

LIMITATIONS OF BLACKMAN'S LAW OF LIMITING FACTORS  
AND HARDER'S CONCEPT OF RELATIVE MINIMUM AS  
APPLIED TO PHOTOSYNTHESIS

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(WITH NINE FIGURES)

**Problem and mode of attack**

The universality of the photosynthetic process and its fundamental nature and importance have long been recognized, and little is known regarding the systems involved and their exact working in consonance with both external and internal factors. From the time it came to be recognized that CO<sub>2</sub> was absorbed by the leaves during photosynthesis and that with an increase in its concentration the rate of assimilation increased, attempts have been made to determine an optimum concentration of carbon dioxide, a percentage which may induce rapid photosynthesis, leading to increased growth in plants.

With the formulation of LIEBIG'S law of the minimum (7) and with the advance in our knowledge of the subject of photosynthesis, it came to be realized that the process is conditioned by numerous factors. PFEFFER (12) and PANTANELLI (13) realized that the optimum value in photosynthesis was not fixed under the influence of any single factor.

Considerable work has been carried out in recent years on the relationship of photosynthesis to external factors, and several laws indicating the possible relationship between the external factors on the one hand and carbon assimilation on the other have been evolved.

BLACKMAN (1) first called attention to the fact that in a phenomenon such as photosynthesis, the focusing of attention on a single factor in disregard to the influence of others leads to erroneous results. A study of the interrelation of the conditioning factors led him to formulate the principle of limiting factors, which he has stated in the following axiom: "When a 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." This theory stimulated investigations designed to test the validity of BLACKMAN'S proposals.

His observations were confirmed by MATTHAEI (11), BLACKMAN and SMITH (2), and WILMOTT (16), but later workers like HOOKER (5), BROWN (4), BOYSEN JENSEN (3), and HARDER (6) criticized the theory and showed that the law of limiting factors was not infallible. This theory was subsequently modified by HARDER (6) in his concept of relative minimum, but at present both theories seem to be equally supported on an experimental

basis. One of the vital points of difference between the viewpoints of BLACKMAN and HARDER is that while the former holds that there is a sudden change in direction of the ascending curve to a horizontal one when the limiting factor comes into operation, the latter holds that the curves are extremely regular and that there is no sudden change of the ascending curve to a horizontal phase. Moreover, the rate is not governed by one factor and that alone, as was supposed by BLACKMAN, but is also conditioned by the intensity of others present in relative minimum. Both these observations are equally supported by experimental data and the question therefore remains, Is there a sharp break in the curve or is it smooth in form?

The present work is the outcome of a study concerned with the nature of growth in relation to external and internal factors. In trying to study growth with respect to other metabolic activities, more particularly assimilation and respiration, it was necessary to determine under laboratory conditions the relation between CO<sub>2</sub> assimilation and such external factors as temperature, light, and carbon dioxide. Special effort has been made to estimate their effect on the rate of photosynthesis when they are present in concentrations and intensities in which they naturally occur in the field.

The results have thrown considerable light on the nature as well as on the applicability of the law of limiting factors and the theory of relative minimum, and these have therefore received separate treatment in the present paper.

### Investigation

**MATERIAL.**—Material was selected from crops of economic importance such as wheat (var. Pusa 4), linseed (var. 1150 S), and sugar cane (var. Reori) grown both under natural environment and under conditions of optimum nutritional supply at the Experimental Farm of this Research Station.

**APPARATUS.**—The apparatus consists of a carbon dioxide absorbing set, a CO<sub>2</sub> generating tower, a constant temperature bath, and a commutator and clockwork arrangement identical in most respects with the one designed by BLACKMAN for investigations on vegetable assimilation. The plant chamber is of special construction, with inlet and exit tubes, an opening at the top for the insertion of a thermometer, and a depression for the attachment of a small funnel containing water into which the leaf base is dipped. The front and back sides of the chamber are of glass, one pane of which can easily be removed for admitting the experimental leaf.

Half-watt 220-volt Phillips bulbs of various intensities were used as the source of illumination. The exact intensity in each case was determined by comparison with standard bulbs and the values expressed in candle

power. A screen of running water intervened between the bulb and the plant chamber in order to prevent heat from the bulb reaching the bath.

The temperature of the bath was maintained constant for each series of experiments by means of thermo-gas regulators. The experimental temperature varied between 23° and 40° C., and never fluctuated more than  $\pm 0.05^\circ$  C.

The CO<sub>2</sub> concentration used was between 0.0 and 0.5 per cent., according to the requirement of the experiment. The percentage of carbon dioxide by volume was calculated by the formula:

$$\text{percentage CO}_2 = \frac{\text{wt. of gas in control tubes per hr. in gm.} \times 500}{1500} \times 100,$$

where 500 is the approximate volume of the gas weighing 1 gm. and 1500 the speed of dropping in the aspirators per hour.

The details of temperature, light intensity, and CO<sub>2</sub> concentration in a particular experiment are given in the tables.

#### EXPERIMENTAL PROCEDURE

The general procedure consisted in the selection of mature healthy normal green leaves from an average plant of known age a day previous to the time of the experiment. These leaves were kept near a north window, covered with an open belljar with their ends in water and left over night to attain laboratory conditions. The leaves were regularly collected at 4 P. M. after a period of rapid assimilation and the experiment was started each day at about 10 A. M. after recording the fresh weight and the area of the assimilating leaf surface.

The leaves prepared in this manner were arranged in the assimilation chamber and connections were then made to the CO<sub>2</sub> generating set and the commutator. A current of air containing a definite amount of carbon dioxide was divided into two equal parts. One was passed over the experimental leaf and was then withdrawn through a Pettenkoffer tube containing a measured quantity of standard baryta solution. The other part was passed directly through another Pettenkoffer tube containing the same amount of barium hydroxide. After a definite interval, the two tubes were disconnected, their contents poured into separate beakers, and the solution titrated against standard hydrochloric acid. The difference in the acid value of the two solutions is due to the reduction in the amount of CO<sub>2</sub> absorbed by the assimilating leaf. A preliminary tube was always run for 1.5 hours before taking the actual readings, followed by three consecutive hourly readings for the determination of the apparent assimilation. The respiration of the same material under identical conditions, but in complete darkness, was determined and the real assimilation calculated for 100 sq. cm. of assimilating area per hour. For each experiment a new leaf was used.

Care was taken to determine experimentally the amount of external carbon dioxide absorbed by the solution during the course of filling, fixing, and titrating the solution. This "washing factor" was determined several times during the course of the experiment and due allowance made for it in the readings.

Precaution was also taken to avoid as far as possible the resistances in the diffusion and photochemical phases of the process (8, 9), or to bring them to a common factor throughout the whole series. Variations due to season were avoided by performing the experiment in a single season of the year. The observations were taken at definite hours of the day and under definite artificial illumination, thus reducing variation in resistance due to time of day, intensity of illumination, and time of beginning of the experiment. To avoid the effect of previous history of the leaf only healthy leaves from average selected plants were taken and kept with the leaf base in water for several hours to equalize moisture conditions. Only leaves of the same developmental stage were used.

The course of assimilation at various concentrations and combinations of external factors was followed with only the mature leaves during the adolescent phase of the life cycle.

### Results

The data are mainly confined to three of the most important external factors, light, temperature, and carbon dioxide, which have been shown by previous workers to have considerable influence on the rate of photosynthesis.

#### CO<sub>2</sub> CONCENTRATION AND ASSIMILATION

With the preliminary precautions already described, observations were made on the effect of carbon dioxide on the rate of assimilation. Concentrations both below and above that present in the field were used, the former being maintained by absorbing some of the CO<sub>2</sub> in the current by potash and the latter by providing extra quantities of this gas in the stream by the CO<sub>2</sub> generating tower.

Since the experiments were conducted entirely under laboratory conditions, other factors like light intensity and temperature were also controlled. Under a constant temperature and a constant light intensity, the CO<sub>2</sub> assimilation was determined at 0.02 per cent. external concentration of carbon dioxide. The results are set forth in table I, experiment 1.

Under the conditions of the experiment, it is evident that the green leaf is not in a position to obtain any appreciable quantity of carbon dioxide from its surroundings. It has naturally to depend on the CO<sub>2</sub> evolved during respiration for the synthesis of carbohydrates. The quantity of

TABLE I

EFFECT OF CO<sub>2</sub> CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.

ILLUMINATION, 90 C.P. HALF-WATT PHILLIPS BULB AT 16 CM. DISTANCE

DATE (1932)	EXPERIMENT NO.	CO <sub>2</sub> CONCENTRATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
		%	mg.
July 1 .....	1	0.02	0.403
“ 2 .....	2	0.051	1.050
“ 3 .....	3	0.0806	1.150
“ 4 .....	4	0.1280	1.005
“ 5 .....	5	0.3740	- 0.960

respiratory carbon dioxide for the entire leaf area being small, the values of assimilation are naturally low.

The carbon assimilation under slightly greater concentration of carbon dioxide, *viz.*, equivalent to that of the atmosphere, was then determined. The results are shown in table I, experiment 2. In this case assimilation is higher than the preceding, indicating that with increase in the percentage of carbon dioxide the value of assimilation increased.

To test whether an increase in assimilation will follow a further increase in the percentage of carbon dioxide, the concentration was raised to 0.0806 per cent. Experiment 3 indicates that the intensity of assimilation does increase.

Experiments 4 and 5 were designed to study the effect of still higher concentrations of carbon dioxide on the rate of photosynthesis. Light intensity and temperature being kept the same as before, the variations found in the readings should be due to the increase in the concentration of carbon dioxide.

The rates of assimilation obtained under different concentrations of carbon dioxide (table I) show that up to 0.08 per cent. of carbon dioxide the velocity of the reaction increases, and any further increase is accompanied by a decrease in the intensity which finally attains a negative value under 0.374 per cent. It would thus follow that after 0.08 per cent. carbon dioxide concentration any extra quantity of carbon dioxide produces some sort of inhibitory effect on the process, the intensity of inhibition increasing with an increase in its concentration.

It has just been shown that assimilation under the particular temperature and light intensity is highest at 0.08 per cent., the readings falling

gradually on both sides of this maximum. Why should the intensity of photosynthesis rise for some time and then decline with increasing concentration of carbon dioxide? In the light of BLACKMAN'S concept we can explain it as due to the fact that the carbon dioxide supply up to this stage is limiting; hence an increase in the limiting factor, the pace of the slowest factor, increases the rate of assimilation. Following HARDER, the same phenomenon may be explained on the basis that the carbon dioxide is at first lesser in quantity or in relative minimum than other conditioning factors which are exerting greater influence over the process. And as its concentration is increased the intensity becomes still greater, finally equaling that of other factors in which the readings are maximum. The subsequent fall with increase in CO<sub>2</sub> concentration may be due to the deleterious effect of excessive carbon dioxide.

Whatever explanation we may give to the nature of the phenomenon, it is clear from the data obtained (fig. 1, 90 c.p.) that with increasing concentration of CO<sub>2</sub> the intensity of photosynthesis increases in regular sequence. The sudden transference of the ascending phase into a horizontal one, as was observed by BLACKMAN and his co-workers, is not apparent in the present case. Instead the curve shows a gradual rounding, thus probably answering one of the vital questions raised in our introduction. Further, the stationary phase when the limiting factor comes into operation as observed by BLACKMAN is not marked in the present case. If it is present at all, it may be found only within a small range of concentration. The decline phase on the other hand is characteristically evident.

In order to test the validity of these results obtained for sugar cane, inquiry along strictly similar lines was conducted on wheat and flax plants grown side by side under similar conditions. The gradations of the carbon

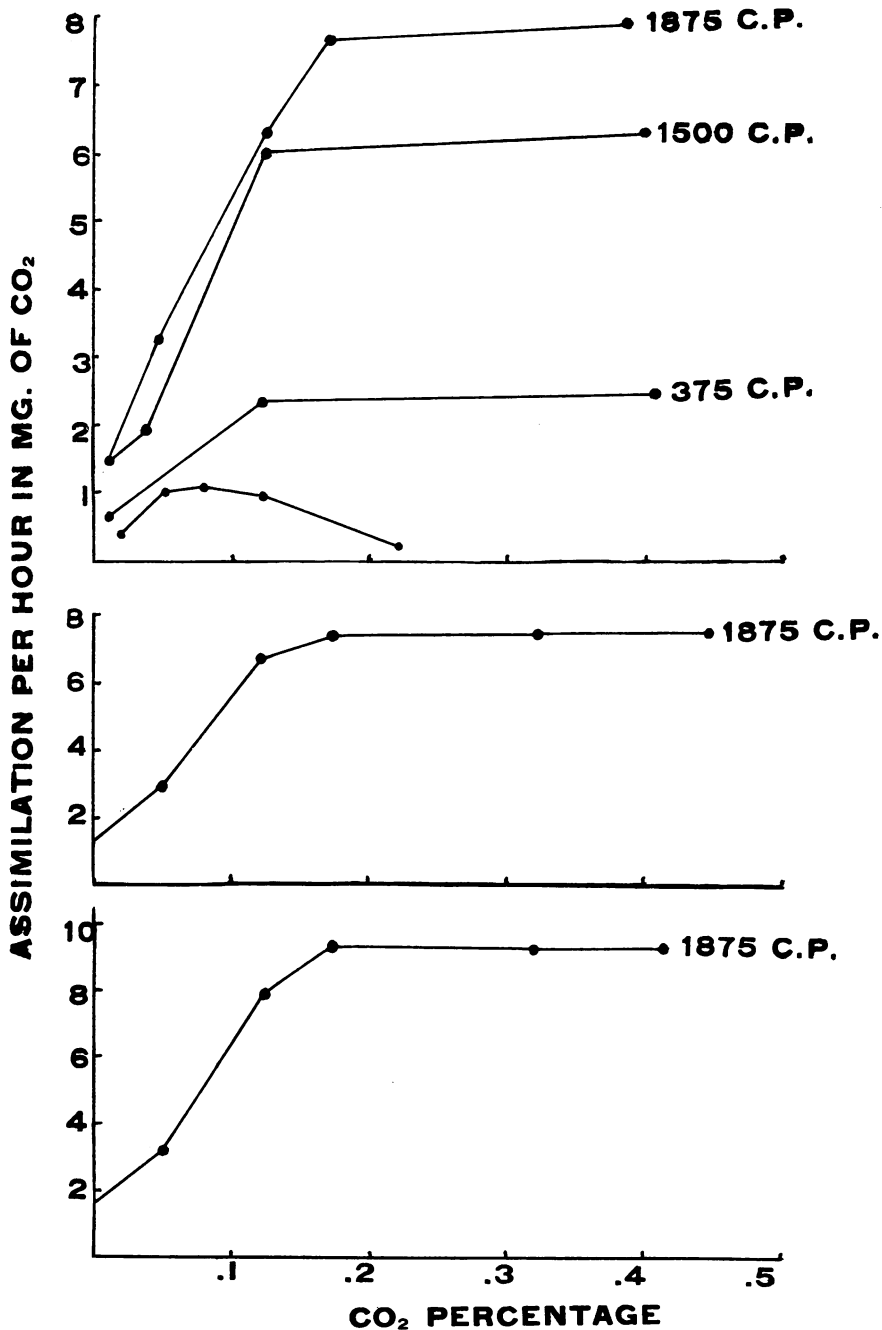
TABLE II

EFFECT OF CO<sub>2</sub> CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF WHEAT, PUSA 4

TEMPERATURE, 30.0° C.

ILLUMINATION 1875 C.P. HALF-WATT PHILLIPS BULB AT 16 CM. DISTANCE

DATE (1932)	EXPERIMENT NO.	CO <sub>2</sub> CONCENTRATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
March 11 .....	22	% 0.05	<i>mg.</i> 2.82
“ 11 .....	29	0.124	6.75
“ 12 .....	30	0.172	7.37
“ 13 .....	31	0.320	7.40



Figs. 1-3. CO<sub>2</sub> and assimilation curves. Above, sugar cane; middle, wheat; below, flax. Illumination in candle power. Lowest curve for sugar cane, 90 c.p.

dioxide range as well as the constancy of temperature and light intensity were maintained, although the magnitude of each was purposely raised in order to compare our data at higher intensities of the factors.

In spite of material difference in the leaves which were gathered from plants of three different types, curves (figs. 2, 3) differing only in magnitude but essentially similar in nature to the one obtained for sugar cane were secured in these two cases as well (tables II and III).

TABLE III

EFFECT OF CO<sub>2</sub> CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF LINSEED, 1150 S

TEMPERATURE, 30.0° C.

ILLUMINATION, 1875 C.P. HALF-WATT PHILLIPS BULB AT 16 CM. DISTANCE

DATE	EXPERIMENT NO.	CO <sub>2</sub> CONCENTRATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
March 8, 1932	25	% 0.05	<i>mg.</i> 3.24
February 1, 1933	26	0.124	7.94
“ 2, 1933	27	0.172	9.23
February 3, 1933	28	0.32	9.23

Medium and high intensities of light were also used on sugar cane to test the nature of the reaction as affected by increased illumination. Table IV includes three experiments with medium light intensity.

TABLE IV

EFFECT OF CO<sub>2</sub> CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.

ILLUMINATION, 375 C.P. HALF-WATT PHILLIPS BULB AT 16 CM. DISTANCE

DATE (1932)	EXPERIMENT NO.	CO <sub>2</sub> CONCENTRATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
May 7 .....	6	% 0.011	<i>mg.</i> 0.680
“ 8 .....	7	0.121	2.391
“ 9 .....	8	0.407	2.510

The readings (table IV) show an initial rapid increase in the rate of assimilation, followed by a more or less stationary phase. In this case



there is a wider range in assimilation than in the previous case. The intensity of the reaction is greater in the present case, however, because of the greater supply of energy now available.

A series of experiments was performed also with higher light intensity. In this series, as is evident from the curves shown in figure 1 and the values in tables V and VI, the nature of the reaction as judged by the rate of assimilation remains the same, differing only in intensity and magnitude.

TABLE V

EFFECT OF CO<sub>2</sub> CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.

ILLUMINATION, 1500 C.P., HALF-WATT PHILLIPS BULBS AT 16 CM. DISTANCE

DATE (1932)	EXPERIMENT NO.	CO <sub>2</sub> CONCENTRATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
		%	<i>mg.</i>
July 12 .....	10	0.011	1.48
“ 11 .....	9	0.036	1.964
“ 13 .....	11	0.124	6.100
“ 18 .....	12	0.403	6.38

TABLE VI

EFFECT OF CO<sub>2</sub> CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.

ILLUMINATION, 1875 C.P., HALF-WATT PHILLIPS BULBS AT 16 CM. DISTANCE

DATE (1932)	EXPERIMENT NO.	CO <sub>2</sub> CONCENTRATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
		%	<i>mg.</i>
July 19 .....	13	0.0476	3.303
“ 20 .....	14	0.124	6.290
“ 21 .....	15	0.172	7.770
“ 22 .....	16	0.388	7.920

It is thus seen (table VI) that a higher light intensity increases the rate of photosynthesis several-fold. The higher rate is possibly correlated with the greater supply of available energy, and in fact it was not practicable within the limited time at our disposal actually to determine the concentra-

tions at which the toxic or inhibitory effect comes into prominence, since the leaves could endure sufficiently high  $\text{CO}_2$  concentration, up to ten times higher than that in the field, under very high intensities. Several readings were not possible for each light intensity since the investigations necessarily had to be finished within a limited stage in the life cycle of crops in order to avoid the effect of age and developmental stage on assimilation.

The curves of figure 1 indicate that with the higher intensities of light, the ascending phase becomes more and more characteristic and prominent. The magnitude of the ascending phase in contradistinction to the level phase is greater as we use higher light intensities, and it may therefore be expected that if an unlimited supply of light energy be available, together with a continuous and increasing supply of carbon dioxide, the inflexion phase may be entirely lost, the reaction proceeding in a linear fashion. But how far such a type of curve can be expected in practice is a matter for experimentation. It is just possible that, even with all the external factors supplied at maximum level, the nature of the reaction may remain the same on account of the control of the process by physiological or internal factors, namely, the specificity of the plant, the nature of the products formed, the chlorophyll apparatus, the water content, etc.

#### LIGHT INTENSITY AND ASSIMILATION

That light is essential for photosynthesis is well known, but owing to the complexities introduced by the simultaneous influence of other factors, carbon dioxide and temperature, it becomes difficult to gauge the relationship at its correct value. For example, as already shown, the influence of carbon dioxide on the rate of assimilation is found to be dependent upon light, the rate rising with increased intensity. It is also known that temperature influences the rate, a rise being obtained by raising the temperature until a certain degree is reached followed by a decline with further rise in temperature. These facts lead one to conclude that in investigating the influence of light, the simultaneous influence of two other factors, carbon dioxide and temperature, must be taken into consideration.

In the present study, which does not aim at a detailed investigation of the problem but is simply concerned with the determination of the light intensity required to deplete the normal concentration of carbon dioxide, the effect of increasing light intensity on the rate of photosynthesis has been tested at four different concentrations of  $\text{CO}_2$  both below and above that normally present in air.

**ZERO PERCENTAGE EXTERNAL  $\text{CO}_2$  CONCENTRATION.**—Under such a condition the plant is not in a position to utilize any other  $\text{CO}_2$  except that which it is itself evolving during the downgrade process of respiration. The readings obtained under such conditions are given in table VII.

TABLE VII

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.

CO<sub>2</sub> CONCENTRATION, 0.0 PER CENT.

DATE (1932)	EXPERIMENT NO.	ILLUMINATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
July 1 .....	1	<i>c.p.</i> 90	<i>mg.</i> 0.403
“ 7 .....	6	375	0.680
“ 12 .....	10	1500	1.480
“ 13 .....	40	1875	1.480

It is seen that the rates of assimilation at 90, 375, 1500, and 1875 c.p. are 0.4, 0.68, 1.48, and 1.48 mg. respectively per hour. This shows that the respiratory carbon dioxide, which is the only carbon dioxide available for photosynthesis under such circumstances, is not all used at lower light intensities. Experiments 1 and 6, dealing with 90 and 375 c.p. respectively, show that under these light intensities the plant is not in a position to synthesize what it respire. As the light intensity is increased the photosynthetic value rises, and ultimately the leaf is able to utilize the total output of carbon dioxide (experiment 10). When the two processes balance each other, *i.e.*, when the compensation point is reached, no more increase in the readings is obtained with further increase in light intensities.

What is the nature of the reaction at this stage, namely, how far does the photosynthetic chain of reactions proceed and how are they correlated with the opposite series of reactions connected with the respiratory process? Answers to these questions may throw light on the nature of the two inter-related reactions.

ATMOSPHERIC CONCENTRATIONS OF CO<sub>2</sub>.—Since all the plants grew under this concentration it was thought advisable to investigate the effect of increasing illumination on the rate of photosynthesis under such atmospheric percentage of CO<sub>2</sub> in the case of different plants. Besides the sugar cane leaves which were subjected to detailed investigation, leaves of wheat and flax were experimented upon also.

The readings obtained for sugar cane leaves under this concentration and different light intensities are shown in table VIII.

It is evident that with higher light intensities (90 to 1875 c.p.) the rate of assimilation increases from 1.05 to 2.23 mg. Even at the high light intensity of 1500 c.p. the readings show an increase when the intensity is

TABLE VIII

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.  
CONCENTRATION, 0.05 PER CENT.

DATE (1932)	EXPERIMENT NO.	ILLUMINATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
July 2, 1932 .....	2	<i>c.p.</i> 90	<i>mg.</i> 1.05
March 6, 1933 .....	17	375	1.52
“ 7, 1933 .....	18	1500	2.1
“ 8, 1933 .....	19	1875	2.23

increased to 1875 c.p. The constant phase of the activity could not be determined on account of lack of higher light intensities. The rates of assimilation of wheat leaves under the different light intensities and atmospheric concentration of CO<sub>2</sub> are shown in table IX.

TABLE IX

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF WHEAT, PUSA 4

TEMPERATURE, 30.0° C.  
CO<sub>2</sub> CONCENTRATION, 0.05 PER CENT.

DATE (1933)	EXPERIMENT NO.	ILLUMINATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
March 9 .....	20	<i>c.p.</i> 375	<i>mg.</i> 2.05
“ 10 .....	21	1500	2.74
“ 11 .....	22	1875	2.82

On comparing the rates (table IX), it is clear that the results with wheat are rather similar, the rate rising with the light intensity. The readings at 1500 and 1875 c.p. are 2.74 and 2.82 mg. There is not much difference between the two, however, and we can say that 1875 c.p. is sufficient to deplete all the carbon dioxide normally present in the atmosphere.

Linseed plants showed contrasting results. The readings at 375, 1500, and 1875 c.p. are shown in table X. The rate of assimilation does not rise with increase in light intensity beyond 1500 c.p.

TABLE X

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF  
LINSEED, 1150 S

TEMPERATURE, 30.0° C.

CO<sub>2</sub> CONCENTRATION, 0.05 PER CENT.

DATE (1932)	EXPERIMENT NO.	ILLUMINATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
March 16 .....	23	<i>c.p.</i> 375	<i>mg.</i> 2.72
“ 17 .....	24	1500	3.24
“ 18 .....	25	1875	3.24

From the results obtained with regard to the influence of light on the photosynthetic rate of the three crop plants, it is seen that while light intensity of 1875 c.p. and more is necessary for maximal values in the cases of wheat and sugar cane, an intensity of 1500 is sufficient for linseed. In other words, linseed plants under normal conditions of CO<sub>2</sub> require less light intensity for attaining their optimum value than do sugar cane and wheat. Whether such differences are in any way connected with the nature of the end product of the upgrade process (sugar in sugar cane, starch in wheat, and oil in linseed) is a problem requiring further investigation.

MEDIUM CO<sub>2</sub> CONCENTRATION.—Only the sugar cane leaves were the subject of experimentation under medium concentration of carbon dioxide. The rates of assimilation are shown in table XI.

TABLE XI

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF  
SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.

CO<sub>2</sub> CONCENTRATION, 0.12 PER CENT.

DATE (1932)	EXPERIMENT NO.	ILLUMINATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
July 4 .....	4	<i>c.p.</i> 90	<i>mg.</i> 1.005
“ 8 .....	7	375	2.391
“ 13 .....	11	1500	6.10
“ 20 .....	14	1875	6.29

There is a clear and characteristic rise similar to that observed under normal concentrations above, but differing in magnitude. Under such a concentration the rise is more or less proportional to light intensity (fig. 4). The stationary phase could not be secured, owing to the lack of sufficiently strong light which we had not at our disposal when the investigations were in progress.

OPTIMUM CO<sub>2</sub> CONCENTRATION.—In this case the values as shown in table XII indicate the rate of photosynthesis of sugar cane leaves.

TABLE XII

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF SUGAR CANE, VAR. REORI

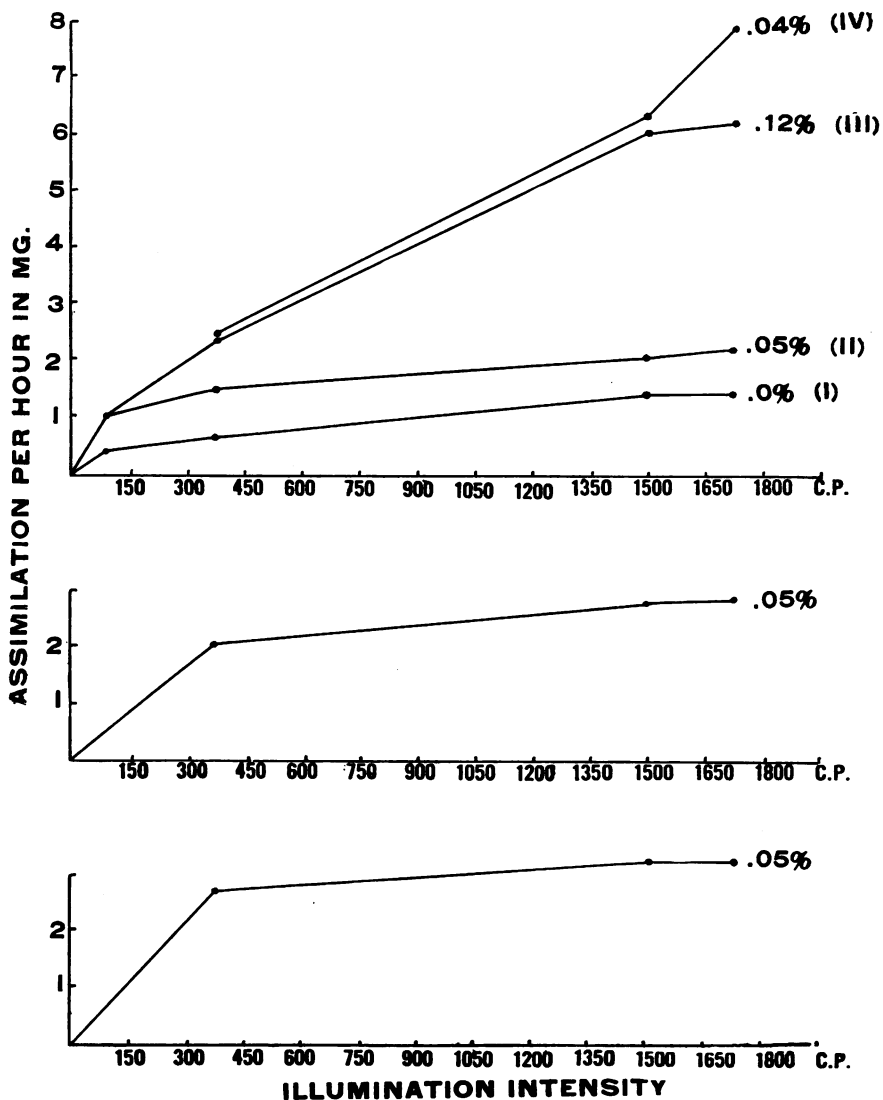
TEMPERATURE, 30.1° C.

CO<sub>2</sub> CONCENTRATION, 0.4 PER CENT.

DATE (1932)	EXPERIMENT NO.	ILLUMINATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
July 5 .....	5	<i>c.p.</i> 90	<i>mg.</i> - 0.96
“ 9 .....	8	375	2.51
“ 18 .....	12	1500	6.38
“ 22 .....	16	1875	7.92

The curves shown in figure 4 display a direct proportionality between light intensity and the rate of assimilation from 375 c.p. onward, showing that light governs the reaction under conditions of optimum CO<sub>2</sub> concentration. At 90 c.p. the intensity of light is too feeble for this high concentration of CO<sub>2</sub>, under which circumstances the carbon dioxide exerts its toxic effect as shown by negative photosynthesis.

The nature of the reaction as measured by the CO<sub>2</sub> intake under different concentrations of CO<sub>2</sub> at different light intensities (fig. 4) shows at first an ascending phase but the steepness of the curve depends upon the concentration of carbon dioxide. The richer the environment the higher is the angle of curvature and the higher the values. In the case of a zero percentage CO<sub>2</sub> concentration the curves become flattened when light intensity is raised from 1500 to 1875 c.p., showing that under these conditions CO<sub>2</sub> is limiting the rate. But such a stationary phase was not observed at 1500 and 1875 c.p. in the case of higher concentrations. This shows that under higher concentrations of carbon dioxide the existence of the stationary phase requires very high light intensity, a theory which has still to be experimentally supported.



FIGS. 4-6. Light and assimilation curves. Above, sugar cane; middle, wheat; below, flax.

In the light of BLACKMAN'S theory, curve no. 1 (fig. 4) can be explained as consisting of two parts, an ascending phase when light is limiting and a stationary one when CO<sub>2</sub> is limiting. In the case of other curves we have only the ascending phase, indicating a stage when light is always limiting. The curves can equally be explained on the basis that during the ascending

phase the light intensity is in relative minimum and during the stationary phase the other factor,  $\text{CO}_2$ , falls short of the requirement.

We shall discuss critically the relative importance of the theory of limiting factors and that of relative minimum after we have dealt with the third factor, namely, temperature.

#### TEMPERATURE AND ASSIMILATION

Under ordinary conditions in the field, the  $\text{CO}_2$  concentration during the day does not increase to a great extent. The values are more or less stationary, but so far as the temperature is concerned it shows decidedly wide fluctuations. In order to determine the optimum temperature under normal concentration of carbon dioxide, a series of experiments as shown in table XIII was performed.

TABLE XIII

EFFECT OF TEMPERATURE ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF SUGAR CANE, VAR. REORI

$\text{CO}_2$  CONCENTRATION, 0.05 PER CENT.

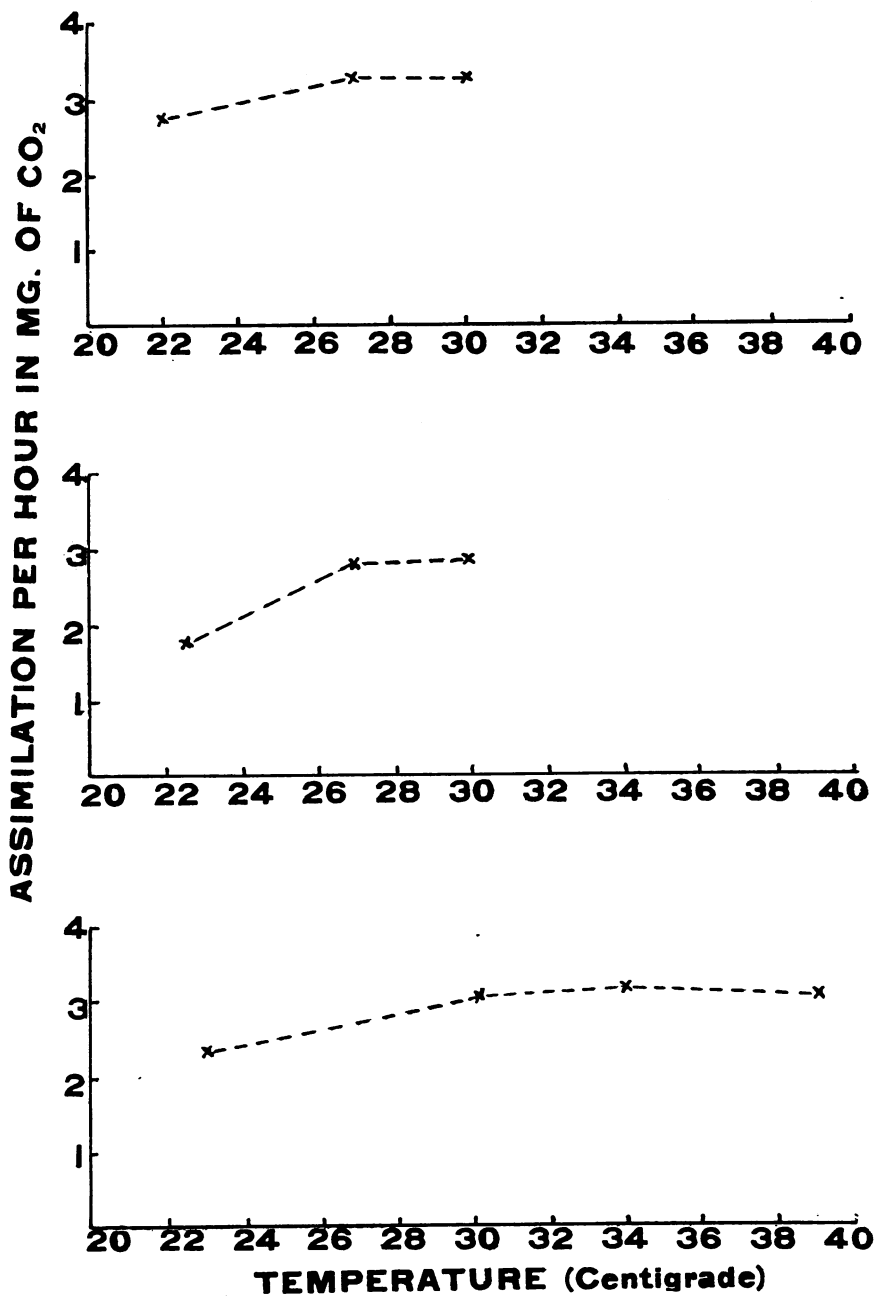
LIGHT INTENSITY, 1875 C.P.

DATE (1932)	EXPERIMENT NO.	TEMPERATURE	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
July 19, 1932 .....	13	°C. 30.1	mg. 3.03
March 9, 1933 .....	36	23.0	2.3
“ 11, “ .....	38	34.0	3.2
“ 12, “ .....	39	39.0	3.05

Only four temperatures, which came within the purview of fluctuations within these regions and to which the plants are subjected at this time of the year, were selected: 23°, 30°, 34°, and 39° C., it being considered unnecessary to go above or below these temperatures. In each case light intensity sufficiently high so as not to limit the rate was used. The concentration of carbon dioxide was the same as that obtained in the field.

From the values shown (figs. 7-9), it is clear that the rate of assimilation rises with rise in temperature for some time, followed by a decline. Two influences seem to be at work: first, the effect of temperature in raising the amount of assimilation of which the leaf is capable; second, an injurious effect which becomes more and more prominent with high temperature. The rate at any point is therefore the result of an accelerating as well as of a depressing effect of temperature. But at higher temperatures a





FIGS. 7-9. Temperature and assimilation curves. Above, flax; middle, wheat; below, sugar cane.

point is reached when the acceleration due to increased temperature is balanced by the depressing action. This point gives the maximum assimilation and lies for sugar cane leaves near 34° C.; but, as may be seen from the individual readings in experiment 38, the initial value is not maintained very long.

At temperatures higher than 34° C. the injurious effect gains ascendancy and there is a fall in the rate of photosynthesis. The initial values are not maintained as can be seen from the individual readings in experiment 39. There is a rapid fall from hour to hour. The time effect sets in very soon at higher temperatures.

A temperature near the optimum most suitable for study of photosynthesis would be about 30° C. At such a temperature the time effect is not marked for a sufficient period, as shown by the constancy of the readings in experiment 37.

TABLE XIV

EFFECT OF TEMPERATURE ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF  
WHEAT, PUSA 4  
CO<sub>2</sub> CONCENTRATION, 0.05 PER CENT.  
LIGHT INTENSITY, 1875 C.P.

DATE (1933)	EXPERIMENT NO.	TEMPERATURE	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
March 14 .....	32	22.5	1.72
“ 15 .....	33	27.0	2.8
“ 11 .....	22	30.0 °C.	2.82 mg.

TABLE XV

EFFECT OF TEMPERATURE ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF  
LINSEED, 1150 S  
CO<sub>2</sub> CONCENTRATION, 0.05 PER CENT.  
LIGHT INTENSITY, 1875 C.P.

DATE (1932)	EXPERIMENT NO.	TEMPERATURE	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
February 9 .....	34	°C. 22.0	mg. 2.72
“ 10 .....	35	27.0	3.22
March 18 .....	25	30.0	3.24

With regard to the nature of the reaction, the studies show that VAN'T HOFF's temperature coefficient between 23° and 33° C. would be in the vicinity of 1.4, characteristic of photochemical reactions. As to the exact nature of this reaction and the part it has in the chain of reactions in the photosynthetic process, we are not in a position to say much at present.

Similar responses are found in the case of wheat and linseed leaves (tables XIV, XV).

In these cases the optimum lies near 28° C., a lower temperature than that for sugar cane, probably owing to the comparatively cooler season in which flax grows and the consequent adaptability of the material to temperature.

### Discussion

In the preceding pages we have briefly presented a quantitative study of assimilation under various light intensities and carbon dioxide concentration in the light of limiting factors and also of the relative minimum theories. How far these two conceptions hold good under different conditions we shall now discuss.

#### LIMITATIONS OF LAW OF LIMITING FACTORS

The essence of this law is that only one factor, that which is slowest, governs the rate of the process, which can be accelerated only by increasing the intensity of this factor. For example, if the rate of the reaction is limited by CO<sub>2</sub>, only increase in its concentration will increase the photosynthetic rate. Taking a concrete case, we find that under atmospheric concentrations of 0.05 per cent. CO<sub>2</sub> and light intensity of 90 c.p. the rate of assimilation is 1.05 mg. (experiment 2). If we increase the concentration to 0.08 per cent. the rate of assimilation also increases to 1.15 mg. (experiment 3). Under 0.05 per cent. carbon dioxide concentration, therefore, the CO<sub>2</sub> is the limiting factor, for by an increase in its concentration is the rate accelerated. Inquiry as to whether an increase in other factors under the same CO<sub>2</sub> concentration brings about greater assimilatory activity was made, and it was noted that under this limiting condition of CO<sub>2</sub> the rate also could be increased to 1,964 mg. by increasing the light intensity to 1500 c.p. (experiment 9). The readings increase by increasing both the concentration of CO<sub>2</sub> and the light intensity.

Several other experiments were conducted to see whether the peculiarity observed at this light intensity and CO<sub>2</sub> concentration holds good under other conditions. Taking a few examples, the rate of assimilation at 1500 c.p. and 0.12 per cent. CO<sub>2</sub> concentration is 6.1 mg. (experiment 11). When the CO<sub>2</sub> concentration was raised to 0.403 per cent. under the same light intensity the rate of assimilation rose to 6.38 mg. (experiment 12). On the other hand, when under approximately similar concentrations of

0.12 per cent. the light intensity was increased to 1875 c.p. the rate rose to 6.29 mg. (experiment 14). Here also we notice a rise in the activity by changing either the CO<sub>2</sub> concentration or the light intensity to higher units.

These experiments indicate that it is not merely one factor that determines the rate, as BLACKMAN claims, but that the pace of the reaction is determined by at least two factors. Whether by varying the third, namely, temperature, the rate is increased or not we are not in a position definitely to say.

#### LIMITATIONS OF THEORY OF RELATIVE MINIMUM

As opposed to BLACKMAN's conception, HARDER (6) holds that of the two factors, light intensity and CO<sub>2</sub> concentration, the one that is present in the minimum governs the pace of the reaction to a greater extent. We have explained the nature of the curves showing the relationship of CO<sub>2</sub> concentration and light intensity on the basis of this theory, but to test how far it holds good under different circumstances a number of experiments were conducted.

Experiment 9 shows that the rate of assimilation under 1500 c.p. and 0.036 per cent. CO<sub>2</sub> is 1.964 mg. An increase in CO<sub>2</sub> concentration to 0.124 per cent., *i.e.*, 3.5 times, increases the photosynthesis to 6.1 mg. (experiment 11), *i.e.*, 3.1 times. In other words, the per unit increase in CO<sub>2</sub> concentration increases the rate by 0.88 times. On the other hand, when the light intensity is increased to 1875 c.p. (1.25 times under more or less similar CO<sub>2</sub> concentrations) the readings are increased to 3.303 mg. (experiment 13), *i.e.*, 1.63 times. In other words, with per unit increase in light intensity the assimilation rate increases 1.3 times. Thus under similar conditions (0.03 per cent. CO<sub>2</sub> concentration) unit increase in CO<sub>2</sub> brings about 0.88 times increase and unit increase in light accelerates 1.3 times, showing that the reaction is more controlled by light even at high light intensities of 1500 c.p. Here the CO<sub>2</sub> concentration is apparently very small while the light is sufficiently high, and therefore the rate, according to the theory of relative minimum, should be governed by CO<sub>2</sub> concentration, an idea contrary to the experimental results obtained.

Several experiments were also carried out to test the applicability of the theory to lower light intensities. Experiment 2 shows the assimilation reading at 90 c.p. and 0.051 per cent. CO<sub>2</sub> concentration. The rate of photosynthesis is 1.05 mg. When the CO<sub>2</sub> concentration is increased to 0.08 per cent., *i.e.*, 1.58 times, the assimilation increases to 1.15 mg. (experiment 3), or 1.09 times. For unit increase in CO<sub>2</sub> concentration, therefore, an increase by 0.69 times is marked in the rate of the reaction. On the other hand, when the light is increased to 1500 c.p., *i.e.*, 16.6 times, the rate under approximately similar concentrations of CO<sub>2</sub> is 1.964 mg. (experiment 9),

an increase of 1.87 times. In other words, for unit light increase there is acceleration by 0.11 times only. Under low light intensity, therefore, the rate is controlled by CO<sub>2</sub> concentration and not by light intensity.

A third aspect remains to be settled, to determine how far the relative minimum theory holds good at high concentrations of CO<sub>2</sub>. Several experiments were designed to prove its applicability. Under 375 c.p. and 0.121 per cent. CO<sub>2</sub> concentration the rate of assimilation is 2.391 mg. (experiment 7). This is increased to 2.51 mg., *i.e.*, 1.08 times when the CO<sub>2</sub> concentration is raised to 0.407 per cent., by 3.36 times. On the other hand, when light is raised to 1500 c.p., *i.e.*, by four times under the same concentration, the rate is 6.1 mg., an increase by 2.55 times. For unit increase in CO<sub>2</sub> concentration and light intensity, therefore, there is an increase of 0.32 and 0.637 times respectively. In this case also light is the determining factor.

These observations indicate that the relative minimum theory of HARDER does not hold good under all conditions. In the majority of cases light, whether of low or high intensity, controls the rate of the reaction. The main reaction, namely, the relationship of unit CO<sub>2</sub> increase to the rate of assimilation, remains practically the same, the results under low CO<sub>2</sub> concentration and high and low intensities of light being 0.88 and 0.69 times. The increase is practically constant under the different conditions mentioned. Under very high light intensity a slight increase is marked, probably connected with the fact that greater energy is available under the circumstances, which leads to a slight increase in the velocity of the photochemical phase.

We have discussed the limitations of the limiting factor concept as well as of the relative minimum. The evidence relating to interaction of factors favors the view that the rate of photosynthesis is controlled neither by one factor alone (as was held by BLACKMAN) nor by one or a set of two factors present in relative minimum (as claimed by HARDER). Most probably each of the factors affecting the rate of photosynthesis has some definite influence on the rate of assimilation, depending upon the intensity of others, the different velocities of the reaction being brought about by changes introduced in the diffusion, photochemical, and chemical stages of the mechanism. Which of these phases is most affected by a particular factor remains to be determined, but since the whole process consists of a series of interlinked reactions, the effect which a change in one factor brings about in a particular phase may induce slight modification in others as well, thus indicating that there cannot be a sharp line of demarcation between the changes produced by one or the other of the factors.

But with all these minor details, the law of limiting factors in its broad sense appears to be of universal applicability and has therefore been a

means of interpreting simply and logically the phenomenon of photosynthesis in its various aspects. To us it seems that any relationship that is traced between the external factors alone, without taking into consideration the internal changes that take place in the mechanism itself, will not hold good under all circumstances. This is well supported by the present observations, and since the actual process is intracellular, the relationship if any has to be sought in the internal concentrations that these factors bring about rather than in the actual external intensities of the factors.

### Summary and conclusions

As a preliminary step toward the analysis of growth in terms of assimilation and respiration, it was considered of importance to study the factors affecting these two processes. The present paper is an attempt to elucidate the relationship between the external factors light, temperature, and carbon dioxide and photosynthesis, special attention being paid to the concentrations and intensities in which they interact under ordinary natural conditions in the subtropics.

The investigations have been mainly confined to wheat, linseed, and sugar cane plants and the rate of assimilation was determined by the continuous current methods of BLACKMAN. The following conclusions are the outcome of the present work:

1. Under low light intensities the curves showing the relationship of  $\text{CO}_2$  and assimilation are smooth, with no sharp break as observed by BLACKMAN and his coworkers. The stationary phase is either absent or if present extends only to a small range.
2. With higher light intensities also the curves in wheat and linseed are extremely regular, the only difference being that there is a big range of stationary assimilation with increasing concentrations of  $\text{CO}_2$ , the toxic effect not being so easily marked.
3. The concentration of  $\text{CO}_2$  at which the readings are maximum depends upon light intensity. The higher the light intensity the higher is this optimum. These facts are probably correlated with greater availability of energy under high intensities.
4. Under zero percentage of  $\text{CO}_2$  the rate of assimilation increases with increasing  $\text{CO}_2$ , until the respiratory output balances the assimilatory intake of  $\text{CO}_2$ , namely, the compensation point is reached.
5. Light requirements of different crops vary, probably owing to the nature of the end products formed.
6. Increase in temperature accelerates the rate of photosynthesis, the rise being the result of the accelerating effect of temperature on the one hand and its depressing effect on the other. The readings show a rise until the former is greater than the latter, an optimum being obtained when the two balance each other.

7. The time effect is earlier at higher temperatures.
8. The optimum in the case of wheat and linseed lies at a lower temperature than in the case of sugar cane, probably owing to the colder climate in which these grow and their consequent adaptability toward temperature.
9. It is not one factor that limits the rate of photosynthesis under any set of conditions; the velocity of the reaction is governed by at least two factors.
10. Whether the CO<sub>2</sub> be in high or low concentrations under high intensities of light, the rate is always controlled by light. The theory of relative minimum is limited in application, partially holding good under very low intensities of light and low concentration of CO<sub>2</sub>.
11. Any relationship that is traced between the external factors alone, without taking into consideration the internal changes that take place in the process as well as the intensities in which the external factors reach the internal tissues, will not hold good under all circumstances.

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#### LITERATURE CITED

1. BLACKMAN, F. F. Optima and limiting factors. *Ann. Bot.* **19**: 281-295. 1905.
2. ———, and SMITH, A. M. Experimental researches on vegetable assimilation and respiration. IX. On assimilation in submerged water plants and its relation to the concentrations of carbon dioxide and other factors. *Proc. Roy. Soc. London* **83 B**: 389-412. 1910.
3. BOYSEN JENSEN, P. Studies in the production of matter in light- and shadow-plants. *Bot. Tidssk.* **36**: 219-262. 1918.
4. BROWN, H. T. The theory of limiting factors. *Philippine Jour. Sci. C. Bot.* **13**: 345-350. 1918.
5. HOOKER, H. D. Liebig's law of the minimum in relation to general biological problems. *Science n. s.* **46**: 197-204. 1917.
6. HARDER, R. Kritische Versuche zu Blackmans Theorie der "begrenzenden Faktoren" bei der Kohlensäureassimilation. *Jahrb. wiss. Bot.* **60**: 531-571. 1921.
7. LIEBIG, J. Chemistry in its application to agriculture and physiology. 3d. ed. Peterson, Philadelphia. 1843.
8. MASKELL, E. J. Experimental researches on vegetable assimilation and respiration. XVIII. The relation between stomatal opening and assimilation. A critical study of assimilation rates and por-

- ometer rates in leaves of cherry laurel. Proc. Roy. Soc. London **102 B**: 488-533. 1928.
9. ————. Experimental researches on vegetable assimilation and respiration. XVII. The diurnal rhythm of assimilation in leaves of cherry laurel at "limiting" concentrations of carbon dioxide. Proc. Roy. Soc. London. **102 B**: 467-487. 1928.
  10. MATTHAEI, G. L. C. The effect of temperature on carbon dioxide assimilation. Ann. Bot. **16**: 591-592. 1902.
  11. ————. Experimental researches on vegetable assimilation and respiration. III. On the effect of temperature on carbon dioxide assimilation. Phil. Trans. Roy. Soc. London. **197 B**: 47-105. 1904.
  12. PFEFFER, W. The physiology of plants. Eng. trans. Vols. I and II. Clarendon Press, London. 1871.
  13. PANTANELLI, E. Abhängigkeit der Sauerstoffausscheidung belichteter Pflanzen von äusseren Bedingungen. Jahrb. wiss. Bot. **39**: 167-228 c. 1904.
  14. SPOEHR, H. A. Photosynthesis. Chem. Catalog Co., New York. 1928.
  15. STILES, W. Photosynthesis: The assimilation of carbon by the green plant. Longmans, London. 1925.
  16. WILMOTT, A. J. Experimental researches on vegetable assimilation and respiration. XIV. Assimilation by submerged plants in dilute solutions of bicarbonate and of acids: an improved bubble-counting technique. Proc. Roy. Soc. London. **92 B**: 304-327. 1921.