

LIMITING FACTORS IN PHOTOSYNTHESIS: LIGHT AND CARBON DIOXIDE

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I

INTRODUCTION

It was F. F. Blackman (1905) who first recognized that in photosynthesis where the "process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the 'slowest' factor".¹ In terms of this idea it was possible to identify two processes in photosynthesis, one, a photochemical reaction, and the other, a temperature-sensitive (Blackman) reaction (Warburg, 1919, 1920; Emerson and Arnold, 1932), both involving chlorophyll in a cycle. Using this cycle as a first approximation, kinetic descriptions have been developed for some of the properties of photosynthesis (*e.g.*, Baly, 1935; Burk and Lineweaver, 1935; Smith, 1937). However, no complete description, either experimental or theoretical, has yet been given of the interrelationships of the different factors which may limit the photosynthesis rate.

The present paper deals with light intensity and CO₂ concentration as limiting factors. We intend first, to show that this relationship may be derived from the equations which we have used to describe other properties of photosynthesis; and second, to present a series of measurements which have been made to test the validity of these ideas.

II

Theoretical

We have shown (Smith, 1937) that the measurements of photosynthesis rate (p) as a function of light intensity (I) or of CO₂ con-

¹ Full accounts of the controversy over Blackman's ideas are given by both Stiles (1925) and Spoehr (1926) in their monographs.

centration at the stationary state can be described by the expression:

$$p = k_1 I (a^2 - x^2)^{1/2} = k_2 [\text{CO}_2] x \quad (1)$$

where a may be regarded as representing the total concentration of chlorophyll, and x the amount of chlorophyll activated by light. The terms containing I and $[\text{CO}_2]$, describe the velocities of the light and dark processes. If x is eliminated and equation (1) is solved for p as a function of I at constant $[\text{CO}_2]$, or as a function of $[\text{CO}_2]$ at constant I , we obtain equations which describe accurately the available data. In logarithmic form, these equations are:

$$\log p = \log p_{ma} - 1/2 \log \left(1 + \frac{1}{K_1^2 I^2} \right) \quad (2)$$

and

$$\log p = \log p_{mb} - 1/2 \log \left(1 + \frac{1}{K_2^2 [\text{CO}_2]^2} \right) \quad (3)$$

where the maximum photosynthesis rates, $p_{ma} = k_2 [\text{CO}_2] a$ and $p_{mb} = k_1 I a$; $K_1 = k_1/k_2 [\text{CO}_2]$ and $K_2 = k_2/k_1 I$. If $\log p$ is plotted against $\log I$ (or $\log [\text{CO}_2]$), the shape of the curve obtained is independent of the constants K and p_m .

This curve is linear at low intensities, gradually curving to a maximum photosynthesis rate at high intensities. This maximum varies with the CO_2 concentration. A precise way of determining the limiting conditions is to secure a family of curves relating photosynthesis and intensity at different CO_2 concentrations and from them to find the relationship between the intensity and the CO_2 concentration required to produce a definite photosynthesis rate. A family of CO_2 -photosynthesis curves at different intensities can be treated similarly. If equation (1) has more than *ad hoc* value, it should be possible to predict from it the nature of the relationship to be expected.

Starting with equation (1), x may be eliminated by substituting $p/k_2 [\text{CO}_2]$. The expression is then solved for $[\text{CO}_2]$ as a function of I when p is constant. This yields in logarithmic form

$$\log A_2 [\text{CO}_2] = -1/2 \log \left(1 - \frac{1}{A_1^2 I^2} \right) \quad (4)$$

where $A_1 = k_1a/p$ and $A_2 = k_2a/p$. Equation (4) may be plotted as $\log [\text{CO}_2]$ against $\log I$ giving a curve whose shape is independent of the constants A_1 and A_2 which define the asymptotes. Reversing the position of I and $[\text{CO}_2]$ in the equation yields the same function, so that either variable can be considered as dependent or independent.

Equations similar to (4) but having somewhat different shapes may be obtained by changing the exponents in equation (1). Where the terms for the light and dark processes are those of a simple first order nature, as in

$$p = k_1I(a - x) = k_2[\text{CO}_2]x \quad (5)$$

solving at constant p yields

$$\log A_2[\text{CO}_2] = -\log \left(1 - \frac{1}{A_1I} \right) \quad (6)$$

Where the exponents are second order, as in

$$p = k_1I(a - x)^2 = k_2[\text{CO}_2]x^2 \quad (7)$$

solving as before, gives

$$\log A_2[\text{CO}_2] = -2 \log \left(1 - \frac{1}{A_1^{1/2} I^{1/2}} \right) \quad (8)$$

The properties of equations (6) and (8) are similar to those of (4) and can be treated in the same way. The curves obtained from the three equations are drawn for comparison in Fig. 1. In addition to these three curves, many others of different curvature may be obtained by changing the exponents for the light and dark processes. Thus, all curves obtained on the basis of a two process cycle are in agreement with the idea of limiting factors; the form of the curve depends on the nature of the functions which describe the light and dark reactions.

The $\log I$ and $\log [\text{CO}_2]$ asymptotes represent the minima necessary to produce a definite photosynthesis value. When either of these two variables is greater than the necessary minimum, the magnitude of the other factor can be reduced accordingly, until finally its minimum is reached. Since the rate of curvature depends on the kinetic properties of the light and dark processes, information on this point can be obtained from the data. It should be emphasized that such data present information somewhat different from that given by an

investigation of the effect of a single variable. For the effect of light intensity (or $[\text{CO}_2]$) on photosynthesis, equations (1) and (4) give curves which have the same slope at low intensities. The two equations differ only in their rate of curvature at high photosynthesis

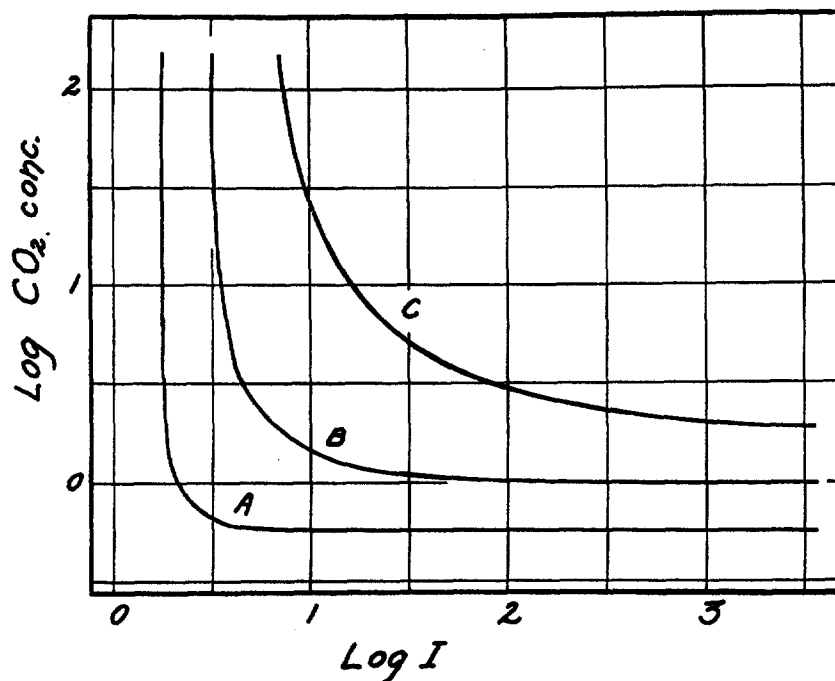


FIG. 1. The relation between light intensity and CO_2 concentration necessary for a constant amount of photosynthesis. Curves *A*, *B*, and *C* represent equations (4), (6), and (8). The three curves are drawn to asymptotes 0.25 log units apart.

values as they approach the maximum rate. By using the data of CO_2 concentration *versus* light intensity, the kinetics of the process can be independently evaluated at all measured values of the photosynthesis rate.

III

RESULTS

In order to test the theoretical curves developed in section II, it is necessary to have families of curves for photosynthesis at different CO_2

concentrations and light intensities. The data for four different photosynthesis values taken from earlier measurements (Smith, 1936; 1937) are presented in Fig. 2 and Table I. The curve for equation (4) has been drawn through the data.

Fig. 2 shows good general agreement with the theoretical expectation at high photosynthesis values, but the range which these measurements cover yields insufficient information in the transitional region

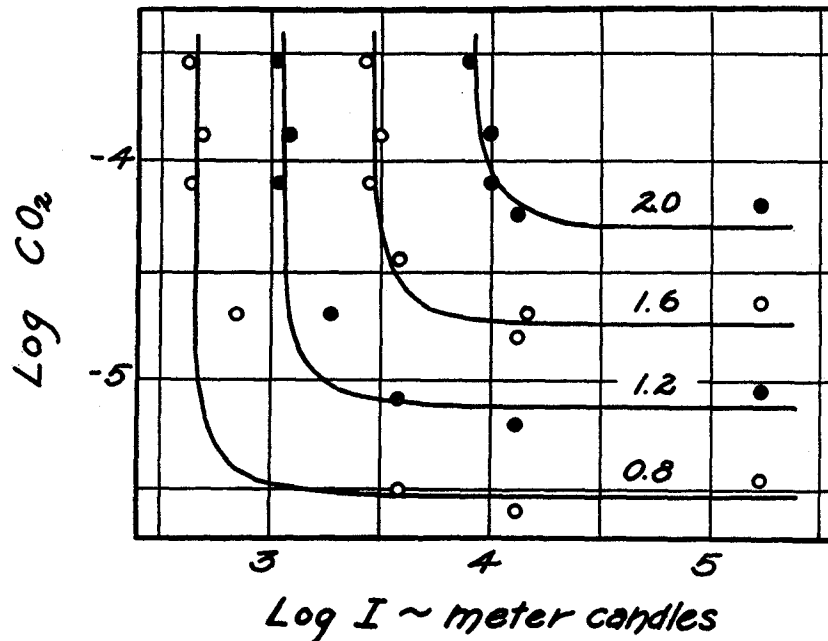


FIG. 2. The intensity and CO₂ concentration at four log photosynthesis values given by the numbers on the curves. The curve drawn is theoretical and is from equation (4). The data are taken from Smith (1937) and are in Table I.

between the asymptotes at low photosynthesis values; this is precisely where the least decisive evidence is given by the curves relating intensity (or [CO₂]) and photosynthesis. In addition, these data are expressed in terms of wet weight of tissue, and there may be 20 to 30 per cent variation in photosynthesis rate, thus affecting the relative position of each curve on the ordinate.

A new series of measurements to eliminate these two objections was

therefore undertaken. The photosynthesis of the fresh water plant *Cabomba caroliniana* was studied as in the previous investigation, using the Warburg apparatus with the same methods for the control of light intensity and CO₂ concentration. All of the measurements were made at 25.3°C.

In order to eliminate the variation caused by the use of different fronds, a measurement of the photosynthetic activity of each frond was made under standard conditions: [CO₂] = 2.90 × 10⁻⁴ moles per liter (Warburg buffer No. 11), *I* = 123,000 meter candles. All of the

TABLE I

Intensity and CO₂ Concentration for Constant Photosynthesis

These data drawn in Fig. 2 represent interpolated values from the measurements given in Tables III and IV of an earlier publication (Smith, 1937). The intensities in Table IV of that paper have been corrected for the absorption of the red filter (Corning No. 246) as determined by measuring photosynthesis-intensity curves on the same plant with white and with red light. The effective absorption of the filter as determined twice was 0.22 log units. Bold-face values are for the factor that was constant in the measurements.

log <i>p</i> = 0.8		log <i>p</i> = 1.2		log <i>p</i> = 1.6		log <i>p</i> = 2.0	
log [CO ₂]	log <i>I</i>	log [CO ₂]	log <i>I</i>	log [CO ₂]	log <i>I</i>	log [CO ₂]	log <i>I</i>
-5.50	3.58	-5.08	3.58	-4.45	3.58	—	—
-5.59	4.12	-5.20	4.12	-4.80	4.12	-4.24	4.12
-5.45	5.23	-5.05	5.23	-4.64	5.23	-4.20	5.23
-4.69	2.84	-4.69	3.27	-4.69	4.17	—	—
-4.10	2.63	-4.10	3.03	-4.10	3.45	-4.10	4.00
-3.88	2.68	-3.88	3.08	-3.88	3.49	-3.88	4.00
-3.54	2.62	-3.54	3.02	-3.54	3.43	-3.54	3.90

data were then corrected in terms of an assigned arbitrary photosynthesis value of 200 c. mm. of oxygen produced per hour per 100 mg. wet weight of tissue for the standard determination. This value is within 5 per cent of the average actually found.

To cover a sufficient range, measurements were made at five light intensities and six CO₂ concentrations. Within a single experiment, the photosynthesis of a frond was investigated as a function of light intensity at a constant CO₂ concentration, and then repeated for one or two additional CO₂ concentrations. Three runs were made at

each CO_2 concentration, a total of eighteen for the series, and the data averaged. While the data were all obtained as photosynthesis at different intensities, they may also be used to obtain the CO_2 curves at constant intensity. Two complete series of such measurements were made; they are presented in Table II.

To find the light intensity necessary to attain a definite amount of photosynthesis at a constant CO_2 concentration, or the converse, it is

TABLE II

Photosynthesis at Different Intensities and CO_2 Concentrations

Data of Figs. 3 and 4. Photosynthesis in cubic millimeters of oxygen evolved per hour per 100 mg. wet weight of material corrected for respiration. Temperature = 25.3°C . CO_2 concentrations $\times 10^6$ in moles per liter. All of the data are in terms of a standard value of 200 when the $[\text{CO}_2] = 290 \times 10^{-6}$ and $I = 123,000$ meter candles. Each set of data represents the averages of three similar experiments.

Series	Intensity	Photosynthesis rate					
		$[\text{CO}_2] = 4.48$ Buffer No. 5	$[\text{CO}_2] = 8.67$ Buffer No. 6	$[\text{CO}_2] = 20.5$ Buffer No. 7	$[\text{CO}_2] = 37.5$ Buffer No. 8	$[\text{CO}_2] = 78.7$ Buffer No. 9	$[\text{CO}_2] = 290$ Buffer No. 11
I	<i>meter candles</i>						
	407	2.89	3.03	3.03	3.36	4.08	3.66
	1,740	7.59	9.62	13.7	16.5	17.0	18.7
	6,310	9.75	19.2	33.7	45.9	62.1	62.5
	21,900	9.91	21.0	43.8	66.4	119	150
	123,000	10.4	21.1	46.5	71.5	145	200
II	407	2.06	3.21	3.40	3.74	3.67	4.49
	1,740	7.16	11.2	14.2	19.5	17.4	20.5
	6,310	10.8	20.4	35.9	54.7	55.2	68.4
	21,900	11.9	23.2	48.3	79.1	119	159
	123,000	11.6	23.5	48.4	84.9	146	200

necessary to interpolate between the measured values. To do this, there was drawn through all of the data, the smooth curve of equation (2). That this curve gives a satisfactory description of these data is shown in Figs. 3 and 4. In Fig. 3 are presented the data of series I for photosynthesis as a function of intensity. All of the data have the curve of equation (2) drawn through them.

The mass plots of Fig. 4 contain all of the data in Table II. The

curve of equation (2) was drawn through all of the log photosynthesis versus log I data at the different CO_2 concentrations. These curves

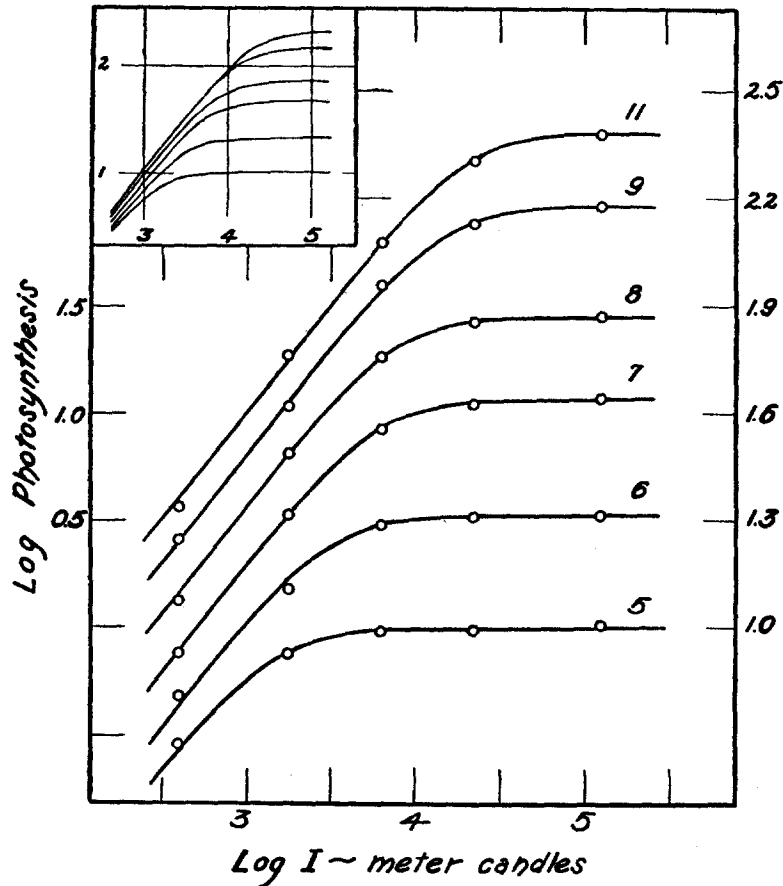


FIG. 3. Photosynthesis as a function of light intensity for different CO_2 concentrations indicated on each curve by the Warburg buffer number. The photosynthesis ordinates are correct only for the uppermost curve; the others have been displaced downwards in steps of 0.2 of a log unit, with their correct positions given on the right side of the figure. The insert shows the absolute positions of the six curves drawn to exactly half the ordinates. All of the curves are drawn from equation (2). These are from the data of series I given in Table II.

were then superimposed and the points traced on a single graph. This is possible because the shape of the curve is invariant in form.

The same procedure was also used for the log photosynthesis—log CO_2 data, except for the measurements at the lowest light intensity (407 meter candles) which are omitted because they were not sufficiently precise to determine the position of the curve. The excellent fit of these new data incidentally confirms and strengthens the validity of equations (2) and (3) as quantitative descriptions of the effect of these two variables on photosynthesis.

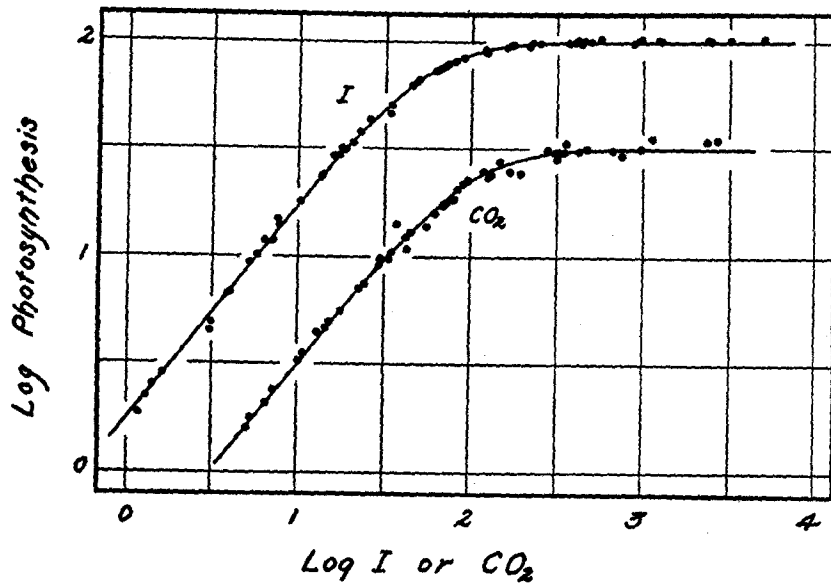


FIG. 4. Photosynthesis as a function of intensity (upper curve) and of CO_2 concentration (lower curve). The points represent all of the data in Table II. The curve from equation (2) was drawn through all of the different sets of data. These were then superimposed and traced on a single graph.

In Fig. 5 and Table III are presented the data obtained for the log CO_2 concentration—log intensity relationship at four log photosynthesis values. These four values were chosen to express best the actual data obtained. The lowest photosynthesis value is near the lower limit of the actual measurements, and in a few cases represents a small extrapolation. The higher values of the log photosynthesis rate were selected to eliminate successively one or more curves from consideration, thus giving whatever real changes in form occur in low as compared with higher photosynthesis rates.

Equation (4) has been drawn through the data in Fig. 5. A good description of the data is obtained at the three higher photosynthesis rates, as is also the case for the older data given in Fig. 2. However, at the lowest photosynthesis value, a much better description is given by equation (6), which is drawn in broken lines.

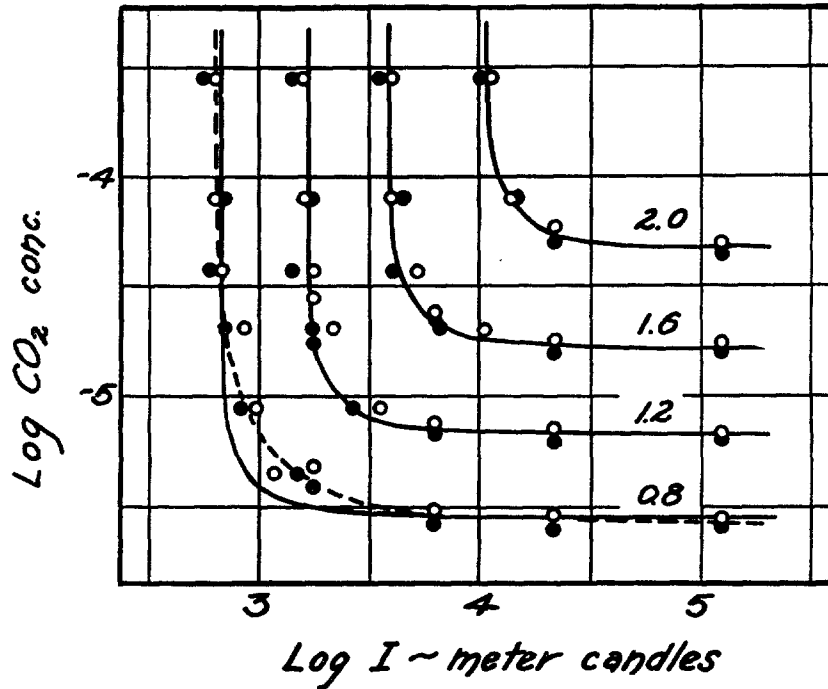


FIG. 5. Intensity and CO_2 concentration at four log photosynthesis values indicated on each curve. The curves are theoretical and represent equation (4). At the lowest photosynthesis value, a better fit of the data is given by equation (6) indicated by dashed lines. The open circles are from series I, and the solid circles are from series II. The numerical values are given in Table III.

After the first series of measurements had been completed, it was thought that the difference in curve form might be due to large errors or variation at low photosynthesis rates, and it was for this reason that a second series of measurements was undertaken. The data show no significant difference between the two series. The average difference in the determination of $\log I$ or of $\log [\text{CO}_2]$ between the two

series is about 0.07 log units. This value is actually an exaggeration of the uncertainty in placing the curve, because there is a distinct shift in the best position of the curves independently drawn through the two series. This shift is about 0.05 log units with respect to both

TABLE III

Intensity and CO₂ Concentration for Constant Photosynthesis

Data of Fig. 5. These are graphically interpolated values from the measurements in Table II. The numbers in bold-face type represent the constant factor.

Series	log $p = 0.80$		log $p = 1.20$		log $p = 1.60$		log $p = 2.00$	
	log [CO ₂]	log I	log [CO ₂]	log I	log [CO ₂]	log I	log [CO ₂]	log I
I	-5.32	3.24	-4.55	3.24	—	—	—	—
	-5.52	3.80	-5.12	3.80	-4.62	3.80	—	—
	-5.54	4.34	-5.14	4.34	-4.74	4.34	-4.23	4.34
	-5.56	5.09	-5.17	5.09	-4.76	5.09	-4.31	5.09
	-5.35	3.07	—	—	—	—	—	—
	-5.06	2.98	-5.06	3.55	—	—	—	—
	-4.69	2.92	-4.69	3.33	-4.69	4.02	—	—
	-4.43	2.83	-4.43	3.24	-4.43	3.72	—	—
	-4.10	2.80	-4.10	3.20	-4.10	3.61	-4.10	4.14
	-3.54	2.80	-3.54	3.20	-3.54	3.60	-3.54	4.06
II	-5.41	3.24	-4.75	3.24	—	—	—	—
	-5.58	3.80	-5.17	3.80	-4.66	3.80	—	—
	-5.61	4.34	-5.21	4.34	-4.81	4.34	-4.30	4.34
	-5.59	5.09	-5.20	5.09	-4.80	5.09	-4.35	5.09
	-5.35	3.17	—	—	—	—	—	—
	-5.06	2.92	-5.06	3.43	—	—	—	—
	-4.69	2.84	-4.69	3.24	-4.69	3.82	—	—
	-4.43	2.77	-4.43	3.16	-4.43	3.61	—	—
	-4.10	2.84	-4.10	3.24	-4.10	3.65	-4.10	4.17
	-3.54	2.75	-3.54	3.15	-3.54	3.55	-3.54	4.01

ordinates and may be due to some specific difference in the plants used, since the two series were run some 6 months apart.

It is possible that the curve obtained at low photosynthesis represents some specific kinetic difference as compared with those at high photosynthesis values. If this is so, then the curves for photosynthesis as a function of intensity should have different shapes at low and at high CO₂ concentrations; or the photosynthesis—CO₂ curves should

vary with light intensity. The published data on these relationships (Smith, 1936; 1937) as well as those given in this paper do not show such variation except where a CO_2 diffusion factor is involved. Such measurements, however, have been usually made with the constant factor at moderate or high values. In order to test this possibility, new measurements were undertaken.

For the photosynthesis-intensity measurements, a buffer (No. 2) of low CO_2 concentration was selected which gave measurements of photosynthesis below the compensation point even at high intensities.

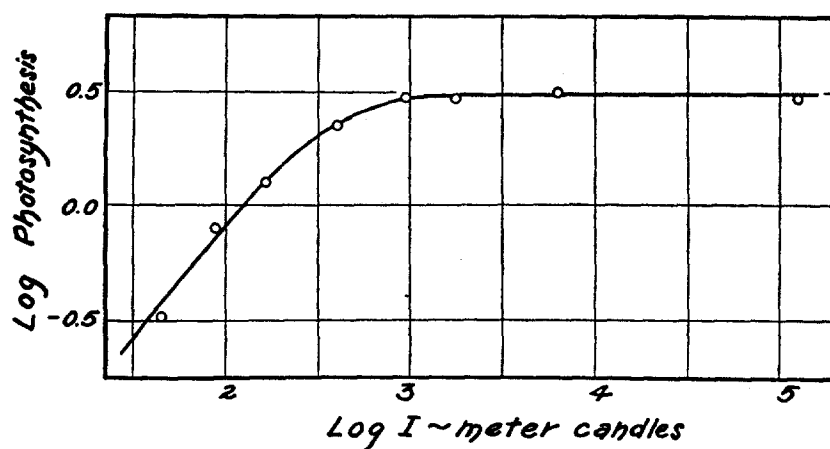


FIG. 6. Photosynthesis as a function of intensity at a low constant CO_2 concentration (0.902×10^{-6} moles per liter). The curve is from equation (2) and is of the same form as those obtained at higher CO_2 concentrations. The data are given in Table IV.

For such low photosynthesis rates, the readings had to be of long duration; respiration was measured for at least 1 hour, and the determinations at each light intensity for 30 minutes. Three fronds were used in each run to increase the accuracy of the measurements.

The data which are given in Fig. 6 and Table IV represent the averages of three similar runs. These measurements are adequately described by equation (2) and therefore do not differ from the results obtained when higher CO_2 concentrations are used.

It is more difficult to determine the curve for photosynthesis as a function of CO_2 concentration at low intensity. The tissue has to be

removed from the manometer vessel after each determination and replaced in a buffer mixture of a different CO₂ concentration. Such handling has little effect on measurements made at high intensities, but at low intensities it is sufficient to invalidate the measurements obtained. Moreover, one cannot use several fronds because they cannot be replaced in the vessel without altering the overlapping and partial shading. The form of the photosynthesis - CO₂ curve at low intensities can be determined only with the use of more suitable material.

TABLE IV

Photosynthesis and Intensity at Low CO₂ Concentration

Data of Fig. 6. Measurements at constant CO₂ concentration = 0.902×10^{-6} , Buffer No. 2. Averages of three similar runs, using three large fronds in each run. Temperature = 25.3°C. Respiration determined for at least 60 minutes, other measurements for 30 minutes each. Photosynthesis in cubic millimeters of oxygen per hour per 100 mg. wet weight of tissue.

Intensity	Photosynthesis
<i>meter candles</i>	
45.7	0.32
87.1	0.79
166	1.26
407	2.27
933	2.94
1,740	2.93
6,310	3.13
123,000	2.96

IV

DISCUSSION

In addition to the data presented in this paper, there have been only two attempts to obtain a comprehensive picture of the mutual effects of intensity and CO₂ concentration on photosynthesis, those of Harder (1921) on *Fontinalis*, and those of Hoover, Johnston, and Brackett (1933) on wheat. We have studied their results in the same way that we have done for *Cabomba*.

For the higher photosynthesis values of Harder's measurements, the uncertainty in drawing the individual curves makes difficult a

choice between equations (4) and (6); but at the low values, equation (6) definitely gives a better fit than (4). This is entirely consistent with the results on *Cabomba*.

The two experiments of Hoover, Johnston, and Brackett present a somewhat different picture. The data for their first experiment give curves which change their shape at low and high photosynthesis values in much the same way as those for *Cabomba* and *Fontinalis*. However, for reasons which are at present obscure, the measurements for their second experiment differ considerably from all of the other data. Here the curvature in the $\log \text{CO}_2$ versus \log intensity graphs becomes very gradual and fits equation (8) best.

The explanation for the difference in the limiting factor equations at low and high photosynthesis rates depends on the interpretation of the exponents in these equations. Franck and Herzfeld (1937), after assuming the existence of back-reactions in photosynthesis, arrived at an equation similar to equation (2). While equation (2) gives a slightly more precise description of the data than does their equation, the latter does provide a possible explanation of the change in curve form at high photosynthesis rates in terms of an appreciable back-reaction at high intensities and a negligible back-reaction at low intensities. In the latter situation, the light-limiting reaction (assuming that there must be four) would be first order, and a scheme such as given by equation (5) would hold. With appreciable amounts of energy loss caused by back-reaction, the data would fit the descriptions given by (1).

Whatever may be the eventual explanation for this change in kinetic properties at low and high photosynthesis rates, the general form of the limiting factor relationship seems clear. This relationship follows from the relative effects of the light and dark processes in the photosynthetic cycle; in fact, such a relationship must obtain whenever such a cycle occurs.

SUMMARY

1. Extensive measurements have been obtained (*a*) relating photosynthesis and light intensity for a large range of CO_2 concentrations and (*b*) relating photosynthesis and CO_2 at different light intensities. From these families of curves, the limiting factor relationship can be secured for any value of the photosynthesis rate.

2. In terms of previous work an equation has been derived for describing these relations between the intensity and CO_2 concentration necessary to produce a definite amount of photosynthesis. This equation furnishes an exact description for all the data, except those for low rates of photosynthesis where a slightly different equation is required. The nature of the two equations suggests that a simple first order reaction determines the velocity of the light process at low photosynthesis rates, but that at high rates the mechanism is complicated by another factor.

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