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(With Two Plates)

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E. D. McALISTER
Division of Radiation and Organisms,
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(Publication 3410)

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A spectrographic (infrared) method of carbon dioxide determination of unique speed and sensitivity has been developed. It has the additional merits of being independent of water vapor and of having small pressure and temperature corrections. At low concentrations the method will detect one part of carbon dioxide gas to a million parts of air. Without a great loss in sensitivity these measurements may be made in a fraction of a second. By changing the wave length of radiation used, other gases may be similarly observed. For example, by using radiation of wave lengths absorbed by water vapor the transpiration of a plant may be studied with equal success. Because of its speed, the method is particularly useful in following the time course of a gaseous exchange. In this connection it could be arranged to measure the metabolic ratio for each breath taken by an animal.

The purpose of this paper is to report results obtained by the application of this method to measurements of the carbon dioxide exchange between a higher plant (wheat, variety Marquis) and its surroundings.

The data obtained by Hoover, Johnston, and Brackett (1933) on the carbon dioxide assimilation of young wheat plants (variety Marquis) as a function of light intensity and carbon dioxide concentration are the most accurate and self-consistent so far reported. Figure 1 is one of their families of assimilation curves. The precision and reproducibility here shown justify their concluding sentence: "These experiments indicate that a wide range of critical experiments upon photosynthesis may be carried out with higher plants. . . . ." The present experiments on the same organism bear out this conclusion and bring forth much new information.

It is a pleasure for the writer to acknowledge his indebtedness to Dr. C. G. Abbot for his constant interest and enthusiastic support; to Dr. Earl S. Johnston for many helpful suggestions on the plant physiological side of this work; and to Dr. Dean Burk for many stimulating discussions.

EXPERIMENTAL

Since the method of measuring carbon dioxide used in the present experiments is new, it will first be very briefly described. The method is based upon the experimental fact that carbon dioxide gas is a very powerful absorber of a certain band of infrared radiation. Two millimeters thickness of pure carbon dioxide at N. T. P. absorb 78 percent of the radiation in a band from 4.2 to 4.3 μ. Thus in a long optical path, small concentrations of carbon dioxide will cause marked absorption of this radiation. No other gas or vapor, including water

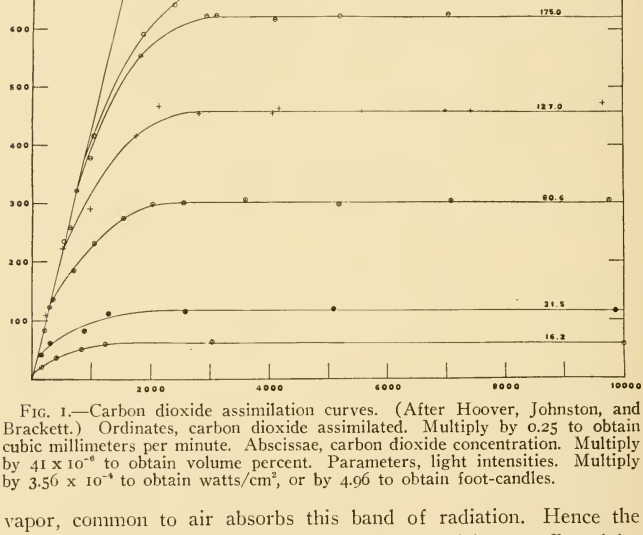

FIG. 1.—Carbon dioxide assimilation curves. (After Hoover, Johnston, and Brackett.) Ordinates, carbon dioxide assimilated. Multiply by 0.25 to obtain cubic millimeters per minute. Abscissae, carbon dioxide concentration. Multiply by 41×10^{-6} to obtain volume percent. Parameters, light intensities. Multiply by 3.56×10^{-4} to obtain watts/cm^2, or by 4.96 to obtain foot-candles.

vapor, common to air absorbs this band of radiation. Hence the method is specific for carbon dioxide alone, and is not affected by humidity changes. Because of its nature, the method has almost negligible temperature and pressure corrections. It is as sensitive as the best chemical methods since at small concentrations it will detect one part in a million (by volume) of carbon dioxide. It is practically instantaneous, the response of the galvanometer-thermocouple system (about 5 seconds) determining this. Because of this latter fact, for most purposes it is many times more sensitive than any chemical method, since it will detect this one-millionth part of carbon dioxide in 5 seconds.

Figure 2 shows a diagram, and plate 1 a photograph, of the plant-growth chamber, air-conditioning system, and optical absorption tube wherein the carbon dioxide is measured. This tube provides an optical path of about 1 meter. The growth chamber has a water jacket which provides constant "surroundings" for the plant. The water in this jacket has the same temperature as the incoming air, since it

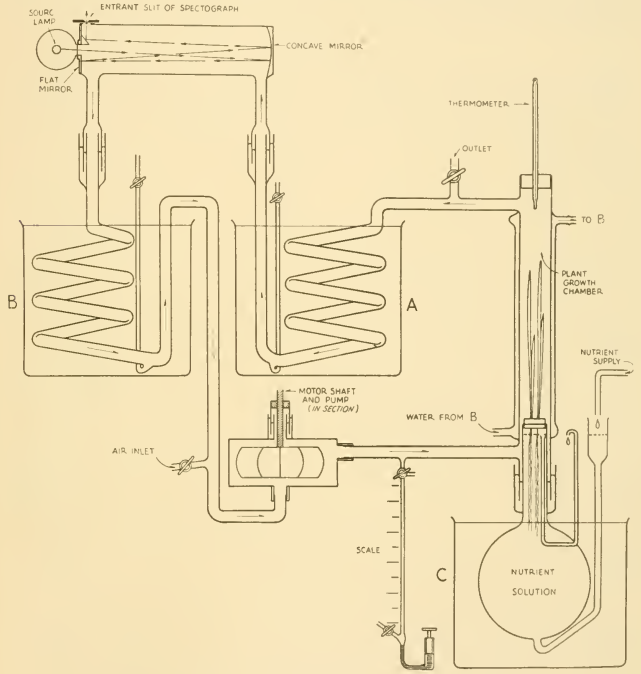

FIG. 2.—Plant growth chamber and accessory system.

flows from bath B. Bath A is held 3° C. below B to provide constant humidity. For simplicity's sake the temperature controls in baths A, B, and C (C provides root temperature control) are not shown. The spectrograph used has halite (rock salt) optics and is of numerical aperture about F-8. The vacuum thermocouple (located at the exit slit of the spectrograph and reading on the 4.2-4.3 μ band) and galvanometer used provide a sensitivity of from 2 to 5 ten-thousandths

of 1 percent carbon dioxide, depending on the concentration in the system, for 1 millimeter deflection. Calibration is accomplished by inserting known amounts of pure carbon dioxide into the system by

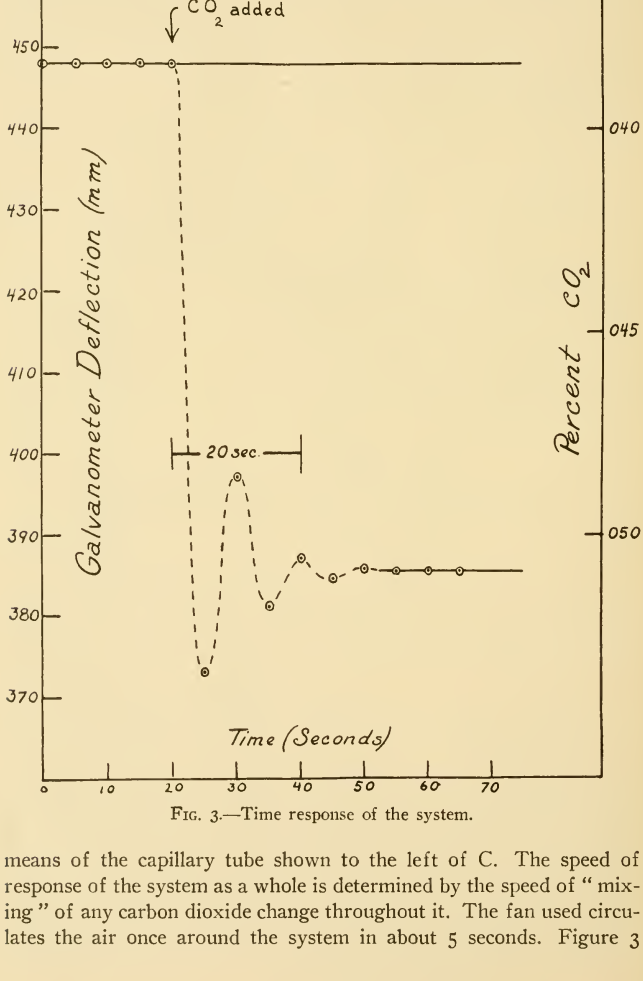

Fig. 3.—Time response of the system.

means of the capillary tube shown to the left of C. The speed of response of the system as a whole is determined by the speed of "mixing" of any carbon dioxide change throughout it. The fan used circulates the air once around the system in about 5 seconds. Figure 3

shows that after about four " trips " around the system, i. e., in about 20 seconds, complete mixing is accomplished. In following the response of a plant, galvanometer readings are taken every 30 seconds, including a " zero " reading. These readings are timed with a shutter operated by a synchronous motor. The " zero " readings are necessary to eliminate slow " zero drifts " that are inevitable in a galvanometer system of this sensitivity—1 millimeter scale corresponding to 10^{-9} amperes.

RESULTS

With this speed of response and sensitivity in mind, let us examine the reactions of three young wheat plants to light and darkness.

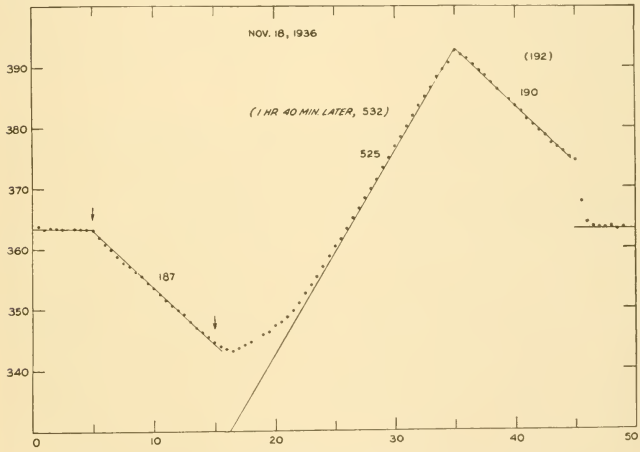

FIG. 4.—Response of plant after a 10-hour period of darkness.

Figure 4 shows a typical set of data. Ordinates, which are roughly inversely proportional to the carbon dioxide concentration in the system, are galvanometer deflection in millimeters. Abscissae are time in minutes. At the beginning of the experiment (plant in darkness), air from a supply tank is blown rapidly through the system and readings are taken every 30 seconds for 5 minutes. Then the system is closed (time=5), and we see respiration building up the carbon dioxide concentration. This is followed for 10 minutes to establish the rate, i. e., slope of the line. Next the plants are illuminated with white light of 500 foot-candles from a Mazda lamp (time=15). They had been in darkness for the previous 10 hours. During the

next 12 to 15 minutes we see the establishment of the assimilation rate. The light is left on for 20 minutes, then is turned off (time= 35), and we see the immediate termination of assimilation and appearance of respiration with practically no time lag. This respiration rate is the same as before the plant was illuminated. This sharp break which has always been found under all conditions indicates the reality of our belief that respiration under constant temperature and humidity conditions proceeds during assimilation at the same rate as before or after a period of illumination. If this were not the case, surely the plants would have to readjust themselves to the new rate and that readjustment would appear here as a curve. This method then throws new light on the much-considered question as to the rate of respiration during assimilation. The assimilation and respirational rates were measured 1 hour and 40 minutes later and had the values shown in brackets.

The above example illustrates the response of a plant following a long period of darkness. Let us interrupt it during the middle of its day and see how it responds. Figure 5 shows the type of response following a few hours exposure to light. Here the induction period after 10 minutes previous darkness is only slightly over 2 minutes in length. The sharp break occurs as always when the light is turned off. There is a surprising similarity as to time between this induction period for young wheat to that observed for algae by Warburg (1928, pp. 341-345) and Van der Paauw (1932, pp. 595-598). In this figure we see the induction period observed continuously for the first time. Both Warburg and Van der Paauw used indirect integrational methods. Figure 6, from data by Van der Paauw, shows this induction period for *Hormidium*. The striking similarity between these data for algae and those for young wheat plants in figure 5 shows that we are dealing with a mechanism fundamentally the same in both plants for carbon dioxide assimilation.

In the work of Van der Paauw on the variation of induction with temperature, we note that at a lower temperature more time is necessary to establish the final assimilation rate. Figure 7 shows similar results for measurements made at 12° C. and 31° C. for young wheat plants, i. e., a longer induction period at lower temperatures. Attention is directed to the obvious difference in respiration at these two temperatures. The assimilation rates would be about as different when corrected for respiration.

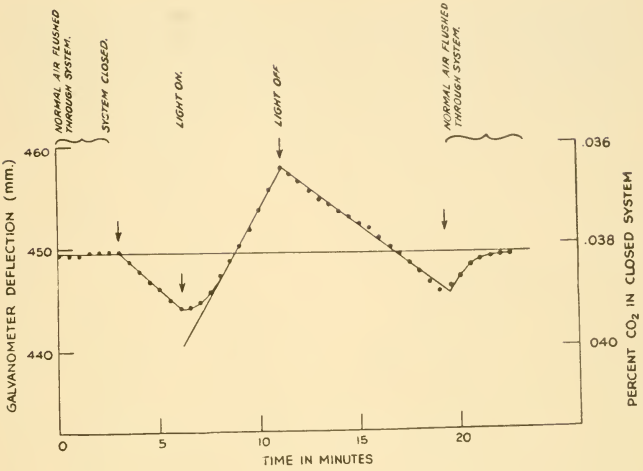

FIG. 5.—Response of plant during the middle of its "day."

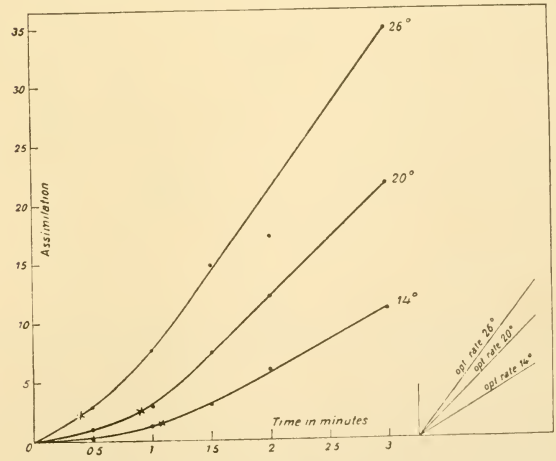

FIG. 6.—Induction period in *Hormidium*. (After Van der Paauw.)

Figure 8 shows Van der Paauw's (1932, pp. 560-564) curve for the respiration of *Hormidium* as a function of temperature. Values (indicated by circles) for the respirational rates of young wheat at

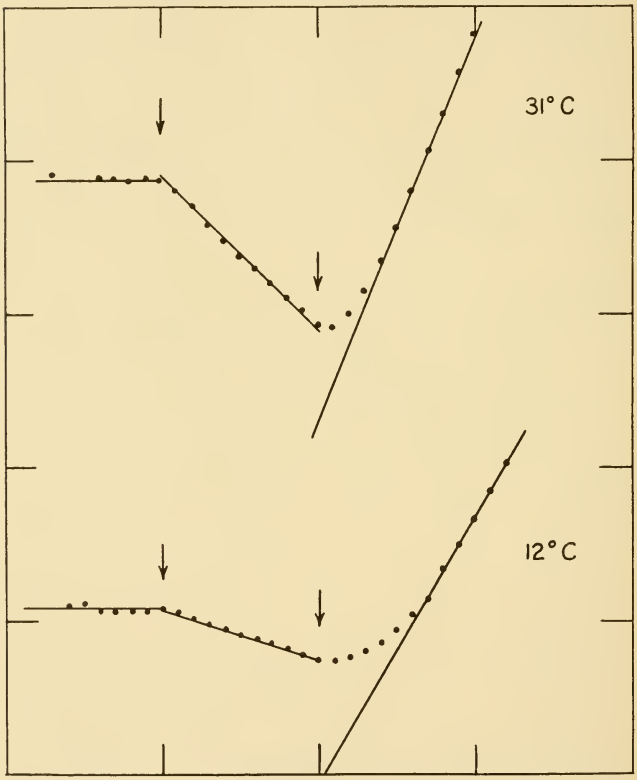

FIG. 7.—Effect of temperature on the induction period in wheat.

12°, 21°, and 31° C. have been placed on this curve and the agreement is striking, though perhaps not unexpected.

The variation of this induction period (for young wheat) with intensity of illumination is shown in figure 9. Note here the constancy of the respirational rates before and after illumination. If respiration

is a function of light, surely it would be a function of light intensity, which here it is not. There is a progressive decrease of induction with decreasing intensity. It should be pointed out that this induction period or lag may be considered as due to a certain amount of carbon dioxide lost to photosynthesis. When these amounts lost to photosynthesis

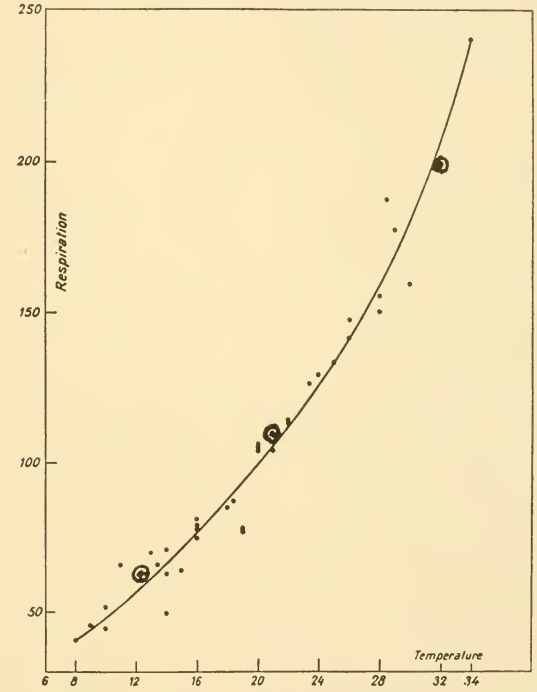

FIG. 8.—Comparison of effect of temperature on the respiration of *Hormidium* (Van der Paauw) and on wheat (circles).

are properly calculated from the true assimilation rates, the values plotted in the inset are obtained. This amount lost is approaching zero at zero light intensity and is apparently approaching an asymptotic value at high intensity. This qualitatively checks with Warburg's (1928) two experiments on *Chlorella* at about the same light intensity.

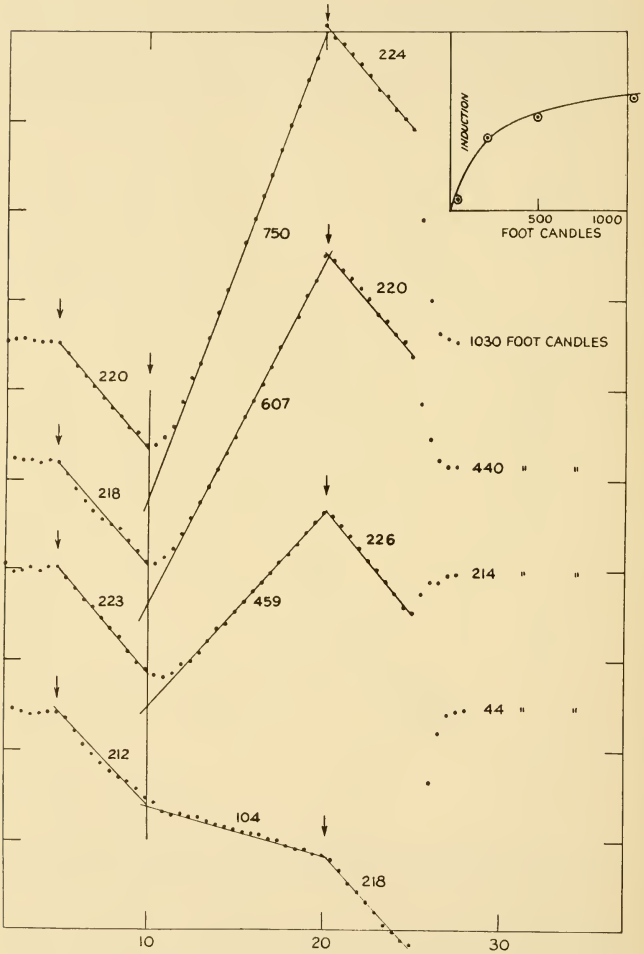

FIG. 9.—Effect of intensity of illumination on the induction period in wheat. The numbers adjacent to the curves give the rates of carbon dioxide respiration or assimilation (true) in cubic millimeters per 10-minute intervals.

Figure 10 shows the results of a 3½-hour " run " wherein the effect of intermittent illumination was studied. At the top we see the effect of continuous illumination for 15 minutes. The next graph shows the effect of equal 1-minute periods of light and darkness. Here the same intensity was used, but only one-half the amount of light for the 15-minute period. The system is fast enough to follow the plant processes. Next in order are shown the effects of equal periods of light and darkness of 15 seconds, 5 seconds, 1/2 second, 1/10 second, and 1/60 second length respectively. It should be noted that the induction period is apparently vanishing at high frequencies and seems to be amplified near the 5- and 15-second periods.

The change in efficiency of carbon dioxide assimilation with frequency of intermittency is clearly shown. The numbers to the right of each curve give the assimilation rate. In all cases the plant received the same quantity of light except the first, which receives twice as much. At one-half the intensity (here light is the limiting factor) the rate for continuous illumination would be 8.1. It is thus seen that the 60-second and 15-second periods give lower rates, while all the shorter intervals give higher rates, the shortest being a 95 percent increase in efficiency of assimilation over the continuous light. Here the rate per unit time is in fact essentially the same as that in continuous light, even though for only half the quantity of light. These results agree almost exactly with Warburg's (1928, pp. 332-334), even to the time relations. In both these experiments and Warburg's, a limiting increase of 100 percent is approached as long as equal periods of light and darkness are used; but if, as in the experiments of Emerson and Arnold (1932) the light periods are shortened with respect to the darkness, the efficiency per unit light may be increased several hundred percent. Data from experiments not shown on this figure give assimilation rates intermediate between that for continuous light and 1-minute intervals for 2-minute and 5-minute periods. Thus we see not only the previously observed increase of assimilation rate with frequency of intermittency, but a minimum of assimilation at about 1-minute periods.

This last-named finding—a minimum of photosynthesis—is strikingly suggestive of Garner and Allard's (1931) results shown in plate 2. This shows the integrated vegetative growth effects obtained by them with higher plants in light of the intermittency periods indicated. Their results have, in this part, been unexplained. It is now indicated that this minimum of growth corresponds to a minimum of photosynthesis because at these rates of intermittency the plant is

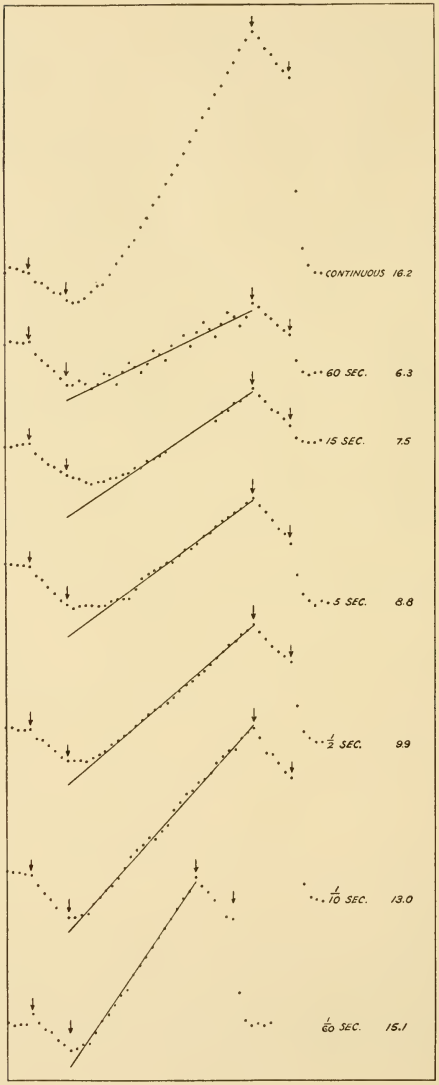

FIG. 10.—Effect of intermittent illumination for equal light and dark periods of the indicated length on the carbon dioxide assimilation of wheat plants.

most of the time in the induction phase. The increased growth toward higher frequencies found by Garner and Allard (1931) is a true intermittency effect. The increased growth toward longer periods can be explained as a decreasing percentage effect of the induction period.

It may be stated further that the induction relations found or reported in this paper show again a striking similarity to the results of Franck (1936) and of Kautsky and Flesch (1936), Kautsky and Marx (1936), and Kautsky and Hormuth (1936) on the fluorescence of photosynthesizing plants. Unquestionably, one is studying exactly the same phenomenon by direct fluorescence observations and by measurement of carbon dioxide.

DISCUSSION

Comparison of the induction period in wheat to that in algae may be questioned because of possible stomatal effects in the higher plant. In the present experiments it is believed that these effects—if any—have been eliminated by maintaining a high relative humidity around the plant and by measuring the induction period (except as in fig. 4) after a 20-minute dark period, the plants previously having been illuminated for more than an hour. Thus, presumably, the stomata were kept open during illumination by high humidity and the induction period measured before they had time to close. Attempts were made to close the stomata enough to limit carbon dioxide assimilation by subjecting the plants to a relative humidity of 5 to 10 percent for an hour. No difference could be detected between the assimilation at this humidity and that at the usual high humidity of 70 percent. This agrees with recent work by J. W. Mitchell (1936) and others. It appears then that the induction period in the present experiments (except as in fig. 4) is not affected by stomatal movement.

A calculation of the length of the diffusion path (carbon dioxide through water) in wheat leaves is of interest. Taking a case where the carbon dioxide concentration in the air is the limiting factor (high illumination and normal air concentration), when the thickness of a water film which would have the same diffusion resistance is calculated, this thickness ought to be of the same order of magnitude as the length of the diffusion path in the leaf. Making the same assumptions as Van den Honert (1930) did in his calculations, data from the present experiments give a water film about one-fifth of the leaf thickness. Since the area of both sides of the leaf was considered, this is reasonable. Using this value for the water-film thickness, it is now possible to calculate the time necessary for the carbon dioxide to

diffuse to the chlorophyll. Taking a case where light is the limiting factor (200 foot-candles), and assuming the concentration at the chlorophyll to be one-third that in the surrounding air, we find that 0.9 second is the time required.[1] This is small compared to the length of the induction period (about 1½ minutes) at this intensity (see fig. 9). It appears then that this induction in a higher plant is a process not structural or physiological, but fundamentally chemical.

[1] In figure 9 the numbers written adjacent to the lines showing the respiration and assimilation (true) are cubic millimeters of CO_2 per 10 minutes at 20° C. For example, the value 750 on the top curve means that the uptake of CO_2 (respiration added) is 75 cubic millimeters per minute. The leaf area (both sides) was 360 cm². The leaf thickness was 0.08 to 0.09 millimeters. The diffusion equation is

$$V = \frac{KA}{L}(C_2 - C_1) \cdot t \quad \ldots\ldots\ldots (I)$$

where

V = volume of CO_2, (cm³)
A = cross-sectional area of path, (cm²)
L = length of path, (cm)
C_2 = concentration (volume) at outer surface
C_1 = concentration (volume) at inner surface
K = diffusion constant (CO_2 in water) cm²/sec.
$= 1.8 \times 10^{-5}$ cm²/sec. (from I. C. T.)
t = time in seconds

assuming

$C_1 = 0$ (CO_2 limiting factor)
$C_2 = 0.0003$ (normal air)

and solving (I) for L we have

$$L = \frac{KA}{V}(C_2 - C_1)\, t$$
$$= \frac{1.8 \times 10^{-5} \times 360 \times (0.0003) \times 60}{.075}$$
$$= 15 \times 10^{-4} \text{ cm or } 15 \times 10^{-3} \text{ mm}$$

This is about ⅛ the leaf thickness.

The time calculation is as follows: From figure 9 for an illumination of 214 foot-candles (light limiting factor) there is an assimilation of 46 cubic millimeters per minute, which is about 0.0008 cc per second. Equation (I) solved for the time is

$$t = \frac{LV}{KA(C_2 - C_1)}$$
$$= \frac{15 \times 10^{-4} \times .0008}{1.8 \times 10^{-5} \times 360 \times (.0003 - .0001)}$$
$$= 0.9 \text{ seconds}$$

The dry weight of the leaves of the three plants used in this experiment was 260 milligrams. Assuming 1 percent of the dry weight of the leaves is chlorophyll we have, at 20° C., an assimilation number of

$$\frac{.075 \times .002 \times 60 \text{ (mg of } CO_2 \text{ per hr.)}}{2.6 \qquad \text{(mg of chlorophyll)}} = 3.5$$

At 30° C. this would be about 7.0.

It is sensitive to temperature and is caused by light. Van der Paauw came to this conclusion in studying induction in *Hormidium*.

All these results, taken together with the well-known experiments of Emerson and Arnold (1932) with flashing light, the flashes being very short and of very high intensity, make it evident that between continuous light and intermittencies (equal light and dark periods) of the order of 1/50 to 1/100 second, both light and dark periods are too long for maximum effect, i. e., are being wasted. In intermittencies more frequent than about 1/100 second, light is still being wasted, but the dark, or Blackman reaction is proceeding very efficiently. The quantitative aspects of the situation are markedly affected and complicated by the existence of the induction period.

It is now apparent that the induction period in a higher plant is an important source of information on the mechanism of photosynthesis. In this connection experiments are under way to correlate the amount of carbon dioxide lost to photosynthesis during the induction period with the amount of chlorophyll present in the plant. The asymptotic value this amount approaches at high light intensity may be compared to the total chlorophyll present since presumably at high illumination we have all the chlorophyll working. Further experiments on the effect of intermittent illumination of various ratios of light to dark periods are planned.

SUMMARY

The application of this method of gas analysis to measurements of the carbon dioxide exchange between a higher plant (wheat, variety Marquis) and its surroundings yielded the following results:

1. *Induction period.* The carbon dioxide assimilation measurements herein reported are the first ever made on the time course of photosynthesis during the first few seconds after illumination of a higher plant. The power of the method is evident when it is realized that these measurements are the first ever made continuously on any organism, i. e., by turning on the light and watching what happens. The work on the induction period previously reported (on algae only) has been done by an indirect integrational method. The time relations of this induction period for young wheat and its variation with temperature and intensity of illumination are found to be in excellent agreement with those previously found for algae. Further, it is shown that this induction represents a certain amount of carbon dioxide lost to photosynthesis and that this amount lost approaches zero progressively with decreasing intensities of illumination and is apparently

approaching an asymptotic value at high intensities of illumination. The induction period is prolonged to 12 or 15 minutes after a night of darkness. The striking similarity between the present data for young wheat plants and the previous work on algae shows that we are dealing with a mechanism fundamentally the same in both plants for carbon dioxide assimilation. The importance of this induction period as a source of information on the mechanism of photosynthesis should be emphasized, for it is chemical in nature, is sensitive to temperature, and is produced by light.

Induction in intermittent illumination of equal light and dark periods is shown to be very small at high frequencies—1/60 second length period—and is larger than normal for periods of from 5 to 15 seconds.

2. *Independence of respiration and illumination.* The time relations of respiration, i. e., the immediate appearance of respiration at the termination of illumination with a rate equal to that maintained before illumination, together with its independence of light intensity here reported for young wheat plants, lead to the conclusion that light has no direct effect on respiration.

3. *Intermittent illumination.* A minimum of carbon dioxide assimilation in flashing light (equal light and dark periods) has been found between 15-second periods and continuous illumination. This minimum probably falls between periods of 1 and 5 minutes length. The usual increase in efficiency of assimilation with increasing frequency of intermittency (for periods shorter than 15 seconds) has been found in young wheat plants and is seen to approach a limiting increase of 100 percent over continuous light.

This minimum of carbon dioxide assimilation is strikingly suggestive of Garner and Allard's results on the integrated growth effects of intermittent illumination of equal light and dark periods. For several higher plants they found a minimum of growth in the range of from 1 to 5 minute periods.

4. *Correlation with the fluorescence of chlorophyll.* The short time relations herein reported for the respiration and carbon dioxide assimilation of a higher plant strikingly confirm and correlate with much of the work on the fluorescence of chlorophyll in a higher plant reported by Franck and by Kautsky.

Recapitulating, it may be said that besides these new results, most of the previous work on the time course of photosynthesis with algae has been verified with wheat.

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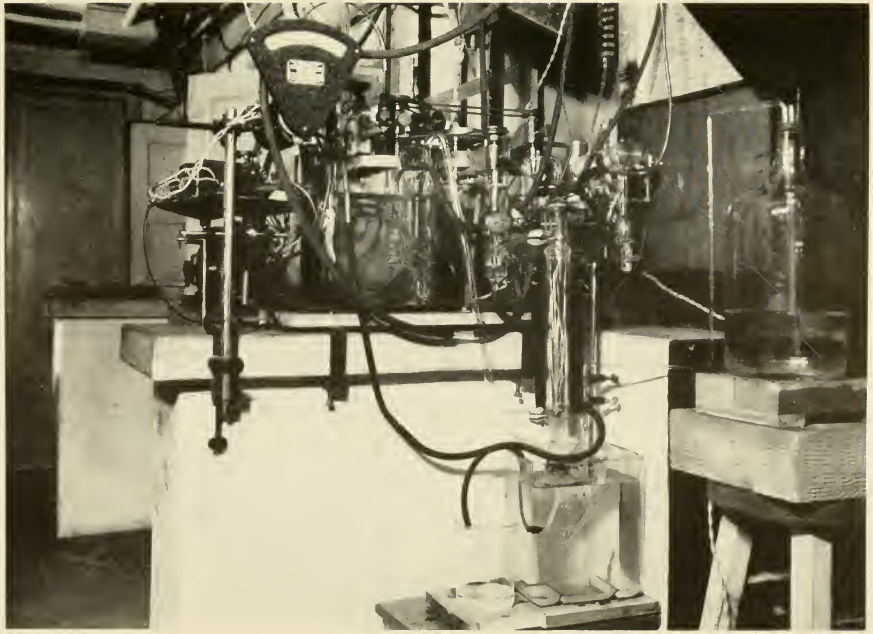

PHOTOGRAPH OF APPARATUS

EFFECT OF INTERMITTENT ILLUMINATION FOR EQUAL LIGHT AND DARK PERIODS OF THE INDICATED LENGTH ON THE GROWTH OF A HIGHER PLANT (YELLOW COSMOS)

(Courtesy of the United States Department of Agriculture.)