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Effect of Temperature, CO_2 Concentration, and Light Intensity on Oxygen Inhibition of Photosynthesis in Wheat Leaves[1]

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Abstract. The effect of 21 % O_2 and 3 % O_2 on the CO_2 exchange of detached wheat leaves was measured in a closed system with an infrared carbon dioxide analyzer. Temperature was varied between 2° and 43°, CO_2 concentration between 0.000 % and 0.050 % and light intensity between 40 ft-c and 1000 ft-c. In most conditions, the apparent rate of photosynthesis was inhibited in 21 % O_2 compared to 3 % O_2. The degree of inhibition increased with increasing temperature and decreasing CO_2 concentration. Light intensity did not alter the effect of O_2 except at light intensities or CO_2 concentrations near the compensation point. At high CO_2 concentrations and low temperature, O_2 inhibition of apparent photosynthesis was absent. At 3 % O_2, wheat resembled tropical grasses in possessing a high rate of photosynthesis, a temperature optimum for photosynthesis above 30°, and a CO_2 compensation point of less than 0.0005 % CO_2. The effect of O_2 on apparent photosynthesis could be ascribed to a combination of stimulation of CO_2 production during photosynthesis, and inhibition of photosynthesis itself.

The apparent rate of photosynthesis, whether it is measured as CO_2 assimilation or O_2 production, is inhibited by oxygen in many plant species (19, 20). This inhibition was first demonstrated by Warburg (21), and it is rapidly reversible by the removal of O_2 (17). Measurements of the magnitude of inhibition are often of the order of 30 to 40 % in air, although wide variations occur, depending on plant species and environmental conditions.

The degree of inhibition of photosynthesis increases in a non-linear fashion with increasing O_2 concentration in higher plants (8), *Chlorella* (17) and in isolated chloroplasts (7). Inhibition by O_2 is often absent at high CO_2 concentrations (3), and is greater at CO_2 concentrations below atmospheric (17). In *Chlorella* at low CO_2 concentration, temperature has no effect on the degree of inhibition of photosynthesis over the range 4° to 25° (17). Similar results are found with the moss *Funaria* between 20° and 30° (19). In wheat, cotton, and tobacco, however, recent data indicate that inhibition increases between 30° and 40° (11). Most studies show that the degree of O_2 inhibition is not affected by moderate to saturating light intensities (2, 13, 19, 22). In several cases, inhibition has been found to increase with increasing light intensity (19, 22). Little is known about the influence of very low light intensity.

It is generally accepted that the inhibitory effect of O_2 on apparent photosynthesis cannot be ascribed to a stimulation of dark respiration in the light. Respiration by leaves in the dark is almost completely saturated by about 2 % O_2 (1, 8), while the O_2 inhibition of photosynthesis is more pronounced at much higher O_2 concentrations.

In the present investigation, the effect of O_2 on photosynthesis in wheat was examined at different temperatures, CO_2 concentrations and light intensities to determine the magnitude and significance of the inhibition of photosynthesis.

Materials and Methods

Seeds of *Triticum sativum* L. were planted in vermiculite, watered daily, and grown in a growth room at 2000 ft-c light, 16 hour day, and 21 to 26/18 to 22° day/night temperature. In most experiments, about twenty-five 10 to 14 day-old shoots were detached, immediately immersed in water, and recut 5 mm below the node. The cut shoots were then transferred to a plexiglas chamber. When intact plants were used, the seeds were planted in a row so that shoots of the same age could be sealed into a plexiglas chamber by a rubber gasket coated with silicone grease. An 860 ml closed system was used for all of the experiments, and a constant flow rate of 2 liters per minute was maintained by a gas pump. The CO_2 concentration in the gas stream was measured from 0 to 0.060 % CO_2 by a Beckman 215 Infrared Gas Analyzer. To measure O_2 concentration, a Clark oxygen electrode was used with a Beckman Oxygen Adapter and a millivoltmeter. O_2 concentration measurements were accurate to ± 1.0 %. Initial O_2 and CO_2 concentrations were adjusted by flushing with N_2, or with 21 % O_2 and 0.033 % CO_2 in N_2. A thermistor was placed between 2 leaves in the chamber and temperature was indicated on a Yellow Springs Company Tele-

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thermometer. Temperature was controlled by placing the chamber in a freezer and circulating water through a jacket surrounding the chamber. Illumination was from a General Electric "Cool Beam" lamp and the light was passed through 14 cm of water to remove infrared radiation. Light intensity in the chamber was varied by changing the position of the lamp and by interposing sheets of Whatman No. 1 filter paper. Intensity was measured using a Gossen "Tri Lux" foot candle meter (Kling Photo Corporation, New York). An illumination of 1000 ft-c was equivalent to approximately 5.6×10^4 erg cm^{-2} sec^{-1} between 400 and 700 mμ.

Plants were allowed to adjust to experimental conditions for an initial 30 minute period in air. No group of plants was used for longer than 3.5 hours or at more than 1 set of conditions. Every experiment was done with at least 2 different groups of plants. CO_2 assimilation rates in the closed system were calculated from the time intervals required for the plants to decrease the CO_2 concentration by 0.005 %. Rates of photosynthesis given in this paper are apparent CO_2 assimilation rates in all cases. Percent inhibition of photosynthesis by O_2 was calculated using the method of Bjorkman (2), and carbon deficit was calculated using: C.D. = $12(P_3-P_{21})/44$. P_3 and P_{21} represent apparent photosynthesis rates at 3 ± 1 % O_2 and 21 % O_2 respectively; 12/44 represents the proportion of carbon in CO_2.

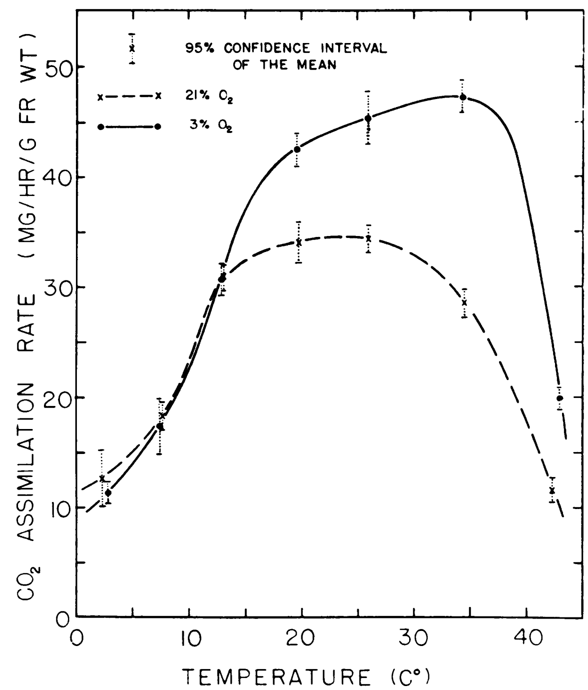

FIG. 1. Effect of temperature and O_2 concentration on the apparent rate of photosynthesis in 0.03 % CO_2 and 1000 ft-c light.

Results

Effect of Temperature on the Oxygen Inhibition of Photosynthesis at Normal CO_2 Concentration. An initial experiment was carried out to determine how temperature affected the apparent rate of photosynthesis in 21 % and 3 % O_2. Rates were measured at 7 temperatures ranging from 2° to 43°. Light intensity was 1000 ft-c, and the CO_2 concentration was 0.030 %. There was no significant difference between apparent rates of photosynthesis at 21 % and 3 % O_2 below 13° (fig 1). Above this temperature, however, the rate was always less in 21 % O_2 than in 3 % O_2. The temperature optimum was also different. In 21 % O_2, photosynthesis was most rapid between 20° and 26°, while in 3 % O_2, 30° to 36° was optimum. Thus, the inhibitory effect of oxygen on the apparent rate of photosynthesis in air is modified by temperature and can be eliminated by low temperature.

Effect of Temperature and Oxygen Concentration on the Carbon Dioxide Compensation Point. When plants are placed in a sealed chamber in the light, CO_2 is assimilated until an ambient CO_2 concentration is reached at which CO_2 assimilation is in equilibrium with CO_2 evolution. This concentration, the CO_2 compensation point, is approximately 0.004 % CO_2 for most plants and varies with temperature and light intensity (6, 23), figure 2 shows the effect of temperature and 21 % and 3 % O_2 on CO_2 compensation point at 1000 ft-c light. Compensation point increased with increasing temperature at both O_2 concentrations. The ratio of compensation point in 3 % O_2 to that in 21 % O_2 was

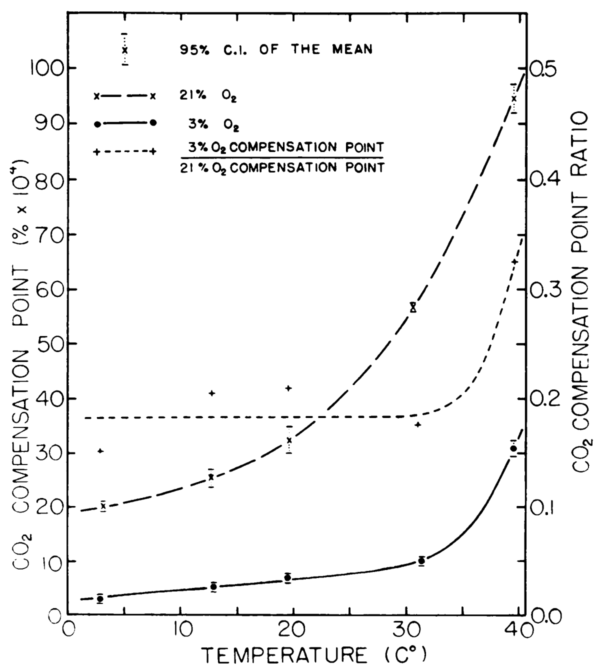

FIG. 2. Effect of temperature and O_2 concentration on the CO_2 compensation point in 1000 ft-c light.

constant between 2° and about 35° and was equivalent to the ratio of the O_2 concentrations used. Above 35°, however, the ratio increased rapidly. This increase corresponded to the sharp increase in CO_2 compensation point in 3 % O_2, and also to the decrease in the rate of CO_2 assimilation in 3 % O_2 which was seen in figure 1. It appears therefore, that above about 35° the balance between CO_2 assimilation and CO_2 production in the light is altered by a process which is active in 3 % and 21 % O_2.

Effect of Carbon Dioxide Concentration on the Oxygen Inhibition of Photosynthesis at Different Temperatures. At temperatures below 13°, it was evident that CO_2 compensation point was proportional to O_2 concentration, but the apparent rate of photosynthesis at 0.030 % CO_2 was not affected by the O_2 concentration used. Because of this anomaly, a thorough study of the changes in O_2 inhibition at different CO_2 concentrations was carried out. Rates of CO_2 assimilation in detached wheat plants were measured at 4 temperatures between 13° and 35° at CO_2 concentrations between 0.050 % and the CO_2 compensation point. Light intensity was maintained at 1000 ft-c. Results of this study are given in figures 3 and 4. Above 0.030 % at 13° and above 0.040 % CO_2 at 19.6°, the 95 % confidence intervals overlap indicating that there was no significant difference between the rates of CO_2 assimilation at the two O_2 concentrations. In all other conditions, the apparent rate of photosynthesis was less in 21 % O_2. Measurements made with intact plants at 13° and 34° showed a response to O_2 and CO_2 concentration identical to the response of detached leaves.

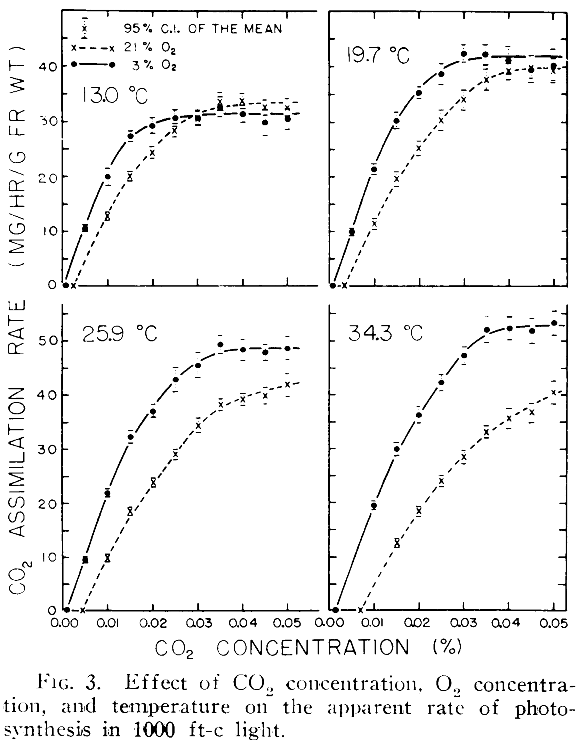

FIG. 3. Effect of CO_2 concentration, O_2 concentration, and temperature on the apparent rate of photosynthesis in 1000 ft-c light.

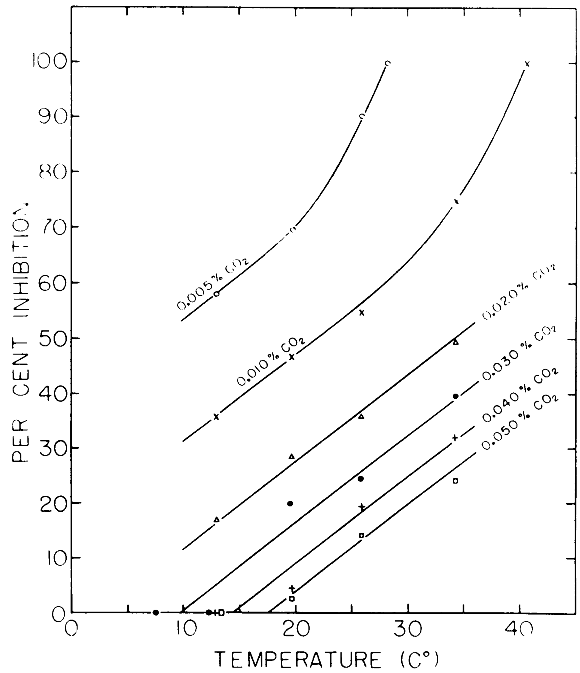

FIG. 4. Effect of temperature on the percent inhibition of apparent photosynthesis by oxygen at different CO_2 concentrations in 1000 ft-c light.

Percent inhibition of apparent photosynthesis by O_2 is given in figure 4. The data were calculated from the values shown in figure 3. 100 % inhibition of apparent photosynthesis was equivalent to CO_2 compensation point in 21 % O_2. It is clear that the degree of inhibition increased with increasing temperature. Except where the CO_2 concentration approached compensation point, the increase in inhibition appeared to be linear with the same slope at all CO_2 concentrations. Also, at any 1 temperature, the percent inhibition decreased in a non-linear fashion with increasing CO_2 concentration. O_2 inhibition of apparent photosynthesis is therefore most pronounced at high temperature and low CO_2 concentration.

Effect of Light Intensity. In addition to these studies at 1000 ft-c, the O_2 effect was also measured at 400 ft-c and 40 ft-c. Observations were made at 14° and 34° and at CO_2 concentration from 0.005 % to 0.050 %. CO_2 compensation point was elevated at low light intensity resulting in an increase in percent inhibition at CO_2 concentrations near compensation. Otherwise, light intensity did not significantly alter the magnitude of the O_2 effect.

Carbon Deficit. Percent inhibition is a relative measure of the O_2 effect and does not indicate changes in the quantity of carbon assimilated. Table

Table I. *Effect of Temperature, CO_2 Concentration, and Light Intensity on Carbon Deficit Caused by O_2 in Detached Wheat Leaves*

Temp	CO_2 conc	Light intensity	Carbon deficit
°	%	*ft-c*	*mg C hr⁻¹ g fr wt⁻¹*
13	0.030	1000	−0.05[1]
13	0.015	1000	1.99
13	0.030	400	0.23[1]
13	0.015	400	1.81
34	0.030	1000	5.13
34	0.015	1000	4.76
34	0.030	400	3.98
34	0.015	400	4.14

[1] Not significantly different from zero.

I shows the difference in carbon gain between 3 % O_2 and 21 % O_2 in various environmental conditions. It is apparent that high temperature greatly increases the carbon deficit. The effects of CO_2 concentration and light intensity are smaller and irregular. Thus, the inhibition of apparent photosynthesis by 21 % O_2 can substantially reduce photosynthetic productivity in wheat, particularly at high temperatures.

Discussion

The data presented above indicate that the inhibition of apparent photosynthesis by O_2 in young wheat shoots increases with increasing temperature and decreasing CO_2 concentration. Light intensity has no effect except at the compensation point. In air, the inhibition is insignificant below 13°. These results extend and are in accord with previous studies using higher plants (2, 8, 11, 13). A similar effect of CO_2 concentration has been observed in the alga *Chlorella* (17) and in the moss *Funaria* (19). In *Chlorella* and *Funaria* however, the O_2 effect was not temperature sensitive and the influence of light intensity varied.

The CO_2 compensation point results confirm earlier studies which demonstrated that CO_2 compensation point is directly proportional to O_2 concentration (8, 18). Above 35° in wheat, however, this relationship was disturbed in a way which can be ascribed to either a decrease in the true rate of photosynthesis or to an increase in the rate of CO_2 production in the light. Whether one or both of these alternatives occurred cannot be concluded from the results obtained.

Many tropical grasses (10), such as corn, have very high rates of apparent CO_2 assimilation (11, 16) and CO_2 compensation points less than 0.0005 % CO_2 (5, 15). Most other plants, like wheat, normally have lower apparent rates of photosynthesis and CO_2 compensation points of about 0.004 % CO_2. It is interesting to note how wheat resembles tropical grasses when the O_2 concentration is low. At 3 % O_2, the rate of CO_2 assimilation approaches that of corn (11), and the CO_2 compensation point is decreased to close to 0.0005 % CO_2. Also, it was observed in the present study (fig 1) that the optimum temperature for photosynthesis increased from 20–26° to 30–36° when the O_2 concentration was decreased from 21 % to 3 %. Murata, Iyama, and Honma (16) have previously found that the optimum temperature for apparent photosynthesis in wheat and most forage crops was 10 to 25°, while in tropical grasses photosynthesis was most rapid at 25 to 40°. At low O_2 concentration, therefore, gas exchange in wheat was comparable to that in tropical grasses with respect to CO_2 compensation point, rate of photosynthesis, and optimum temperature for photosynthesis. While CO_2 fixation in wheat and most other species seems to occur by the Calvin cycle (4), a different carboxylation pathway has been demonstrated in tropical grasses (9, 10). It will be interesting to know whether the carboxylation pathway in wheat is altered at low O_2 concentration.

Several suggestions have been advanced concerning the manner in which O_2 inhibits apparent photosynthesis (19), and one possibility which is receiving much current attention is that of photorespiration. Recent evidence has indicated that CO_2 production during photosynthesis differs in magnitude and mechanism from dark respiration (14, 18, 23). In particular, photorespiration is enhanced by much higher O_2 concentrations than are required to saturate dark respiration. O_2-stimulated photorespiration could depress the apparent rate of photosynthesis through competition for the CO_2 acceptor or a precursor of it, and by the production of CO_2 in the light.

Figure 2 and previous studies of CO_2 compensation (8, 18) demonstrate that either photosynthesis is inversely proportional or photorespiration is directly proportional to O_2 concentration, or there is a mixed effect. In figure 3, the response of photosynthesis to CO_2 concentrations just above compensation is similar in 3 % and 21 % O_2. From these observations it can be concluded that at CO_2 concentrations near the compensation point, the major portion of the O_2 effect is on photorespiration and not on true photosynthesis.

In conditions where CO_2 does not limit the apparent rate of photosynthesis, the absence of an O_2 effect may indicate an absence of photorespiration. It is possible, however, that at CO_2 saturation, photorespiration will not influence the apparent rate of photosynthesis. This situation would occur if photorespiration oxidizes an intermediate between the CO_2 fixation step and the limiting step of photosynthesis. These considerations are interesting in the light of recent evidence demonstrating that the substrate of photorespiration is a very early photosynthetic product (12). At high CO_2 concentrations, therefore, the absence of an O_2 effect does not necessarily establish the absence of photorespiration.

Literature Cited

1. BEEVERS, H. 1960. Respiratory metabolism in plants. Row, Peterson, and Company, White Plains, New York.
2. BJÖRKMAN, O. 1966. The effect of oxygen concentration on photosynthesis in higher plants. Physiol. Plantarum 19: 618–33.
3. BRIGGS, G. E. AND C. P. WHITTINGHAM. 1952. Factors affecting the rate of photosynthesis of *Chlorella* at low concentrations of carbon dioxide and in high illumination. New Phytologist 51: 236–49.
4. CALVIN, M. 1962. The path of carbon in photosynthesis. Science 135: 879–89.
5. DOWNTON, W. J. S. AND E. B. TREGUNNA. 1968. CO_2 compensation—Its relation to photosynthetic carboxylation reactions, systematics of the *Gramineae,* and leaf anatomy. Can. J. Botany. 46: 207–15.
6. EGLE, K. AND W. SCHENK. 1953. Der Einfluss der Temperatur auf die Lage des CO_2—Kompensationspunktes. Planta 43: 83–97.
7. GIBBS, M., E. S. BAMBERGER, P. W. ELLYARD, AND R. G. EVERSON. 1967. Assimilation of carbon dioxide by chloroplast preparations. In: Biochemistry of Chloroplasts. T. W. Goodwin, ed. Academic Press, New York. II: 3–38.
8. FORRESTER, M. L., G. KROTKOV, AND C. D. NELSON. 1966. Effect of oxygen on photosynthesis, photorespiration and respiration in detached leaves. I. Soybean. Plant Physiol. 41: 422–27.
9. HATCH, M. D. AND C. R. SLACK. 1966. Photosynthesis by sugarcane leaves. A new carboxylation reaction and the pathway of sugar formation. Biochem. J. 101: 103–11.
10. HATCH, M. D., C. R. SLACK, AND H. S. JOHNSON. 1967. Further studies on a new pathway of photosynthetic carbon dioxide fixation in sugarcane and its occurrence in other plant species. Biochem. J. 102: 417–22.
11. HESKETH, J. D. 1967. Enhancement of photosynthetic CO_2 assimilation in the absence of oxygen, as dependent upon species and temperature. Planta 76: 371–74.
12. LUDWIG, L. J. AND G. KROTKOV. 1967. The kinetics of labeling of the substrates for CO_2 evolution by sunflower leaves in the light. Plant Physiol. 42: S-47.
13. MCALISTER, E. D. AND J. MYERS. 1940. The time course of photosynthesis and fluorescence observed simultaneously. Smithsonian Inst. Misc. Collections 99(6): 1–37.
14. MEIDNER, H. 1967. Further observations on the minimum intercellular space carbon-dioxide concentration of Maize leaves and the postulated roles of "photo-respiration" and glycollate metabolism. J. Exptl. Botany 18: 177–85.
15. MOSS, D. N. 1962. The limiting carbon dioxide concentration for photosynthesis. Nature 193: 587.
16. MURATA, Y., J. IYAMA, AND T. HONMA. 1965. Studies on the photosynthesis of forage crops. IV. Influence of air-temperature upon the photosynthesis and respiration of alfalfa and several southern type forage crops. Proc. Crop. Sci. Soc. Japan 34: 154–58.
17. TAMIYA, H. AND H. HUZISIGE. 1949. Effect of oxygen on the dark reaction of photosynthesis. Stud. Tokugawa Inst. 6: 83–104.
18. TREGUNNA, E. B., G. KROTKOV, AND C. D. NELSON. 1966. Effect of oxygen on the rate of photorespiration in detached tobacco leaves. Physiol. Plantarum 19: 723–33.
19. TURNER, J. S. AND E. G. BRITTAIN. 1962. Oxygen as a factor in photosynthesis. Biol. Rev. 37: 130–70.
20. TURNER, J. S., M. TODD, AND E. G. BRITTAIN. 1956. The inhibition of photosynthesis by oxygen. I. Comparative physiology of the effect. Australian J. Biol. Sci. 9: 494–510.
21. WARBURG, O. 1920. Über die Geschwindigkeit der Photokemischen Kohlensäurezersetzung in lebenden Zellen. II. Biochem. Z. 103: 188–217.
22. WASSINK, E. C., D. VERMEULEN, G. H. REMAN, AND E. KATZ. 1938. On the relation between fluorescence and assimilation in photosynthesizing cells. Enzymologia 5: 100–09.
23. ZELITCH, I. 1966. Increased rate of net photosynthetic carbon dioxide intake caused by the inhibition of glycolate oxidase. Plant Physiol. 41: 1623–31.