Effect of Oxygen on Photosynthesis, Photorespiration and Respiration in Detached Leaves. I. Soybean[1]

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Summary. The effect of O_2 on the CO_2 exchange of detached soybean leaves was measured with a Clark oxygen electrode and infrared carbon dioxide analysers in both open and closed systems.

The rate of apparent photosynthesis was inhibited by O_2 while the steady rate of respiration after a few minutes in the dark was not affected. Part of the inhibition of apparent photosynthesis was shown to be a result of increased photorespiration. This stimulation of photorespiration by O_2 was manifested by an increase in the CO_2 compensation point.

The differential effects of O_2 on dark respiration (no effect) and photorespiration (stimulation) indicated that these were 2 different processes.

Moreover the extrapolation of the CO_2 compensation point to zero at zero O_2 indicated that dark respiration was suppressed in the light at least at zero O_2 concentration.

The rate of apparent photosynthesis has been shown to be inhibited by O_2 in a wide range of plant species (13). Until recently, however, no satisfactory explanation of this phenomenon has been proposed. The possibility that part of this inhibition might be due to a stimulation of respiration was not considered because A) the respiratory process which operates in the dark was thought to continue during photosynthesis (3, 13), and B) O_2 has no effect on the dark respiration of green leaves (7, 9, 13).

Recent evidence suggests that dark respiration is inhibited in the light in green leaves and algae and is replaced by a different respiratory process, photorespiration (4, 6, 12).

The question now to be answered is whether O_2 has an effect on photorespiration i.e. on the evolution of CO_2 in light.

In a previous communication from this laboratory, Tregunna et al. (12) showed that photorespiration was stimulated by O_2. This stimulation was manifested by an increased CO_2 compensation point, a decreased rate of apparent photosynthesis, and an increase in the magnitude of the dark CO_2 burst, which had been suggested to represent the last remnant of photorespiration (9, 10, 11).

From these results it was concluded that there were 2 different effects of O_2 on apparent photosynthesis: A) a stimulation of photorespiration; B) an inhibition of photosynthesis.

Because in the above experiments a closed system was used to measure the CO_2 exchange, difficulties resulting from continuously changing CO_2 concentrations did not allow a direct comparison of the rates of apparent photosynthesis at different O_2 concentrations. In the experiments reported below these effects of O_2 were investigated more fully, using a different plant species, a wider range of O_2 and CO_2 concentrations and steady state conditions.

Materials and Methods

Soybean plants, *Glycine max* Merr. var. Comet, were grown in pots of vermiculite in a growth chamber. The light intensity was 1500 ft-c, and the day length 16 hours. The temperature was 22.5° during the day and 19° at night. The plants were watered twice daily with tap water and once every 2 weeks with a solution of Plant Prod 20:20:20 (manufactured by the Plant Products Corporation, Blew Point, New York). Fully expanded trifoliate leaves were used 3 weeks from the date of planting.

Two methods were used to measure CO_2 exchange. A closed system apparatus was used to determine CO_2 compensation points during illumination and the CO_2 burst immediately after the light was turned off. An open system was used to determine the steady rates of CO_2 absorption in the light and production in the dark.

Closed System. The closed system apparatus incorporating a Beckman Infrared CO_2 analyser (IRCA) was that described by Lister et al. (5). To this was added a Clark oxygen electrode, as described

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by Tregunna et al. (12). The plexiglass plant chamber was of a design similar to that described by Tregunna et al. (10) but of smaller dimensions. The volume of this chamber was 250 ± 2 ml.

The O_2 content of the system was so large that its O_2 concentration was not appreciably changed during the experiments. Thus, the oxygen electrode was used only to measure the prevailing O_2 concentration in the system. Whenever the O_2 concentration was to be changed, the system was opened and a mixture of gases from tanks of N_2, O_2, and 5 % CO_2 in air was flushed through it for 2 or 3 minutes and then the system was closed again.

Open System. The open system apparatus is shown diagrammatically in figure 1. Gases from tanks of N_2 and O_2 were bubbled through distilled water in the wash towers (A) and (B). CO_2 from a tank of 5 % CO_2 in air was introduced into the gas stream through a fine capillary tube (D). The wash tower (C) served as a safety valve in case of the build up of excess pressures in the system. The gas stream was then passed through a flowmeter (E), the first IRCA (F), the oxgyen electrode (H), the plant chamber (I), the second IRCA (N), and finally through a second flowmeter (P) into the laboratory air. The readings from the IRCAs were recorded on the Texas Instrument Dual Recorders (G) and (O), while O_2 concentrations from the oxygen electrode (H), and temperature readings from the thermocouple (K), were recorded on the Bristol Recorder (J). Light was supplied by four 375-w Sylvania photoflood lamps (M) and filtered through 10 cm of water in the tanks (L).

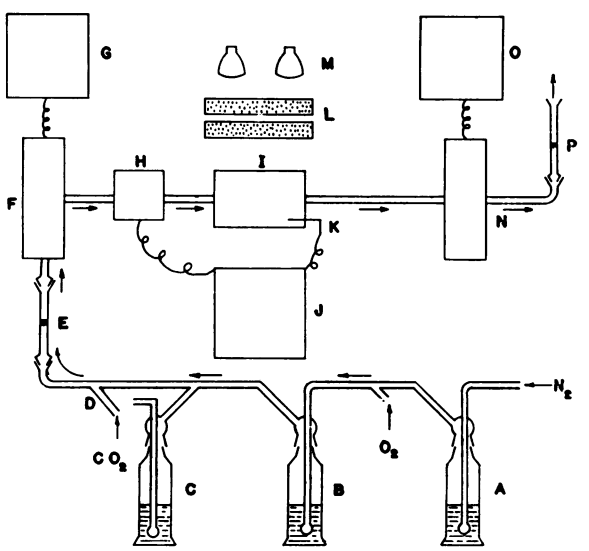

FIG. 1. Diagram of the open system apparatus. A and B, wash towers; C, wash tower as safety valve; D, capillary tube to introduce CO_2; E, first flow meter; F, first infrared CO_2 analyzer; G, first recorder; H, oxygen electrode; I, plant chamber; J, second recorder; K, thermocouple; L, water screen; M, photoflood lamps; N, second infrared CO_2 analyzer; O, third recorder; P, second flowmeter.

Each IRCA was carefully calibrated with standard CO_2 gas mixtures, and N_2, bubbled through distilled water, was used to zero the instruments. The 2 flowmeters, before the first and after the second IRCA, insured accurate flow rate measurements. Any difference in concentrations between the 2 IRCAs multiplied by the flow rate was a measure of the rate of CO_2 exchange. The temperature was always between 20 and 25° and did not vary more than 1° during any experiment. For measurements of CO_2 production in the dark, the lights were turned off and the plant chamber was covered with a black cloth. The maximal observed error of each rate determination was ± 6.4 %.

Experimental and Results

The object of these experiments was to study the CO_2 exchange of detached soybean leaves at various O_2 and CO_2 concentrations and at various light intensities in both open and closed systems. Soybean was chosen since it was previously shown to behave like tobacco with respect to the effect of light intensity on the CO_2 burst (11).

Three samples, each consisting of 4 leaves (total fr wt of each sample approximately 2 g), were studied separately. Leaves were detached at 8:45 AM, their petioles re-cut under water, and placed in the plant chamber in the open system. The leaf samples were then subjected to a number of consecutive light-dark cycles, each of which was at a different O_2 concentration. Each cycle consisted of 15 minutes in light at 600 ft-c, 5 minutes at 1000 ft-c, and 5 minutes in the dark. These were the periods of time required to obtain steady rates of CO_2 exchange at various light intensities. The O_2 concentration was always changed at the beginning of the 600 ft-c light period. Thus, a series of O_2 concentrations ranging between 1 and 100 % was formed with the first and the last concentration always being 21 % O_2. In this manner the reversibility of O_2 effect on CO_2 gas exchange could be observed. For samples 1 and 2, the average CO_2 concentration of the gas stream entering the plant chamber was 275 ppm, and for sample 3, it was 73 ppm.

At the end of these open system measurements, the CO_2 compensation point for each leaf sample was determined in the closed system at a number of different O_2 concentrations.

Finally, for sample 3 only, the magnitude of the dark CO_2 burst was measured (also in the closed system) after 10-minute light periods of 1000 ft-c at 1, 21 and 100 % O_2.

In the open system the concentration of O_2 and CO_2 in the gas stream entering the plant chamber was maintained constant. As shown in figure 2, the steady rate of apparent photosynthesis at both 275 and 73 ppm CO_2 decreased as the O_2 concentration increased. The 2 curves are described by the general equation: $y = ae^{-bx} + K$. At 275 ppm the equation for the curve was $y = 56.5e^{-0.02x}$; at 73 ppm the equation was $y = 24.3^{-0.02x} - 10$.

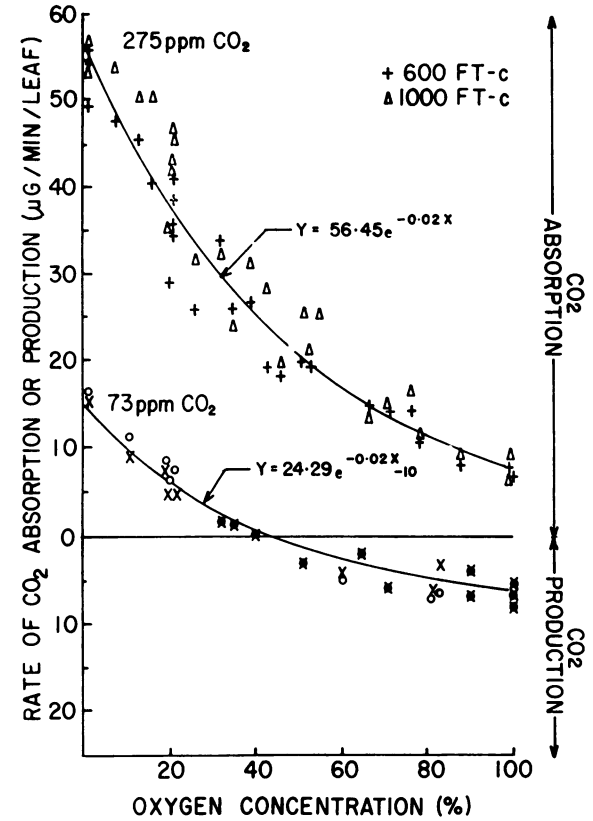

FIG. 2. Effect of O_2 on the rate of apparent photosynthesis in detached soybean leaves.

The effect of O_2 was reversible since approximately the same rates were obtained at 21 % O_2 after either high or low O_2 treatments. Since there was little effect of light intensity, CO_2 and not light was limiting the rate at both CO_2 concentrations.

At 73 ppm CO_2 and 43 % O_2, the rate of apparent photosynthesis was zero; that is, the CO_2 compensation point was 73 ppm at 43 % O_2. Above 43 % O_2 there was a negative rate; that is, below the CO_2 compensation point there was a net CO_2 evolution.

Dark Respiration. The steady rates of dark respiration at various O_2 concentrations are plotted in figure 3. The scale is the same as that in figure 2. O_2 had little effect on the rate of dark respiration.

CO_2 Compensation Point. As seen from figure 4, the CO_2 compensation point increased linearly from 1 to 100 % O_2. The linear regression for the points is $y = 1.63x + 2.8$, where y = CO_2 compensation point in ppm, and x = % O_2. The 2.8 was not significantly different from zero. As noted above in figure 2, the CO_2 compensation point was about 70 ppm at 40 % O_2.

CO_2 Burst. As seen from figure 5 the CO_2 burst also increased with increasing O_2 concentration. In leaves kept at 1 % O_2 the burst was completely lacking, while it was much higher at 100 % O_2 than at 21 %. The rates of CO_2 production after 300 seconds in the dark were about the same for all 3 O_2 concentrations, as already observed in figure 3.

Inhibition of Apparent Photosynthesis by O_2. These effects of O_2 on the CO_2 exchange of detached soybean leaves are essentially in agreement with those described by Tregunna et al. (12) for tobacco.

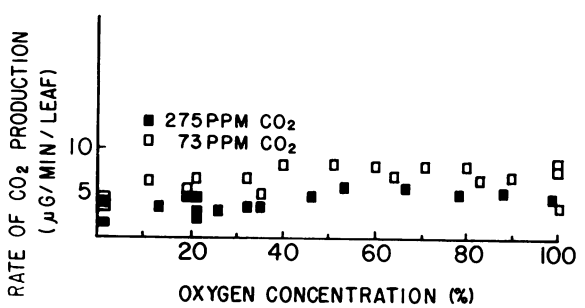

FIG. 3. Effect of O_2 on the steady rate of dark respiration in detached soybean leaves.

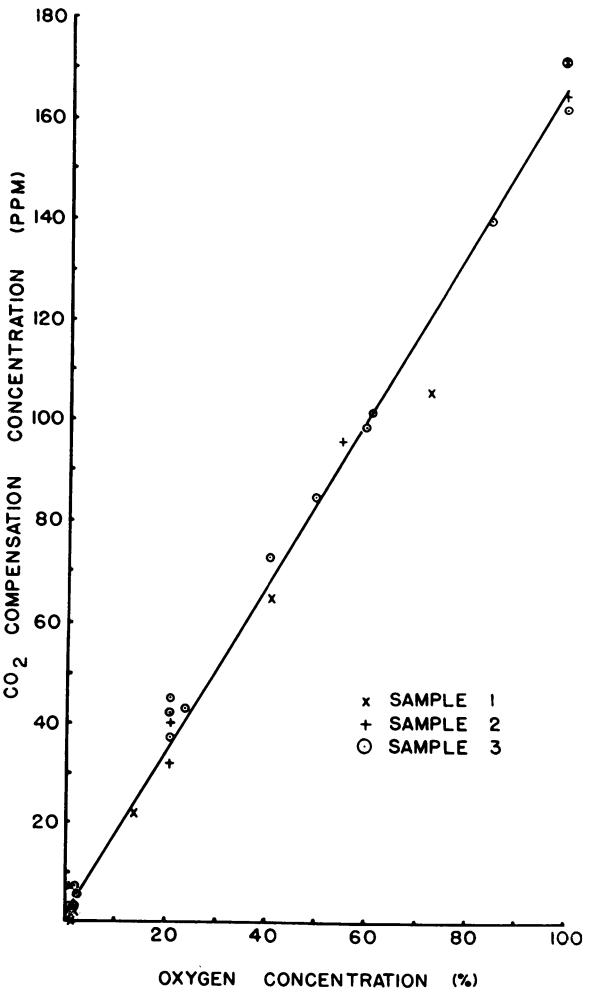

FIG. 4. Effect of O_2 on the CO_2 compensation point in detached soybean leaves.

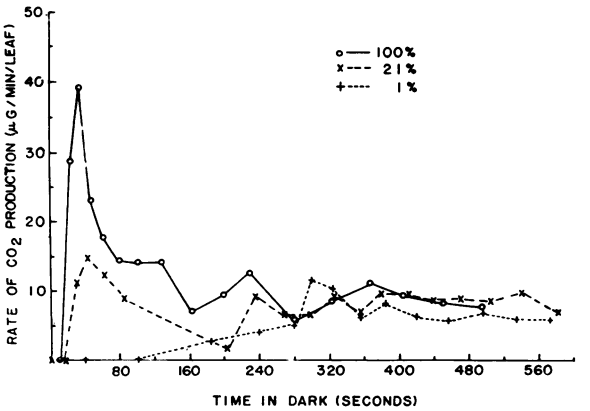

FIG. 5. Effect of O_2 on the CO_2 burst at 1, 21 or 100 % O_2 after previous illumination of detached soybean leaves. (CO_2 evolution during the first 60 seconds in the dark is the CO_2 burst).

The total inhibition of apparent photosynthesis (I_T) can now be calculated directly from the data of figure 2, and expressed as a percentage of the noninhibited value:

$$I_T = \frac{(APS_{max} - APS)}{APS_{max}} \times 100 \qquad I$$

where APS_{max} = the rate of apparent photosynthesis extrapolated to zero O_2 concentration (noninhibited).

APS = the observed rate of apparent photosynthesis at various O_2 concentrations.

The results of such a calculation are plotted in figure 6. The lower curve represents the data obtained directly from the 275 ppm graph of figure 2 and the upper curve from the 73 ppm graph. The percent inhibition was always higher at lower CO_2 concentration.

Photorespiration. At the CO_2 compensation point the rate of CO_2 evolution is equal to the rate of its absorption. Tregunna et al. (9, 12) showed that the rate of CO_2 production during photosynthesis at the CO_2 compensation point could be expressed as follows:

$$PR \text{ (photorespiration)} = CE\ [CO_2 \text{ comp.}] \qquad II$$

where CE(carboxylation efficiency) $= \frac{APS}{[CO_2] - [CO_2 \text{ comp.}]}$

and $[CO_2 \text{ comp.}]$ = CO_2 concentration at the CO_2 compensation point.

Figure 7 will serve to clarify the rationale behind the derivation of equation II. In this figure the rate of apparent photosynthesis (APS) is plotted against CO_2 concentration in air, with different percentage of O_2 in it. The slope of each graph represents the rate at which CO_2 is absorbed at the same percent of O_2 but at different CO_2 concentrations. This slope has been designated as a carboxylation efficiency (CE) (9, 12). The x-intercepts of various graphs with the abcissa represent the CO_2 compensation points (i.e. $[CO_2$ comp.]) at various O_2 concentrations.

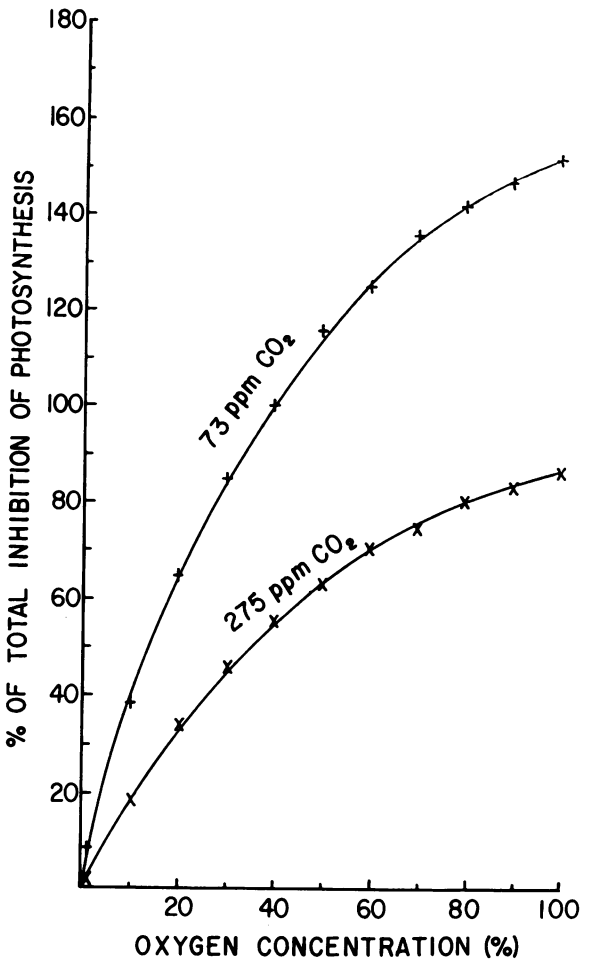

FIG. 6. Total inhibition of apparent photosynthesis by O_2 in detached soybean leaves.

From the examination of figure 7 it is clear that at each concentration of O_2 apparent photosynthesis (APS) is expressed by the slope of the graph (called Carboxylation Efficiency or CE) multiplied by the concentration of CO_2 above the compensation point. The slope of the graph is decreasing and the compensation point is increasing with increasing percentage of O_2 in the air.

True photosynthesis at each O_2 concentration, being apparent photosynthesis corrected for the amounts of CO_2 respired, would be represented by a line drawn parallel to each of these graphs and intercepting abcissa at zero CO_2 concentration. The magnitude of true photosynthesis (TPS) at compensation point is equal to $CE \times CO_2$ concentration at compensation point. Since at compensation point true photosynthesis is equal to CO_2 evolution or photorespiration, we arrive at the following expression of photorespiration: Photorespiration (PR) = $CE \times CO_2$ at compensation point, or stated briefly: Photorespiration (PR) = $CE \times [CO_2$ comp.]. A detailed de-

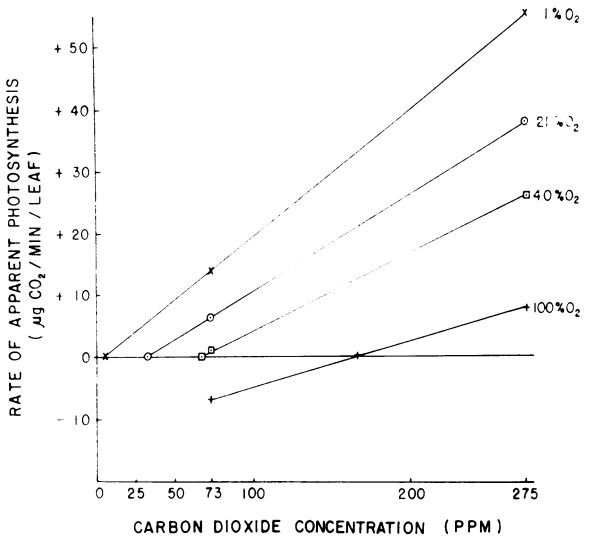

FIG. 7. Effect of CO_2 on the rate of apparent photosynthesis at various O_2 concentrations in detached soybean leaves.

scription for the derivation of this equation is given elsewhere (12).

Equation II was used to calculate the rate of photorespiration for the soybean leaves in this experiment. The results of this calculation are shown in figure 8 where the rate of photorespiration (PR) is compared with that of dark respiration (R_D) at various O_2 concentrations.

Photorespiration was less than steady dark respiration from 1 to 10 % O_2, but became greater above 10 %. Dark respiration was only slightly affected by O_2, while photorespiration increased 12 times with the increase of O_2 from 1 to 100 %.

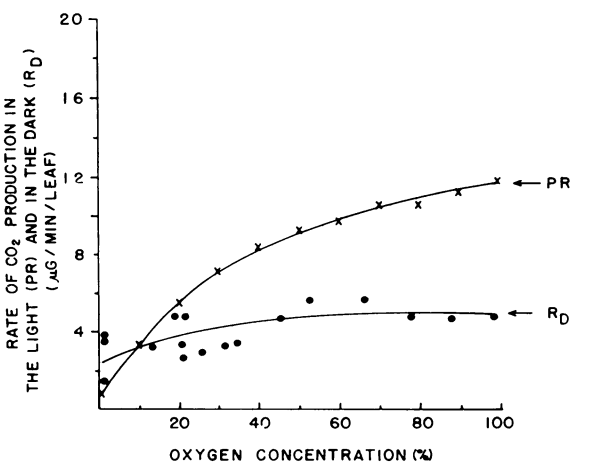

FIG. 8. Effect of O_2 on the rate of photorespiration (PR) and dark respiration (R_D) in detached soybean leaves.

Discussion

The results presented in this paper have confirmed and extended the studies of Tregunna et al. (9, 12) on the effects of O_2 on photosynthesis, photorespiration, and dark respiration. The most important conclusion from these results is that dark respiration is inhibited during photosynthesis and is replaced by a different process: photorespiration. Hoch et al. (4), measuring the oxygen-18 exchange of green and blue-green algae, and Osbun et al. (6) measuring the oxygen-18 and carbon-13 dioxide exchange of excised leaves of *Phaseolus vulgaris*, reached similar conclusions.

Tregunna et al. (9, 10, 11) initially assumed that the CO_2 burst represented the overshoot of increased respiration in the light. They observed that in tobacco leaves the CO_2 burst increased with increasing light intensity (10) and O_2 concentration (12), whereas the steady rate of CO_2 production, measured after a few minutes in the dark, was not affected. These differential effects of O_2 and light intensity on the initial CO_2 burst and the final steady rates of dark respiration indicated that these 2 processes are different. These workers also concluded that dark respiration was inhibited in the light (11, 12). This was based on the observations that in tobacco CO_2 production at zero O_2 was zero (i.e., the CO_2 compensation point extrapolated to zero at zero O_2).

These earlier observations on tobacco leaves were confirmed in the present investigation of soybean. Thus, both the CO_2 burst (fig 5) and the CO_2 compensation point (fig 4) increased with increasing O_2 from 1 to 100 %, the line extrapolated to zero at zero O_2 (fig 4) and there was no effect of O_2 on dark respiration over the same range of concentrations (fig 3). Moreover, the linear relationship between the CO_2 compensation point and O_2 concentration persisted from 0 to 100 %, whereas in the experiments using tobacco leaves (10, 12) observations were carried out only up to 50 % O_2.

The effect of O_2 on photorespiration and dark respiration were compared in figure 8. It is obvious that the value for the photorespiration will be affected by the O_2 concentration and, also, by the light intensity (9, 12). The conflicting results reported for the measurements of the rate of respiration during photosynthesis can probably be explained on the basis of the differing experimental conditions used in these various investigations (1, 2, 3).

The percent inhibition of apparent photosynthesis was higher, the lower was the CO_2 concentration (fig 6). This was also observed previously by Tamiya and Huzisige (8) and Turner and Brittain (13). The reason for this is evident from table I. As is seen from this table the rate of photorespiration was not affected by the CO_2 concentration, while the rate of photosynthesis was. As a consequence, photorespiration accounted for a greater percentage of the inhibition at the lower CO_2 concentration when the rate of photosynthesis was low, than at the

Table I. *Effect of CO_2 on the Inhibition of Photosynthesis in Detached Soybean Leaves by O_2*

The data are given as μg of CO_2 absorbed or produced per minute per leaf.

% O_2		1	21	100
Apparent photosynthesis	*Low	13.8	6.3	−6.6
	**High	55.4	37.8	7.9
Photorespiration	Low	0.89	5.94	11.8
	High	0.91	5 58	11.9
True photosynthesis	Low	14.7	12.2	5.2
	High	56.3	43.4	19.8
% Inhibition of apparent photosynthesis	Low	0	54.4	148 0
	High	0	31.8	85.8
% Inhibition of true photosynthesis	Low	0	17.0	64.6
	High	0	22.9	64.8
% Inhibition of apparent photosynthesis due to photorespiration	Low	0	67.4	53.7
	High	0	26.5	23.1

* Low = 73 ppm CO_2.
** High = 275 ppm CO_2.

higher concentration when the rate of photosynthesis was high. On the other hand, photosynthesis corrected for photorespiration (true photosynthesis) was inhibited by about the same extent at both CO_2 concentrations. It is suggested that this inhibition of photosynthesis per se may be due to A) a decreased rate of regeneration of the CO_2 acceptor because of the diversion of part of the absorbed carbon into photorespiration, or B) to a direct effect of O_2 on some component of the photosynthetic carbon cycle, or C) to a direct effect of O_2 on the formation of the primary photosynthetic products. At present there is no evidence which will allow us to distinguish between these alternatives. It might be noted here that these studies were carried out at CO_2 concentrations which limited photosynthesis and, therefore, the conclusions may not hold for saturating CO_2 concentrations.

Further information about the O_2 effects on photosynthesis could be obtained from a similar study of the gas exchange of young corn shoots which lack photorespiration (11). The results of such an investigation are reported in the following paper.

Acknowledgment

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