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Effects of Light Intensity on Photosynthetic Carboxylative Phase Enzymes and Chlorophyll Synthesis in Greening Leaves of Hordeum vulgare L.

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Summary. The effects of various light intensities on in vivo increases in activities of phosphoriboisomerase, phosphoribulokinase and ribulose-1,5-diP carboxylase and on synthesis of chlorophyll were studied in greening leaves of *Hordeum vulgare* L.

Each enzyme was already present in dark-grown plants, but further increases in activities required both a light treatment of the intact plant and a favorable temperature. The amount of enzymatic activity and chlorophyll developed was governed by light intensity.

Measured activities of phosphoriboisomerase and ribulose 1,5-diP carboxylase were highly correlated with synthesis of chlorophyll at all intensities studied. Measured activity of phosphoribulokinase was correlated with synthesis of chlorophyll only at saturating or near saturating light intensities. At decreasing light intensities the response curves of this enzyme differed from those of chlorophyll and of phosphoriboisomerase and ribulose-1,5-diP carboxylase. A lag period of phosphoribulokinase increased with decreasing light intensity. After the lag period a rapid rate of increase occurred which did not level off during 48 hours of illumination. Thus, a different control mechanism may be operative in inducing increased activity of this enzyme.

During greening, higher plants develop many of the biochemical components of the photosynthetic process. Upon exposure to light chlorophyll forms, chloroplasts begin to take on ordered lamellar form, and increases in activity of enzymes in the carboxylative phase of photosynthesis occur.

Tolbert and Gailey (19) reported that CO_2 fixation increased in greening intact wheat plants. Later, Hall et al. (5) showed that fixation of CO_2 by a system requiring the enzymes of the carboxylative phase of photosynthesis, i.e., phosphoriboisomerase (3, 9), phosphoribulokinase (9), and ribulose-1,5-diP carboxylase (15, 22), increased in activity during greening of barley leaves. Increased activity of phosphoriboisomerase (8) and ribulose-1,5-diP carboxylase (8, 13) in greening leaves has been demonstrated, but the response of phosphoribulokinase has not been reported. Also, further investigation was required to determine the response of each of the 3 enzymes in leaves greening at varying light intensity.

The relationship of increasing enzymatic activity to synthesis of chlorophyll in greening leaves is not clear. Several studies indicate a close correlation between the 2. Trown (20) suggests that fraction 1 protein (23), which contains ribulose-1,5-diP carboxylase (17, 18, 20, 21), may be part of the protein constituent of protochlorophyll holochrome. This indicates an intimate relationship between synthesis of chlorophyll and that of ribulose-1,5-diP carboxylase. Another report (7) indicates that increased enzymatic activity may be related more to structural development of the chloroplast.

The object of the present study was to determine how phosphoriboisomerase, phosphoribulokinase and ribulose-1,5-diP carboxylase respond in plants treated with varying light intensities and, concurrently, to investigate the relationship of increasing enzymatic activity to synthesis of chlorophyll.

Materials and Methods

Plant Materials. Hordeum vulgare L. var. Club Mariout was grown without light in vermiculite in plastic pots placed on top of pint jars filled with a half-strength nutrient solution. A cotton wick which connected the vermiculite in the pot with the solution in the jar supplied the developing seedlings with moisture. The plants were grown in aerated light-proof boxes in a growth chamber for 7 days at 24° and then illuminated for various periods of time. Seven-day-old plants were used in the study since at this stage growth of leaves is not significant during 48 hours, the maximum time of illumination. Thus, the increases in activities of the enzyme systems were due primarily to light, and were not confounded by growth. There was only a 3 % decrease in the moisture content of the leaves during the 48 hours of illumination. The first leaf of each plant was harvested for assay following illumination.

Nutrient Solution. The half-strength nutrient solution consisted of the following in meq per liter: $Ca(NO_3)_2$, 5; KNO_3, ½; K_2SO_4, 2; $MgSO_4$, 4; and $NH_4H_2PO_4$, 1; and in μmoles per liter; $MnSO_4$, 18; $ZnSO_4$, 3.8, H_3BO_3, 9.2; $(NH_4)_6Mo_7O_{24}$, 0.10; and Fe as Fe-ethylenediaminedi (*o*-hydroxyphenol) acetic acid, 91.

Light Treatments. Light intensity was varied from 20,900 to 1076 lux by changing the distance of the plants from a white light source consisting of a bank of 8 Sylvania VHO cool white fluorescent tubes, supplemented with five 60-w incandescent bulbs. For intensities of less than 1076 lux, the source consisted of tungsten lamps only.

Assay of Enzyme Systems. Conditions for the assay of each enzyme were so chosen that a doubling in the concentration of enzymatic extract resulted in a 2-fold increase in reaction rate. The coefficient of variability for assay of each enzyme was less than 5 %. The activity of each enzyme was measured at pH 8.0, since preliminary studies showed this value to be optimum for activity.

The activity of phosphoriboisomerase was assayed according to a slight modification of the method of Axelrod (3). The reaction mixture (0.6 ml) contained the following in μmoles: Tris buffer, 120; and ribose-5-P, 6. The reaction was started by the addition of 0.1 ml of a cell-free extract from 1 g of fresh leaf material in 36 ml of 0.2 M Tris buffer, pH 8.0, and stopped with 0.1 ml of 1.0 N H_2SO_4. At each assay period a time course series (20-sec intervals for 3 min) was run at 37°. Ribulose-5-P was followed by the cysteine-carbazole method of Dische and Borenfreund (4), as modified by Ashwell and Hickman (2). Activity was determined from the slope of the linear part of the resulting curve.

The activity of phosphoribulokinase was assayed by following the formation of ribulose-1,5-diP which was measured by the rate of acid production (16) with a pH stat (14). The reaction system was automatically maintained at pH 8.0 by recorded additions of 0.01 N NaOH. The reaction was started by the addition of the enzymatic mixture and was run at 28°. Reaction rate was determined from the slope of the recorded curve. Ribose-5-P was used as substrate, since the measured activity of phosphoriboisomerase was in excess in the cell-free extract. This excess was demonstrated by adding additional phosphoriboisomerase, which was purified from spinach (6), to the complete reaction system. No increase in the measured activity of phosphoribulokinase occurred. No activity was observed in the absence of ribose-5-P or ATP.

The reaction mixture (2 ml) contained the following in μmoles: ribose-5-P, 16; ATP, 18; $MgCl_2$, 30; EDTA, 2; GSH, 2; and 0.2 ml of cell-free extract from 1 g of leaf material in 3 ml of 0.2 M Tris buffer, pH 8.0.

The assay of ribulose-1,5-diP carboxylase was done at 28° according to previously established methods (20, 21). The reaction mixture (0.7 ml) contained the following in μmoles: ribulose-1,5-diP, 2; $KH^{14}CO_3$, 5, with a specific activity of 2×10^5 cpm per μmole; $MgCl_2$, 15; Tris buffer, 80; and 0.2 ml of cell-free extract from 1 g of leaf material in 21 ml of 0.2 M Tris buffer, pH 8.0. The reaction was stopped by adding 0.1 ml of 1.0 N HCl to each reaction system. Aliquots (0.1 ml) were dried under vacuum and subsequently counted for ^{14}C content with a gas-flow Geiger-Muller counter. At each assay period a time-course series was run at 3, 5, 7 and 9 minutes.

Preparation of ribulose-1,5-diP. Ribulose-1,5-diP was prepared according to Horecker et al. (6). Phosphoriboisomerase and phosphoribulokinase, used in the preparation of ribulose-1,5-diP were purified as described by Horecker et al. (6).

Preparation of Cell-Free Extracts. One g of leaves was ground with mortar and pestle in 3 ml of 0.2 M Tris buffer, pH 8.0, and then centrifuged at 37,000 × *g* for 15 minutes. After appropriate dilution the supernatant liquid was used as the source of enzymes. All procedures were carried out at 0°.

Chlorophyll and Protein. Chlorophyll content of leaves was determined spectrophotometrically from 80 % (v/v) acetone extracts according to Arnon's (1) modification of the method of Mackinney (12). Protein content of cell-free extracts was determined both by the biuret (10) and Lowry (11) methods. No significant differences in protein content due to light treatments were detected. The average protein contents determined by the Lowry method were 7.1 and 7.8 mg per g fresh weight from levels of dark-grown plants and from leaves of plants receiving 48 hours of illumination, respectively.

Results and Discussion

Changes in enzymatic activities and concentrations of chlorophyll accompanied the adjustment of dark-grown barley plants to varying light intensities. The amounts of enzymatic activity and chlorophyll

developed in the leaves were dependent upon light intensity as well as time of illumination (fig 1–4). At the several exemplary periods of illumination light saturation occurred between 5165 and 10,760 lux for

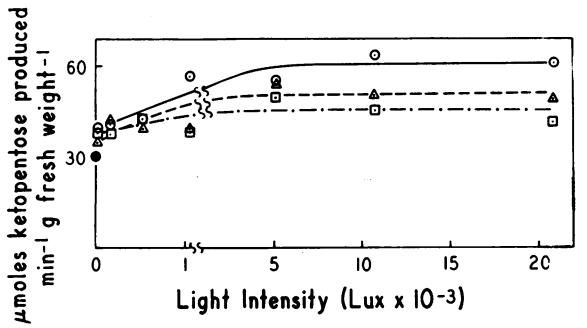


FIG. 1. Measured activity of phosphoriboisomerase vs. light intensity. ●, Dark control; ⊙, 36; △, 24; and ⊡, 12 hours of illumination, respectively. $LSD_{0.05,\ 0.01}$ (within intensities) 4.8, 6.4; (between intensities) 6.3, 8.4.

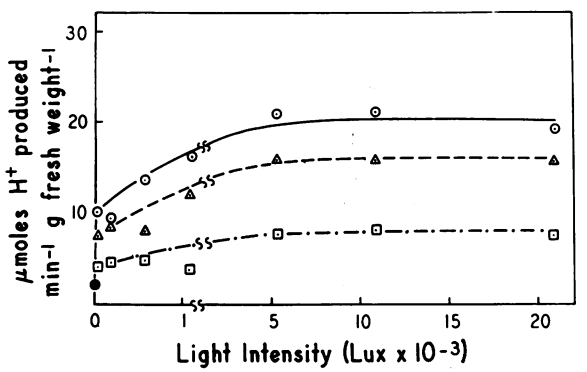


FIG. 2. Measured activity of phosphoribulokinase vs. light intensity. ●, Dark control; ⊙, 36; △, 24; and ⊡, 12 hours of illumination, respectively. $LSD_{0.05,\ 0.01}$ (within intensities) 1.0, 1.3; (between intensities) 1.3, 1.7.

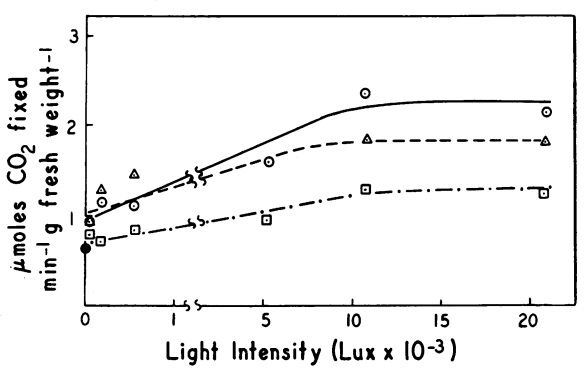


FIG. 3. Measured activity of ribulose-1,5-diP carboxylase vs. light intensity. ●, Dark control; ⊙, 36; △, 24; and ⊡, 12 hours of illumination, respectively. $LSD_{0.05,\ 0.01}$ (within intensities) 0.18, 0.24; (between intensities) 0.25, 0.33.

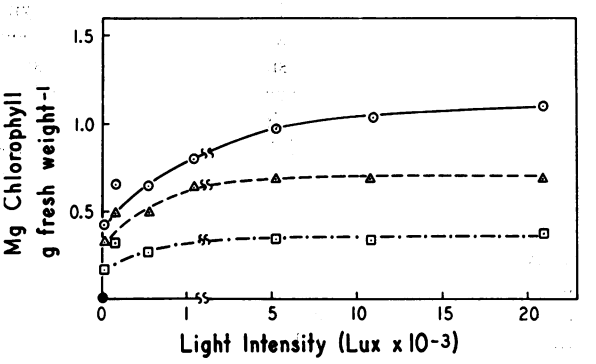


FIG. 4. Chlorophyll synthesis vs. light intensity. ●, Dark control; ⊙, 36; △, 24; and ⊡, 12 hours of illumination, respectively. $LSD_{0.05,\ 0.01}$ (within intensities) 0.20, 0.26; (between intensities) 0.08, 0.11.

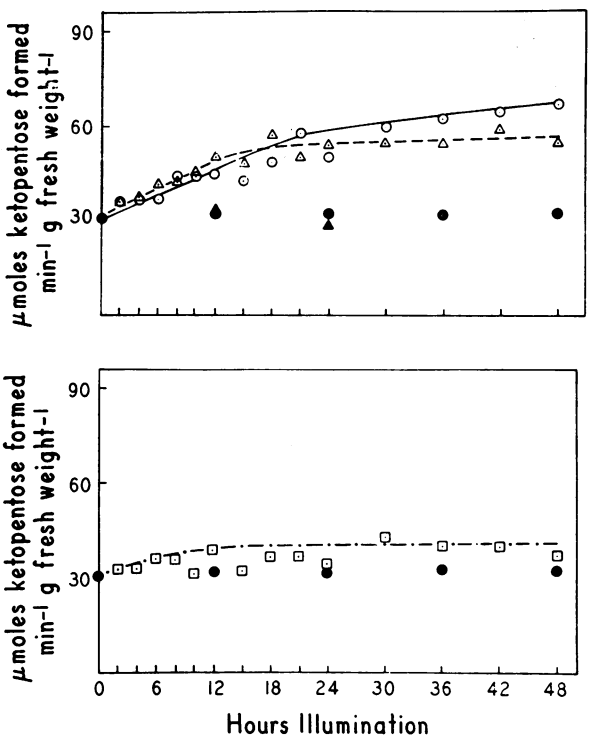


FIG. 5. The time course curves for measured activity of phosphoriboisomerase at various intensities. Upper: ⊙, 10,760 lux; △, 5165 lux; ▲, 10,760 lux at 0°. Both upper and lower: ●, dark control. Lower: ⊡, 32 lux. $LSD_{0.05,\ 0.01}$ (within intensities) 4.8, 6.4; (between intensities) 6.3, 8.4.

increases in the measured activities of phosphoriboisomerase (fig 1), between 1076 and 5165 for phosphoribulokinase (fig 2), and between 5165 and 10,760 lux for ribulose-1,5-diP carboxylase (fig 3). Light saturation occurred for chlorophyll synthesis between 1076 and 5165 lux at periods of illumination of 12 and

24 hours (fig 4). At 36 hours of illumination synthesis of chlorophyll increased only slightly between 5165 and 20,900 lux. No significant increase in synthesis of chlorophyll occurred at an approximate doubling of the light intensity from 10,760 to 20,900 lux. At times of illumination of 42 and 48 hours no significant increases in synthesis of chlorophyll occurred at intensities greater than 5165 lux. After 48 hours of illumination at saturating intensities the measured activities of phosphoriboisomerase, phosphoribulokinase and ribulose-1,5-diP carboxylase had increased about 2-fold, 12-fold, and 4-fold, respectively, above those of the dark controls (fig 5-7).

Activity of each enzyme was detected in dark-grown leaves (fig 5-7). An increase in activity required not only a light treatment of the intact plants but also a favorable temperature since no increases occurred when plants were illuminated at 10,760 lux at 0° (fig 5-7). At 10,760 lux the measured activity of phosphoriboisomerase increased more rapidly during the initial than latter 24 hours of illumination (fig 5). The increase in activity between 24 and 48 hours of illumination was only slight. At 5165 and 32 lux no significant increases in activity occurred after about 18 and 12 hours of illumination, respectively, indicating that steady states of enzymatic activity had occurred.

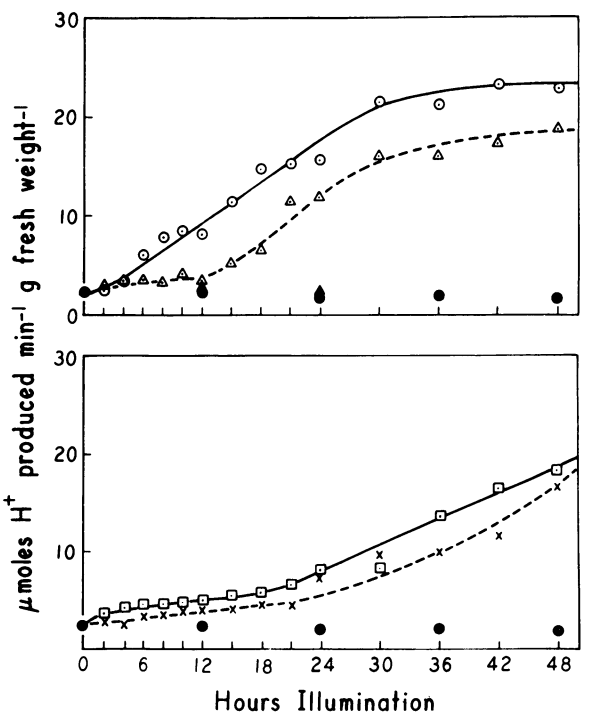


FIG. 6. The time course curves for measured activity of phosphoribulokinase at various intensities. Upper: ⊙, 5165 lux; △, 1076 lux; ▲, 10.760 lux at 0°. Both upper and lower: ●, dark control. Lower: ⊡, 538 lux; x, 32 lux. $LSD_{0.05,\ 0.01}$ (within intensities) 1.0, 1.3; (between intensities) 1.3, 1.7.

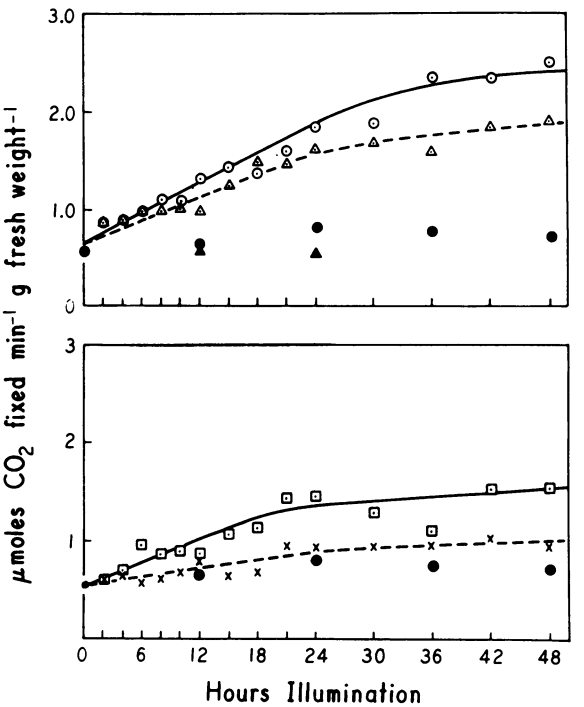


FIG. 7. The time course curves for measured activity of ribulose-1,5-diP carboxylase at various intensities. Upper: ⊙, 10,760 lux; △, 5165 lux; ▲, 10,760 lux at 0°. Both upper and lower: ●, dark control. Lower: ⊡, 538 lux; x, 32 lux. $LSD_{0.05,\ 0.01}$ (within intensities) 0.18, 0.24; (between intensities) 0.25, 0.33.

At 5165 lux no significant increase in the measured activity of phosphoribulokinase occurred after 30 hours of illumination indicating a steady state of enzymatic activity had been achieved (fig 6). At 1076 lux activity increased only slightly between 30 and 48 hours of illumination. At lower intensities an initial lag period was detected in the response pattern. As light intensities were decreased below saturation, the lag period was extended from 12 hours at 1076 lux to about 20 hours at 32 lux. After the long lag period activity increased rapidly without leveling off during the 48 hours of illumination at 538 and 32 lux. In contrast, the measured activities of phosphoriboisomerase (fig 5) and ribulose-1,5-diP carboxylase (fig 7) increased very little at 32 lux before leveling off.

At 10,760 and 5165 lux the measured activity of ribulose-1,5-diP carboxylase increased only slightly after about 36 and 24 hours of illumination, respectively (fig 7). At 538 and 32 lux no significant increases in activity occurred after about 21 hours of illumination indicating that steady states of enzymatic activity had occurred.

Similar to the measured activities of the enzymes, synthesis of chlorophyll increased more rapidly during the initial than final 24 hours of illumination (fig 8). At 1076 and 32 lux no significant increases in synthesis of chlorophyll occurred after 36 and 30

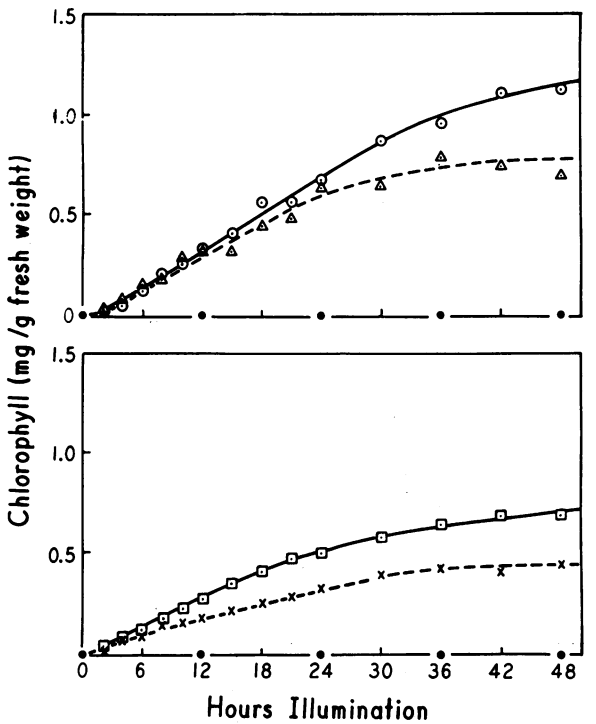


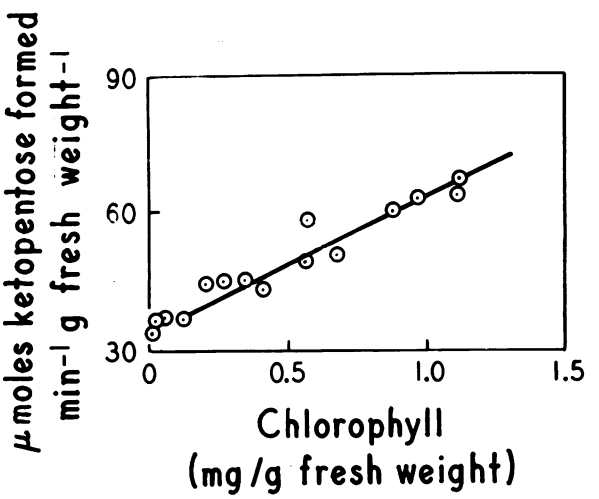
FIG. 8. The time course curves for chlorophyll formation at various intensities. Upper: ⊙, 5165 lux; △, 1076 lux. Both upper and lower: ●, dark control. Lower: ⊡, 538 lux; x, 32 lux. $LSD_{0.05,\ 0.01}$ (within intensities) 0.20, 0.26; (between intensities) 0.08, 0.11.

hours of illumination, respectively, indicating that steady states of chlorophyll synthesis had been achieved. About 20 % as much chlorophyll was synthesized at 32 lux as at 5165 lux.

To determine their correlation enzymatic activity was plotted against chlorophyll. Measured activities of phosphoriboisomerase and ribulose-1,5-diP carboxylase correlated highly (r = 0.93 and 0.96, respectively) with synthesis of chlorophyll at all light intensities studied (fig 9, 10). No significant changes in r values were observed at lower intensities.

The measured activity of phosphoribulokinase was also highly correlated (r = 0.95) with synthesis of chlorophyll at light intensities of 20,900 to 5165 lux. An example of the correlation is shown in figure 11. At lower, limiting light intensities, activity was not linearly correlated with synthesis of chlorophyll (fig 11). At light intensities of 1076, 538, and 32 lux synthesis of chlorophyll initially increased at a much greater rate than did the measured activity of the enzyme. Enzymatic activity at 32 lux started to increase only after synthesis of chlorophyll began to level off. At this point, enzymatic activity increased rapidly in relation to synthesis of chlorophyll. The activity developed during 48 hours at 32 lux was almost equal to that of 538 and 1076 lux, even though much less chlorophyll was present at 32 lux.

Increases in the measured activity of phosphoribulokinase may be regulated by a mechanism different from that of phosphoriboisomerase and ribulose-1,5-diP carboxylase as evidenced by the following: 1) at low light intensities phosphoribulokinase lacks the linear correlation with synthesis of chlorophyll that is shown by phosphoriboisomerase and ribulose-1,5-diP carboxylase; 2) the response curves of phosphoribulokinase to low light intensities are markedly different from those of phosphoriboisomerase and ribulose-1,5-diP carboxylase.



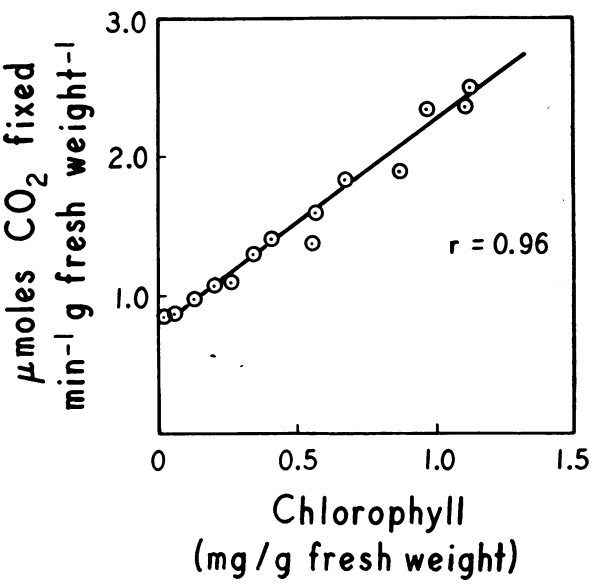


FIG. 9. Measured activity of phosphoriboisomerase vs. chlorophyll formation at 10,760 lux.

FIG. 10. Measured activity of ribulose-1,5-diP carboxylase vs. chlorophyll formation at 10,760 lux.

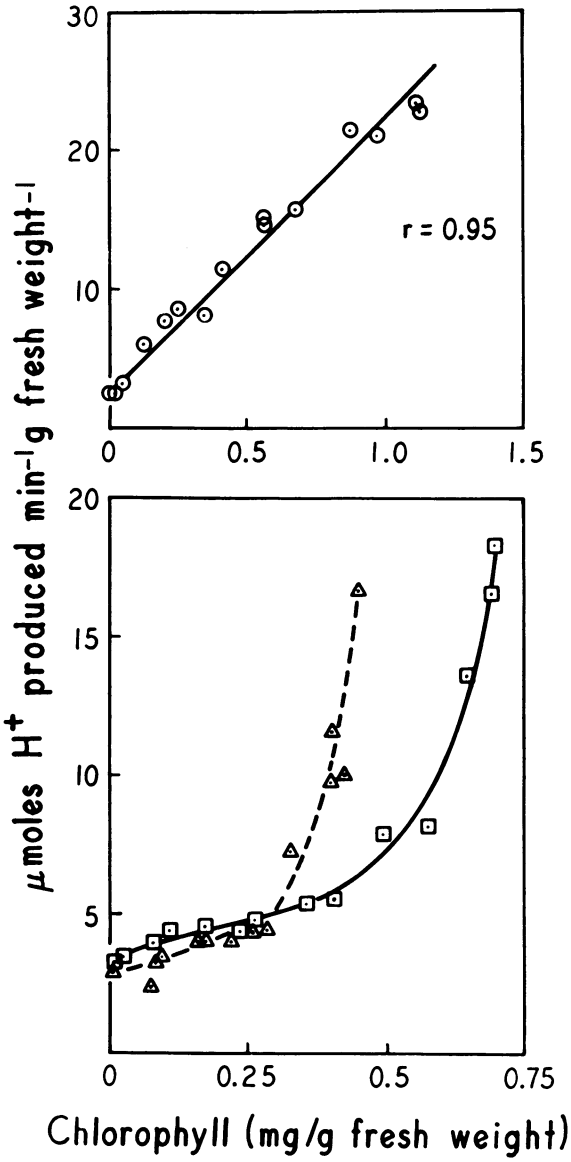


FIG. 11. Measured activity of phosphoribulokinase vs. chlorophyll formation. Upper: ⊙, 10,760 lux. Lower: ⊡, 538 lux; △, 32 lux.

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