered, P_M replaces P in the final equation (see Tenhunen et al., 1976) and net photosynthesis is given by:

$$P_N = P_M - W \quad (12)$$

where: P_M and W are determined in Equations (10) and (11),

P_N is the rate of net photosynthesis in $\text{nM } \text{cm}^{-2} \text{ s}^{-1}$.

Again a three dimensional surface is described that represents the originally observed data. This surface is shown in two dimensions in Figure 7. Net photosynthesis as a function of leaf temperature is shown for several constant light intensity values. On this surface, a shift occurs in the optimum temperature of photosynthesis with increasing light intensity. A similar phenomenon has often been observed for net photosynthesis at normal carbon dioxide and oxygen concentration (Lange, 1969; see especially Pisek et al., 1973). It will be of interest to determine whether the shift under normal conditions can be accounted for by additive effects of photosynthetic and respiratory processes as suggested by the data obtained here.

We are aware of only one other set of experiments that relates to the P_M surface. Using a radiometric method, Chmora and Oya (1967b) described a version of the P_M surface for maize (Figs. 5 and 7 of their work) though not analytically. Chmora and Oya claim that respiration did not affect their measure-

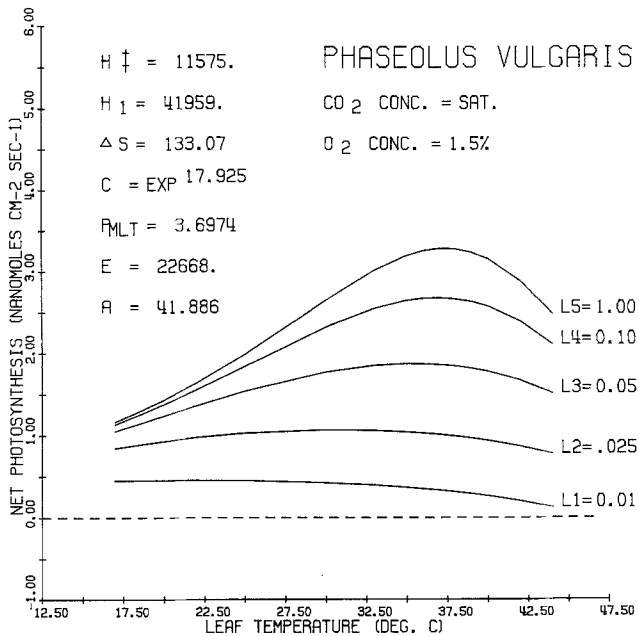


Fig. 7. Net photosynthesis surface (P_M surface minus residual respiration) at saturating CO_2 and 1.5% oxygen projected onto the net photosynthesis and leaf temperature plane. Solid lines are equal photosynthetically active radiant flux lines ($PhAR$) as indicated ($L1, L2 \dots$) in $\mu\text{Einsteins cm}^{-2} \text{ s}^{-1}$. Surface is based on analysis of light curves according to the Smith equation with $\alpha = 50 \text{ nM}/\mu\text{E}$

ments but this is uncertain from the description of methods given. They demonstrate effects that could be interpreted as being caused by changes in stomatal resistance (Chmora and Oya, 1967a; their Fig. 8). It is possible therefore that a pool of respired C^{12}O_2 existed in the intercellular air spaces of their leaves that would introduce an error in measurement. Nevertheless, their results in general support the statements above about Figures 6 and 7.

Acknowledgements. This research was supported by a grant from the Ford Foundation and by contract AT(11-1)-2164 from the Energy Research and Development Administration. Dr. Wayne Adams, Department of Agronomy, Michigan State University, is gratefully acknowledged for providing the selected strain of *Phaseolus vulgaris* var. Charlevoix for this study.

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Received June 29, 1976