PHOTOSYNTHESIS, PHOTORESPIRATION AND RELATED ASPECTS
OF CO₂ EXCHANGE BY WHEAT, CORN
AND AMARANTHUS EDULIS

by

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April, 1970
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ABSTRACT

Certain aspects of CO₂ exchange by wheat (*Triticum aestivum* L.), corn (*Zea mays* L.) and the grain Amaranth (*Amaranthus edulis* Speg.) were investigated.

The effects of O₂ concentration on apparent photosynthesis of juvenile wheat and corn shoots were measured at different temperatures, CO₂ concentrations and light intensities using infra-red CO₂ analysis and both open and closed gas-flow systems. The only condition in which apparent photosynthesis of wheat was not inhibited by O₂ was in 20.8% O₂ when the CO₂ concentration was saturating and the temperature was 30°C or less. The degree of inhibition increased with increasing O₂ concentration, increasing temperature, and decreasing CO₂ concentration and was independent of the light intensity. During some of the growing season in temperate regions, the effect of atmospheric O₂ on the photosynthetic productivity of wheat may be negligible. The effect of O₂ on wheat was ascribed to both a stimulation of photorespiration and an inhibition of photosynthesis by O₂.

In corn, which apparently lacks photorespiration, photosynthesis was also inhibited by 99% O₂. No inhibition occurred at 20.8% O₂, however, and the stability and reversibility of the inhibition observed at 99% O₂ differed from that found with wheat. These differences between wheat and corn are correlated with differences in leaf anatomy and photosynthetic carbon metabolism and with differences in the response of apparent photosynthesis and the CO₂ compensation point to environmental conditions. Many of the gas
exchange characteristics of corn and similar plants seem to be advantageous for the warm dry areas they often inhabit.

Initially high rates of CO₂ production are exhibited by wheat immediately following illumination, and it has been suggested that this post-illumination CO₂ burst is an extension of photorespiration into the dark period. In accord with this, CO₂ concentration was found to influence both the post-illumination CO₂ burst and the inhibition of apparent photosynthesis by O₂ in a similar way. Except for Amaranthus edulis, plants which lack photorespiration also lack significant post-illumination CO₂ bursts. On the basis of its response to O₂ concentration, however, the burst of Amaranthus edulis is concluded to be unrelated to photorespiration.

Measurements of $^{12}\text{CO}_2$ and $^{14}\text{CO}_2$ exchange were used to estimate the quantity of carbon in wheat and corn shoots which was in free exchange with atmospheric CO₂. The free exchange pool size was found to be very small and it cannot be an important factor in the CO₂ concentration response of photosynthesis or in the post-illumination CO₂ burst.
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Finally, this thesis is dedicated to my wife Ann, who assisted in drafting many of the diagrams, and whose encouragement made this thesis possible.
Two relatively recent developments have focused new attention on the CO₂ exchange processes of plants. Starting about 10 years ago, Krotkov, Egle, and others carried out a number of studies on the effects of atmospheric O₂ on plant CO₂ exchange (2, 3, 4, 10, 11). These investigations culminated in the suggestion that many plants release CO₂ during photosynthesis by a process called photorespiration. The distinctive feature of photorespiration is that it is enhanced by O₂ concentrations greater than 2% O₂ while respiration by leaves in the dark is not.

Meanwhile, in 1965 it was reported that malate and aspartate, instead of the phosphorylated sugars typical of the Calvin cycle, were the initial products of photosynthetic CO₂ fixation in sugar cane (9). Hatch, Slack and their collaborators extended these results and developed the C₄-dicarboxylic acid pathway to account for these observations (5, 6, 8). In addition, they discovered numerous other "tropical" species which possessed the same type of photosynthetic carbon metabolism (6, 7).

These two trends in plant research provide the basis for the present studies. They are linked by the observation that plants which exhibit the C₄-dicarboxylic acid pathway apparently lack photorespiration, while plants which possess only the Calvin cycle can rapidly photorespire (1).

This thesis includes experiments designed to indicate the activity of photorespiration in different environmental conditions as well as to indicate the differences in CO₂ exchange characteristics.
of plants which possess and lack photorespiration. In addition, the relationship between photorespiration and post-illumination CO$_2$ exchange transients was studied, and an attempt was made to estimate the quantity of carbon within plants which can exchange with atmospheric CO$_2$. 
LITERATURE CITED


Chapter I

THE EFFECTS OF TEMPERATURE, CO₂ CONCENTRATION AND LIGHT INTENSITY ON THE OXYGEN INHIBITION OF APPARENT PHOTOSYNTHESIS IN WHEAT

INTRODUCTION

The apparent rate of photosynthesis, whether it is measured in terms of CO₂ assimilation or O₂ evolution, is often found to be inhibited in the presence of molecular oxygen. This inhibition was first demonstrated by Warburg (46) and has come to be known as the "Warburg effect". The effect has been observed in many photosynthetic organisms, including algae, mosses, liverworts, ferns, gymnosperms and angiosperms (31, 37, 43, 44, 46).

It is known that the inhibitory effect of O₂ is influenced by the environmental conditions, but information on the environmental relations of the O₂ effect is limited and often inconsistent. The effect of O₂ is reduced and may be absent at high CO₂ concentrations (7, 40, 44), and is increased at CO₂ concentrations below atmospheric levels (40). In Chlorella at low CO₂ concentration, temperature did not affect the degree of inhibition of photosynthesis over the range 4° to 25° C (40). Similar results were obtained with the moss Funaria between 20° and 30° C (43). In wheat, cotton and tobacco, however, recent results indicate that inhibition increases between 30° and 40° C (23). Most studies have shown that the degree of inhibition is not affected by moderate to saturating light intensities (3, 31, 43, 47). In several other cases, the degree of inhibition increased with
increasing light intensity (43, 47).

Numerous suggestions have been advanced concerning the mechanism(s) by which O₂ inhibits apparent photosynthesis (12, 43). Both the light (3, 32, 34, 36) and dark (10, 40, 45) processes of photosynthesis, as well as photorespiration (14, 17, 18, 41) have been implicated as possible sites for the O₂ effect.

In the present investigation, the effect of O₂ concentration on the apparent rate of photosynthesis of wheat shoots in different conditions of temperature, CO₂ concentration and light intensity was examined. The results permit us to assess the significance of the O₂ effect on photosynthetic productivity, and add perspective to current proposals on the mechanism of the O₂ effect.

MATERIALS AND METHODS

Seeds of wheat (Triticum aestivum L. var. Spring Thatcher), obtained from Buckerfield's Seed Co., Vancouver, were planted in flats of vermiculite, watered daily, and grown in a ventilated room at 21,600 lux of fluorescent and incandescent light and 16 hr. photoperiod.

At the start of experiments with excised shoots, 1.5 g. fresh weight of 8 to 14 day-old shoots were detached by severing the bases of the shoots with a razor blade. The cut ends of the shoots were immediately immersed in water and recut beneath the water surface about 5 mm. below the lowest node of the shoot. The excised shoots were then weighed and transferred to one of the plexiglass chambers shown in Figures I-1 and I-2. Within these chambers, the cut ends of the shoots were submerged in approximately 5 mm. of water. After the shoots were enclosed in a chamber, they were allowed to adjust to the
Figure I-1

Plant Chamber Used for Excised Shoots in Experiment I
Inlet
FRONT VIEW
shoot chamber
water jacket
plant shoot
water trough
wing nuts and bolts
air outlet
thermistor
water inlet
AIR OUTLET
0 2 4 cm
WATER INLET AND OUTLET
SIDE VIEW
Figure I-2

Plant Chamber Used for Excised Shoots in Experiment II
experimental conditions for 30 to 45 minutes before CO\textsubscript{2} exchange measurements were started.

The enclosed shoots were illuminated by 1 to 3 General Electric "Cool Beam" 750 watt incandescent lamps. The lamp tower, shown in Figure I-3 was used in experiments with excised shoots. The chamber was placed on the freezer immediately below the lamp tower or, when more efficient cooling was required, within the freezer supporting the lamp tower. Before the light was incident on the shoots, it was passed through 14 cm. of water to remove much of the infra-red radiation. Figure I-4 shows the spectral energy distribution of the unfiltered radiation as measured by an Instrumentation Specialties Co. (ISCO) spectroradiometer. Light intensity was measured by a Gossen "Tri-Lux" footcandle meter and converted to lux by multiplying by 10.764. An illumination of 32,300 lux (3000 ft-c) was equivalent to a radiant intensity of $1.6 \times 10^5$ erg./cm./sec. between 400 and 700 nm. when no water filter was used. The light intensity was varied by changing the number of lamps used, by varying the distance of the lamps from the shoots, and by interposing layers of cheesecloth between the lamps and the chamber.

The temperature within the chambers was detected by a thermistor placed in contact with some of the shoots. A Yellow Springs Instruments Co. Telethermometer was connected to the thermistor and indicated the temperature. The chamber temperatures were controlled by circulating water from a thermoregulator through jackets surrounding the chambers.

A simple closed system was used for the CO\textsubscript{2} exchange studies reported here. This system contained the plant chamber, a CO\textsubscript{2} release
Figure I-3

Lamp Tower and Freezer Assembly Used for Control of Light Intensity and Temperature
Figure I-4

Spectral Energy Distribution of Light from a General Electric "Cool Beam" Lamp as Measured by a ISCO Spectroradiometer
flask, a Beckman IR 215 infra-red gas analyzer (IRGA), a Fisher "Dynapump" air pump, and a Matheson R-2-15-B flowmeter, which were linked in series by Tygon tubing. A Bausch and Lomb VOM-5 strip chart recorder was connected to the IRGA and recorded the CO$_2$ concentration changes in the system.

Two studies were made of the effect of O$_2$ concentration on CO$_2$ exchange by excised wheat shoots. Experiment I was carried out in September 1967 to determine the effects of 20.8% (v./v.) and 3± 1% O$_2$ on the apparent rate of photosynthesis and on the CO$_2$ compensation point of wheat. For this experiment, the plants were grown at 22-26/18-22 °C day/night temperatures. The chamber shown in Figure 1-1 was used, and the volume of the closed system was 860 ml. The air flow rate in the closed system was maintained at 2 l./min., and the light intensity at the chamber surface was 10,760 lux. A Clark oxygen electrode, connected to a Beckman Oxygen Adapter and a millivoltmeter, was included in the system to measure the O$_2$ concentration in the air stream. Laboratory air or N$_2$ from a compressed gas tank was flushed through the system to adjust the O$_2$ concentration in the air stream.

Experiment II was carried out from February to May 1969 to investigate the effects of O$_2$ concentrations higher than atmospheric on CO$_2$ exchange by excised wheat shoots. In this case, the plants were grown at day/night temperatures of 26-31/22-26 °C, and the chamber shown in Figure 1-2 was used. The volume of the closed system was 230 ml., and the air flow rate was 3 l./min. Except for the light intensity study reported in Figure 1-15, the light intensity was 32,300 lux. The O$_2$ concentrations were established by flushing the closed system with laboratory air or with gas from compressed gas tanks. The O$_2$
concentrations in the air stream were not measured during this experiment, but separate measurements with a Picker MS-10 mass spectrometer indicated that the O₂ concentrations used were 1.8%, 20.8%, 60.9%, 78.6% and >99% O₂. Between 11 and 24 measurements of the apparent rate of photosynthesis were made at any one combination of O₂ concentration, temperature, CO₂ concentration and light intensity used in Experiments I and II.

When intact plants were required, the wheat seeds were planted in a row so that a number of shoots could be sealed into the 135 ml. pléxiglass chamber shown in Figure I-5 by a rubber gasket coated with silicone grease. The environmental conditions during the growth of these plants, and during the measurement of their CO₂ exchange were comparable to those described for Experiment I. When intact plants were used, the illumination system was similar to that described above except that the light was directed horizontally at the upright chamber.

The following sequence of operations was carried out during the CO₂ exchange studies reported in this Chapter. After the system was flushed to establish the O₂ concentration, it was closed. A hypodermic syringe was then used to inject less than 0.5 ml. of air containing a high CO₂ concentration into the release flask to elevate the CO₂ concentration in the system above 600 μl/l. The CO₂ concentration in the system then decreased because of net CO₂ assimilation by the enclosed shoots until the CO₂ compensation point was reached. Thereafter, this sequence of operations was repeated until measurements with one sample of shoots was terminated. No sample of shoots was used for more than 3.5 hours following their enclosure in a chamber.
Figure I-5

Plant Chamber Used for Intact Shoots
Apparent rates of photosynthesis were calculated from the time required for the shoots to reduce the CO$_2$ concentration in the system by 50 µl/l. or by 25 µl/l. and from the system volume. The rates were expressed on a per fresh weight basis. Separate measurements of shoot area, using an airflow planimeter similar to that of Jenkins (27), established that the area of 1 g. fresh weight of 10 day-old wheat shoots was 0.40 dm$^2$. Thus, an approximate indication of the apparent rates of photosynthesis on a leaf area basis (mg. CO$_2$/hr/dm$^2$) can be obtained by multiplying the reported rates by 2.50.

RESULTS

1. CO$_2$ Exchange by Intact and Excised Wheat Shoots

To establish whether excised shoots were suitable experimental material for the current studies, preliminary tests were done to compare the CO$_2$ exchange characteristics of intact and excised shoots. Figure 1-6 illustrates the similarity of the CO$_2$ concentration response of apparent photosynthesis by intact and excised shoots at high and low temperatures. The vertical bars which extend above and below the averages at each CO$_2$ concentration indicate the 95 per cent confidence limits about the sample means. Where similar vertical bars occur on other figures in this thesis, they also show the 95 per cent confidence limits about the sample means. If the excised shoots were well-supplied with water, their apparent rates of photosynthesis remained constant between 0.5 and 3.5 hours after excision, even when the temperature was as high as 40°C. Several other investigations have indicated that gas exchange by leaves is little affected for several hours after excision (25, 35, 42), except for transient
Figure 1-6

Effect of CO₂ Concentration and Temperature on the Apparent Rates of Photosynthesis of Intact and Excised Wheat Shoots. The Light Intensity Was 10,760 Lux
changes in CO₂ exchange (8, 28) or transpiration (33) when petioles or leaves are severed. Because their CO₂ exchange behavior resembled that of intact shoots, and because of their convenience, excised shoots were used for the rest of the research reported in this thesis.

2. Effects of Low O₂ Concentration on CO₂ Exchange by Excised Wheat Shoots (Experiment I)

(a) Effects of Low O₂ Concentration and Temperature on Apparent Photosynthesis.

An initial series of measurements was carried out to compare the effects of 3±1% and 20.8% O₂ on the apparent rate of photosynthesis of wheat shoots exposed to 300 μl./l. CO₂ and temperatures ranging from 2°C to 43°C. Figure 1-7 shows that there was an optimum temperature for apparent photosynthesis, below and above which the rate of net CO₂ assimilation declined. It appears from these results that O₂ concentration exerted an influence on the response of apparent photosynthesis to temperature. In 20.8% O₂, the apparent rate of photosynthesis was greatest between 20°C and 26°C, but in 3±1% O₂ the optimum temperature was between 26°C and 34°C. In 20.8% O₂, the apparent rate of photosynthesis at 34°C was significantly less than at 20°C, while in 3±1% O₂, the rate was significantly greater at 34°C than at 20°C. Therefore, a decrease in O₂ concentration from 20.8% O₂ to 3±1% O₂ appeared to cause an increase in the temperature optimum for apparent photosynthesis. In addition, it is noteworthy that at 13°C or lower temperatures, there was no significant difference between the apparent rates of photosynthesis in 20.8% O₂ and 3±1% O₂. At higher temperatures, however, apparent photosynthesis in 20.8% O₂ was greatly inhibited compared to the rates of CO₂ assimilation observed in 3±1% O₂. Thus, these results show that O₂ concentration can alter the temperature response of apparent photosynthesis, and that the
Figure I-7

Effects of Temperature on the Apparent Rates of Photosynthesis of Excised Wheat Shoots in Atmospheres Containing 300 μl./l. CO₂ and 20.8% O₂ or 3± 1% O₂
inhibitory effect of O₂ on apparent photosynthesis is modified by the temperature.

(b) Effects of Low O₂ Concentration and Temperature on the CO₂ Compensation Point.

When plants are placed in a sealed chamber and illuminated, CO₂ is assimilated until a CO₂ concentration is reached at which the rate of CO₂ uptake is equal to the rate of CO₂ production. The CO₂ concentration at which this equilibrium occurs is known as the CO₂ compensation point (15). Figure 1-8 shows the effect of temperature on the CO₂ compensation point of wheat shoots in 3± 1% and 20.8% O₂. The CO₂ compensation point was always much lower in 3± 1% O₂ than in 20.8% O₂, but at both O₂ concentrations the CO₂ compensation point increased with increasing temperature. At 32° C or less, the ratio of the CO₂ compensation point in 3± 1% O₂ to the CO₂ compensation point in 20.8% O₂ was constant. Also, this ratio was equivalent to the ratio of the O₂ concentrations used. Above 32°, however, the ratio increased sharply.

(c) Effects of Low O₂ Concentration, Temperature, and CO₂ Concentration on Apparent Photosynthesis.

It was clear from Figure 1-7 that at 13° C or lower temperatures the apparent rate of photosynthesis was the same in 3± 1% O₂ and 20.8% O₂. At the same temperatures, however, Figure 1-8 indicated that the CO₂ compensation point was proportional to the O₂ concentration. Because of this anomaly, a thorough study was made of the effects of 3± 1% O₂ and 20.8% O₂ on apparent photosynthesis at different temperatures and CO₂ concentrations. Rates of CO₂ assimilation were measured at 4 temperatures from 13° C to 34° C and at CO₂ concentrations between 500 μl./l. CO₂ and the CO₂ compensation
Figure I-8

Effects of Temperature on the CO₂ Compensation Points of Excised Wheat Shoots in Atmospheres Containing 20.8% O₂ or 3± 1% O₂
point.

The results of this study are presented in Figures I-9 and I-10. At CO₂ concentrations just above the CO₂ compensation point, the apparent rate of photosynthesis was limited by the CO₂ since the rate increased rapidly as the CO₂ concentration increased. At more elevated CO₂ concentrations, the response of apparent photosynthesis to a change in CO₂ concentration became less pronounced. Often, if the CO₂ concentration was sufficiently high, the apparent rate of photosynthesis became insensitive to changes in CO₂ concentration. When this occurred, apparent photosynthesis was saturated by CO₂. It is evident from Figure I-9 that the CO₂ concentration required to saturate apparent photosynthesis increased as the O₂ concentration and temperature increased. In 20.8% O₂ at 25.9° C and 34.3° C, apparent photosynthesis was not saturated by CO₂ concentrations below 500 μl/l. Under all other conditions, apparent photosynthesis was saturated by 500 μl/l. CO₂ or less, and the apparent rate of photosynthesis at saturating CO₂ concentrations increased with increasing temperature. Except at high CO₂ concentrations at 13° C and 19.7° C, the apparent rate of photosynthesis was less in 20.8% O₂ than in 3±1% O₂. The CO₂ concentration required to eliminate the inhibitory effect of 20.8% O₂ was higher at 19.7° C than at 13° C and appears to correspond to the minimum CO₂ concentration required to saturate apparent photosynthesis in 20.8% O₂ in these cases. Under no condition of temperature or CO₂ concentration was the apparent rate of photosynthesis significantly enhanced in the presence of 20.8% O₂ compared to the rate in 1.8% O₂. In addition, the slope of the CO₂ concentration response curves immediately above the CO₂ compensation
Figure 1-9

Effects of CO₂ Concentration and Temperature on the Apparent Rates of Photosynthesis of Excised Wheat Shoots in Atmospheres Containing 20.8% O₂ or 3± 1% O₂
points appeared to be less in 20.8% than in 3±1% O₂.

Inspection of Figure I-9 reveals that the inhibitory effects of 20.8% O₂ on apparent photosynthesis were greatest at high temperatures and low CO₂ concentrations. A useful quantitative index of the effect of O₂ on apparent photosynthesis is that of per cent inhibition (\(\% I_p\)), which may be calculated (3, 18, 43) using the expression:

\[
\% I_p = \frac{P_1 - P_2}{P_1} \times 100
\]

(1)

in which \(P_1\) and \(P_2\) represent the apparent rates of photosynthesis at a low (e.g. 3±1% O₂) and a high (e.g. 20.8% O₂) concentration of O₂ respectively. Examination of this formula shows that 100% inhibition must occur at the CO₂ compensation point in the higher O₂ concentration, since at that point \(P_2\) equals zero.

Figure I-10 shows the per cent inhibition of apparent photosynthesis by 20.8% O₂ at different temperatures and CO₂ concentrations. These data are derived from the results presented in Figure I-9. It is clear that the per cent inhibition of apparent photosynthesis increased with increasing temperature. Except where the CO₂ concentration approached the CO₂ compensation point, the increase in degree of inhibition appeared to be linear with the same slope at all CO₂ concentrations. In this linear portion of the figure, the temperature at which a certain per cent inhibition occurred appears to be related to the logarithm of the CO₂ concentration. At any one temperature, the per cent inhibition decreased in a non-linear fashion with increasing CO₂ concentration.
Figure I-10

Effects of Temperature and CO₂ Concentration on the Per Cent Inhibition of Apparent Photosynthesis by 20.8% O₂ in Excised Wheat Shoots
3. Further Studies on the Effects of $O_2$ Concentration on $CO_2$ Exchange by Excised Wheat Shoots \hspace{1em} (Experiment II)

(a) Effects of $O_2$ Concentration and Temperature on Apparent Photosynthesis and the $CO_2$ Compensation Point.

A second series of tests was carried out to extend these results to include higher $O_2$ concentrations. Figure I-11 is comparable to Figure I-7 and shows the temperature response of apparent photosynthesis of wheat shoots in 300 $\mu l./l$. $CO_2$ and $O_2$ concentrations ranging from 1.8% $O_2$ to >99% $O_2$.

Throughout Experiment II, the apparent rates of photosynthesis were much higher than those obtained in Experiment I. This difference can be accounted for largely by the higher light intensity used in Experiment II. In accord with the previous results, at low temperatures there was no significant difference in the apparent rates of photosynthesis in 20.8% $O_2$ and 1.8% $O_2$. In this case, however, the apparent rates of photosynthesis at these $O_2$ concentrations were similar at 20° C as well as at 13° C. At high temperatures apparent photosynthesis in 20.8% $O_2$ was again inhibited compared with the rates observed at the low $O_2$ concentration used. At all the temperatures examined, apparent photosynthesis was inhibited by the presence of $O_2$ concentrations from 60.9% $O_2$ to >99% $O_2$, and the degree of inhibition was enhanced as the $O_2$ concentration and temperature increased. Although this time there was no significant difference in the optimum temperature for apparent photosynthesis in the 20.8% $O_2$ and 1.8% $O_2$, Figure I-11 confirms the earlier indication that the optimum temperature tends to decrease as the $O_2$ concentration increases. In this case, the apparent rate of photosynthesis was greatest at 30° C
Figure I-11

Effects of O₂ Concentration and Temperature on the Apparent Rates of Photosynthesis of Excised Wheat Shoots in 300 μl./l. CO₂
and 35° C when the O₂ concentration was 1.8% O₂ or 20.8% O₂. When the O₂ concentration was raised to >99% O₂, however, the optimum occurred between 13° C and 25° C.

Figure 1-12 shows the effect of O₂ concentrations from 1.8% O₂ to >99% O₂ on the CO₂ compensation point. Once again, the CO₂ compensation point was affected by the O₂ concentration and temperature. At any one temperature, the CO₂ compensation point was directly proportional to the O₂ concentration. The slope of the linear CO₂ compensation point-O₂ concentration relationship increased as the temperature increased. If the data are extrapolated to zero O₂ concentration, the CO₂ compensation points appear to become less than 15 μl./l. CO₂.

(b) Effects of O₂ Concentration, Temperature, CO₂ Concentration and Light Intensity on Apparent Photosynthesis.

Figures 1-13 to 1-18 show the results of additional measurements which were made to assess the effects of O₂ concentrations from 1.8% O₂ to >99% O₂ on apparent photosynthesis in different conditions of CO₂ concentration and temperature. The general pattern of the results obtained at 1.8% O₂ and 20.8% O₂ was quite similar to the earlier results from Experiment I which were presented in Figures 1-9 to 1-10. In Figures 1-13 to 1-18 it can be seen that at higher O₂ concentrations the trends observed between 1.8% O₂ and 20.8% O₂ were continued. At 60.9% O₂ to >99% O₂ the apparent rate of photosynthesis was significantly inhibited in all conditions except at high CO₂ concentrations at 13° C and 60.9% O₂. The degree of inhibition increased as the O₂ concentration and temperature increased and the CO₂ concentration decreased. The slopes of the CO₂ concentration response curves immediately above the CO₂ compensation points
Figure I-12

Effects of O₂ Concentration and Temperature on the CO₂ Compensation Point of Excised Wheat Shoots
Figure I-13

Effects of $O_2$ Concentration and $CO_2$ Concentration on the Apparent Rates of Photosynthesis of Excised Wheat Shoots at $13^\circ C$
Figure I-14

Effects of O₂ Concentration and CO₂ Concentration on the Apparent Rates of Photosynthesis of Excised Wheat Shoots at 20°C
20°C
○ 1.8% O₂
△ 20.8% O₂
△ 60.9% CO₂
□ 73.6% O₂
□ >99% O₂

APPARENT RATE OF PHOTOSYNTHESIS (mg CO₂/hr/g fr wt)

CO₂ CONCENTRATION (µl/l)
Figure I-15

Effects of $O_2$ Concentration and $CO_2$ Concentration on the Apparent Rates of Photosynthesis of Excised Wheat Shoots at $25^\circ C$
25°C

- O 1.8% O₂
- △ 20.8% O₂
- △ 60.9% O₂
- □ 78.6% O₂
- □ >99% O₂

APPARENT RATE OF PHOTOSYNTHESIS (mg CO₂/hr/g fr wt)

CO₂ CONCENTRATION (µl/l)
Figure I-16

Effects of O₂ Concentration and CO₂ Concentration on the Apparent Rates of Photosynthesis of Excised Wheat Shoots at 30°C
28

30°C

- 1.8% O2
- 20.3% O2
- 60.9% O2
- 78.6% O2
- >99% O2

APPARENT RATE OF PHOTOSYNTHESIS (mg CO2/hr/g fr wt.)

CO2 CONCENTRATION (μl/l)

0 100 200 300 400 500 600
Figure I-17

Effects of $O_2$ Concentration and $CO_2$ Concentration on the Apparent Rates of Photosynthesis of Excised Wheat Shoots at 35° C
Figure I-18

Effects of O$_2$ Concentration and CO$_2$ Concentration on the Apparent Rates of Photosynthesis of Excised Wheat Shoots at 40° C
were significantly decreased as the $O_2$ concentration increased from 1.8% $O_2$ to >99% $O_2$. At 13° C and 78.6% $O_2$, and at 20° C and 60.9% $O_2$, apparent photosynthesis was saturated by the highest $CO_2$ concentrations used but was still inhibited to some extent. Therefore it appears that saturating $CO_2$ concentrations may not be able to reverse the inhibitory effects of very high $O_2$ concentrations on apparent photosynthesis.

The data at 25° C were used for Figure I-19 which shows the per cent inhibition of apparent photosynthesis by $O_2$ concentrations ranging from 1.8% $O_2$ to >99% $O_2$ at $CO_2$ concentrations from 100 µl./l. $CO_2$ to 500 µl./l. $CO_2$. At each $CO_2$ concentration, the degree of inhibition increased with increasing $O_2$ concentration. At low $CO_2$ concentrations at least, this increase appears to be non-linear. When the results are extrapolated to zero inhibition, it appears that there would be no inhibition of apparent photosynthesis in 300 µl./l. $CO_2$ below about 14% $O_2$. Similarly, in 400 µl./l. $CO_2$ zero inhibition would occur below about 22% $O_2$, and in 500 µl./l. $CO_2$ below about 30% $O_2$. This lack of inhibition by some $O_2$ concentrations above 1.8% $O_2$ is equivalent to that observed at high $CO_2$ concentrations in Figures I-9 and Figures I-13 to I-17.

Figure I-20 summarizes other measurements which were made to test the influence of light intensity on the per cent inhibition of apparent photosynthesis at 25° C. Although the data are somewhat scattered, light intensity had no apparent effect on the degree of inhibition caused by 20.8% $O_2$ or 60.9% $O_2$. Changes in light intensity between 6460 lux and 107,640 lux affected the apparent rate of photosynthesis (see Chapter II), but the per cent inhibition was not altered because the ratio $P_1/P_2$ was constant over this range in light.
Figure I-19

Effects of O₂ Concentration and CO₂ Concentration on the Per Cent Inhibition of Apparent Photosynthesis in Excised Wheat Shoots at 25°C
Figure I-20

Effects of CO₂ Concentration and Light Intensity on the Per Cent Inhibition of Apparent Photosynthesis in Excised Wheat Shoots at 25⁰ C and 20.8% O₂ or 60.9% O₂
intensity. Figures I-14 and I-15 again show the decrease in per cent inhibition as the CO$_2$ concentration increased.

4. Effect of O$_2$ on Photosynthetic Productivity

Per cent inhibition is a relative measure of the O$_2$ effect and does not directly indicate the influence of O$_2$ on the quantity of carbon assimilated in photosynthesis. The expression:

$$\text{Carbon Deficit} = \frac{12 (P_1 - P_2)}{44}$$  \hspace{1cm} (2)

can be used to calculate the decrease in photosynthetic productivity caused by the inhibitory effect of O$_2$. The data from Experiment I were used to calculate the carbon deficit produced by 20.8% O$_2$ in different conditions of temperature and CO$_2$ concentration, and these results are presented in Figure I-21. It is apparent that there was a general increase in carbon deficit with each rise in temperature.

At 300 µl./l. CO$_2$ or higher CO$_2$ concentrations at 13° C and 450 µl./l. CO$_2$ or above at 19.6° C, the carbon deficit was zero. The absence of a carbon deficit under these conditions is related to the zero per cent inhibition of apparent photosynthesis which was noted in Figure I-9. Also, according to Figure I-21, the carbon deficit tended to decrease as the CO$_2$ concentration approached 50 µl./l. CO$_2$. As we have seen in the preceding sections, the degree of inhibition of apparent photosynthesis increases as the CO$_2$ decreases. The carbon deficit does not parallel this increase in per cent inhibition because the apparent rate of photosynthesis is reduced by low CO$_2$ concentrations.

The carbon deficit values calculated from the results of Experiment II are presented in Figure I-22. Once again, the carbon
Figure I-21

The Carbon Deficit in Excised Wheat Shoots Caused by 20.8% O₂ at Different Temperatures and CO₂ Concentrations (Experiment I)
Figure I-22

The Carbon Deficit in Excised Wheat Shoots Caused by 20.8% O₂ at Different Temperature and CO₂ Concentrations (Experiment II)
deficits were greatest at high temperatures and at CO₂ concentrations intermediate between the CO₂ compensation point and saturating CO₂ concentration. At the CO₂ concentration to which plants are normally exposed, about 300 μl./l. CO₂, the carbon deficit ranged from zero to 0.67 mg. C/hr./g. fr. wt., and there was no significant carbon deficit at 20°C or less. The greatest carbon deficit observed in these studies was 0.76 mg. C/hr./g. fr. wt. and this occurred at 40°C and 450 μl./l. CO₂ in Experiment II. Therefore, the presence of 20.8% O₂ in the atmosphere can greatly reduce the photosynthetic productivity of wheat. It is obvious from the results presented in Figures I-13 to I-18 that the presence of higher O₂ concentrations would reduce photosynthetic productivity even more. High CO₂ concentrations and low temperatures, however, tend to reduce the carbon deficit.
DISCUSSION

These results provide a comprehensive description of the inhibitory effects of O₂ on apparent photosynthesis by wheat under different environmental conditions. The degree of inhibition of apparent photosynthesis by O₂ was enhanced by high O₂ concentrations, high temperatures and low CO₂ concentrations, and under some conditions the presence of O₂ can cause significant reductions in photosynthetic productivity.

These results extend and are in general accord with the findings of most previous studies on the effect of O₂ on apparent photosynthesis. The relationship between the O₂ concentration and the per cent inhibition of apparent photosynthesis, shown in Figure 1-12, is similar to that obtained by other researchers using algae (40) and terrestrial plants (18). For the first time, except in algae (40), it has been demonstrated that high CO₂ concentrations can eliminate the inhibitory effects of 20.8% O₂ on apparent photosynthesis. Some previous investigations have indicated that the degree of inhibition of apparent photosynthesis by O₂ is greatest at low CO₂ concentrations (40, 43), but the details of the response of the O₂ effect to CO₂ concentration are now clearly evident. The observation that light intensity does not affect the per cent inhibition of apparent photosynthesis by O₂ coincides with most other reports (3, 31, 43, 47). A few experiments with algae and mosses (43, 47) which found the per cent inhibition of apparent photosynthesis to increase with increasing light intensity do not agree with the present results. A conflict between the present results and those of others also exists with respect to the influences of temperature on the O₂ effect. In this
study with wheat, temperature had a marked effect on the percent inhibition of apparent photosynthesis by O₂, but early investigations with Chlorella (40) and Funaria (43) revealed no such effect. Some recent results with angiosperms, however, are in agreement with the present observations (23). Whenever 21% O₂ was found to inhibit apparent photosynthesis, the degree of inhibition increased between 30°C and 40°C. There is little previous information on the effect of O₂ concentration on the optimum temperature for apparent photosynthesis. Contrary to the results in Experiment I but in agreement with Experiment II, however, Björkman found no increase in the optimum temperature when Marchantia polymorpha was placed in 2% O₂ (4). The difference between the present observations and those of others cannot now be resolved, but it is possible that some of the discrepancies are due to the different species or different growing conditions utilized in different investigations.

The effects of O₂ on apparent photosynthesis are reflected in the rates of plant growth at different O₂ concentrations. The dry matter productivity of Phaseolus vulgaris, Mimulus cardinalis and Marchantia polymorpha was found to be enhanced when the plants were grown in 2.5 to 5% O₂ compared with those grown in 21% O₂ (5, 6). This enhancement of growth at low O₂ concentrations near the CO₂ compensation point was more than when the CO₂ concentration was 320 µl./l. or 640 µl./l. CO₂. Therefore, the response of plant growth to O₂ and CO₂ concentrations corresponds well to, and may be explained by, the present observations on apparent photosynthesis. It is expected that the present results may be useful in predicting the effect of atmospheric O₂ on the productivity of wheat in conditions where O₂
concentration, temperature, CO₂ concentration and light intensity are limiting productivity through their effects on apparent photosynthesis.

The CO₂ compensation point results confirm earlier studies which demonstrated that the CO₂ compensation point is directly proportional to the O₂ concentration (18, 41). This relationship is now extended to include a wide range of temperatures, and the increase with increasing temperature in the slope of the linear response of the CO₂ compensation point to O₂ concentration is now apparent. In most previous studies, the CO₂ compensation point has been found to extrapolate to zero at zero O₂ concentration (18, 41). In the present investigation, this was observed only at 25° C or lower temperatures. Above 30° C, the extrapolated CO₂ compensation point appears to be significantly greater than zero at zero O₂. Heath and Orchard (22) have reported that the extrapolated CO₂ compensation points of Pelargonium and Hydrangea were much higher than zero CO₂ at zero O₂ when the temperature was 27° C. It would be interesting to learn whether these plants are capable of attaining much lower CO₂ compensation points in the absence of O₂ at lower temperatures.

The linear relationship between the CO₂ compensation point and O₂ concentration and the finding that it extrapolates to zero at zero O₂ has previously been used as evidence that during photosynthesis, dark respiration is replaced by a different process of CO₂ production called photorespiration (18, 41). In contrast to dark respiration, which is saturated by about 2% O₂ (2, 18, 48), photorespiration is distinguished by a lower affinity for O₂ and it continues to increase in rate with increasing O₂ concentration up to 100% O₂ (18, 41). The observations that the CO₂ compensation point-O₂ concentration
relationship sometimes extrapolates to greater than zero at zero $O_2$, however, is consistent with the possibility that some CO$_2$ production by dark respiration may occur during photosynthesis. It is possible that during illumination the rate of dark respiration may appear to be small because much of the CO$_2$ produced is quickly reassimilated by photosynthesis before it can escape from the leaf. At $25^\circ$C or less the rate of CO$_2$ release by dark respiration during photosynthesis may be too low to cause a significant increase in the CO$_2$ compensation point in wheat. The operation of a classical tricarboxylic acid cycle has been demonstrated in the alga *Scenedesmus obliquus* during photosynthesis, but illumination did reduce the quantity of carbon entering the cycle (29, 30). Therefore, CO$_2$ production by dark respiration may occur in illuminated photosynthetic tissue, but probably at a reduced rate.

The CO$_2$ compensation point and the apparent rate of photosynthesis are both determined by the opposing processes of CO$_2$ uptake and CO$_2$ production. It follows from this that the observed response of CO$_2$ exchange to O$_2$ concentration could be due to an inhibition by O$_2$ of photosynthetic CO$_2$ uptake, or to a stimulation by O$_2$ of CO$_2$ production, or to a combination of these two effects.

The results of recent investigations do not support earlier suggestions (3, 43) that O$_2$ inhibits apparent photosynthesis by acting on photosynthetic electron transport in the chloroplast. For example, a typical O$_2$ effect has been observed in CO$_2$ fixation by a chloroplast-free fraction from *Euglena gracilis* (16).

The activities of the isolated Calvin cycle enzymes (NADH and NADPH) glyceraldehyde-3-phosphate dehydrogenase (19, 45) and ribolose-
5-phosphate kinase (19) have been shown to be inhibited by O$_2$. These enzymes could be the primary sites for the O$_2$ effect, but the significance of their inhibition by O$_2$ in terms of the rate of photosynthesis, and whether the inhibition occurs in vivo remains to be determined.

Direct evidence that photorespiration is a component of the O$_2$ effect has been provided by estimates of the individual rates of CO$_2$ uptake and production obtained from simultaneous measurements of $^{14}$CO$_2$ and $^{12}$CO$_2$ exchange by illuminated leaves. Bulley and Tregunna (9) found that, at the CO$_2$ compensation point, about 80% of the total effect of 21% O$_2$ on apparent photosynthesis in soybean was due to photorespiratory CO$_2$ production. The remainder was due to an inhibition of photosynthetic CO$_2$ uptake. In addition, D'Aoust and Canvin (11) have reported that a large portion of the O$_2$ effect in sunflower was due to photorespiratory CO$_2$ production. It is interesting to note that there was a noticeable inhibition at the saturating CO$_2$ concentration in Figure I-13 at 80% O$_2$ at 13° C and at 60% O$_2$ at 20° C. This may correspond to the residual non-photorespiratory portion of the O$_2$ effect.

Further support for the correlation between photorespiration and the O$_2$ effect comes from studies of CO$_2$ production by illuminated plants into a CO$_2$-free atmosphere. The effect of O$_2$ concentration on the rate of CO$_2$ production by illuminated barley leaves (48) is similar to its effect on the percent inhibition of apparent photosynthesis shown in Figure I-19. Also, the large inhibitory effects of O$_2$ on apparent photosynthesis at high temperatures corresponds with the high (about 35° C) optimum temperatures which
have been reported for CO$_2$ evolution by illuminated leaves (24, 26).

The absence of an O$_2$ effect at high CO$_2$ concentrations does not by itself prove the absence of photorespiration under those conditions. It is conceivable that, at CO$_2$ saturation, photorespiration may not influence the apparent rate of photosynthesis. This situation would occur if photorespiration involves the oxidation of an intermediate between the CO$_2$ fixation step and the limiting step in photosynthesis. Results which will be presented in Chapter III, however, demonstrate that the size of the post-illumination CO$_2$ burst, and therefore the rate of photorespiration, is decreased and may be negligible at high CO$_2$ concentrations.

It is possible that the portion of the O$_2$ effect which is not due to photorespiratory CO$_2$ production may be associated in another way with the photorespiratory process. For example, photorespiration could depress the apparent rate of photosynthesis through competition for the photosynthetatic CO$_2$ acceptor or a precursor of it (19) as well as by the production of CO$_2$. Of course, that component of the O$_2$ effect which cannot be accounted for by photorespiratory CO$_2$ production could be entirely unrelated to photorespiration.

It has been suggested that glycolic acid or a related metabolite is the substrate for photorespiration (13, 20, 49, 50, 51). The present results are consistent with this hypothesis. The excretion of glycolate by algae is greatest at low CO$_2$ concentrations and high O$_2$ concentrations (1, 38) and it is in these conditions that the degree of inhibition of apparent photosynthesis by O$_2$ is greatest. Glycolate may be formed from intermediates of the Calvin cycle (1) and its formation may inhibit photosynthesis by lowering the levels
of photosynthetic intermediates as just described above.

An alternative to the glycolic acid hypothesis has recently been advanced by Samish and Koller (39). They suggest that \( \text{O}_2 \) acts to increase the leaf mesophyll resistance to \( \text{CO}_2 \) uptake. As a result, increased quantities of the \( \text{CO}_2 \) produced within the leaf by dark respiration are released from the leaf at high \( \text{O}_2 \) concentrations instead of being reassimilated in photosynthesis. This is an interesting concept and can account for the present data equally as well as the glycolic acid hypothesis. Recent results on the behavior of \( \text{CO}_2 \) uptake and production rates at the \( \text{CO}_2 \) compensation point (9), however, tend to support the hypothesis that the action of \( \text{O}_2 \) is to stimulate internal photorespiratory \( \text{CO}_2 \) production rather than to directly inhibit photosynthetic \( \text{CO}_2 \) fixation.

At the present time, \( \text{CO}_2 \) production by photorespiration does not appear to be beneficial to the plant. Growth can be reduced by this activity and no ATP or reduced pyridine nucleotide formation has been associated with the \( \text{CO}_2 \) production. Goldsworthy (21) has recently speculated that photorespiration has been inherited from primitive photosynthetic and non-photosynthetic microorganisms which underwent symbiotic union early in the history of life. He has suggested that until recently photorespiration was not detrimental to plants because the \( \text{CO}_2 \) concentration of the earth's atmosphere was too high for an \( \text{O}_2 \) effect to occur. Now, however, photorespiration is becoming disadvantageous, especially for plants growing in dense stands in the tropics.

There is much to be learned about photorespiration, however, and I hesitate to condemn as entirely detrimental a plant activity which can involve a carbon flux similar to that involved in
photosynthesis. Nevertheless, the function of photorespiration in the life of a plant is puzzling and requires further investigation. It should be recalled, however, that in air at temperatures of less than 13° C to 20° C, the apparent rate of photosynthesis in wheat was not inhibited. Thus, photorespiration and the O_2 effect may not be disadvantageous to a plant like wheat during some of its life cycle in temperate conditions.


Chapter II

SOME COMPARATIVE ASPECTS OF THE PHYSIOLOGY OF CO₂ EXCHANGE
BY WHEAT AND CORN SHOOTS

INTRODUCTION

In recent years, certain plants have been distinguished from others on the basis of their characteristics of CO₂ exchange, photosynthetic carbon metabolism, and leaf anatomy. The chloridoid-eragrostoid and some panicoid grasses, such as corn, as well as some members of the Cyperaceae, Amaranthaceae, Chenopodiaceae, Portulacaceae and Euphorbiaceae exhibit CO₂ compensation points below 5 μl./l. CO₂ in the presence of 20.8% O₂ (17, 66, 79, 80), and are capable of very rapid photosynthesis at warm temperatures and high light intensities (11, 23, 24, 25, 35, 39, 41, 44, 67, 68). These plants possess the C₄-dicarboxylic acid pathway of photosynthesis (17, 36, 38, 51, 52) in which CO₂ is initially combined with phosphoenolpyruvic acid by the enzyme phosphopyruvate carboxylase. In this thesis, these plants will be called C₄ plants since the four-carbon acids oxaloacetate, malate and aspartate are their initial products of photosynthetic CO₂ fixation (36, 54). The leaves of C₄ plants contain a well-developed parenchyma bundle sheath surrounded by palisade mesophyll tissue. The size, structure, and ability to accumulate starch differs between chloroplasts of the bundle sheath and palisade mesophyll (5, 16, 17, 55, 56, 57).

In contrast, wheat is representative of other plants which exhibit CO₂ compensation points much above 5 μl./l. CO₂ in the
presence of 20.8% \( \text{O}_2 \) (17), and have relatively low apparent rates of photosynthesis at warm temperatures and high light intensities (12, 23, 24, 40, 41, 42, 44, 67, 68). For convenience, plants like wheat which carry out photosynthetic \( \text{CO}_2 \) fixation by the Calvin cycle in which the three-carbon acid 3-phosphoglycerate is the initial product (2) will hereafter be called \( \text{C}_3 \) plants. In leaves of \( \text{C}_3 \) plants, the parenchyma bundle sheath is usually poorly defined and the palisade mesophyll can be much more extensive. Chloroplasts in the palisade mesophyll and the parenchyma bundle sheath are not strikingly different in appearance (5).

The research presented here was carried out to survey the differences in the \( \text{CO}_2 \) exchange characteristics of \( \text{C}_3 \) and \( \text{C}_4 \) plants, as exemplified by wheat and corn. Forrester et al. have shown that \( \text{O}_2 \) inhibits apparent photosynthesis in corn as well as in several \( \text{C}_3 \) plants (28, 29). In the present study, further tests were made to determine whether the inhibitory effects of \( \text{O}_2 \) are similar in wheat and corn. Measurements were made of \( \text{CO}_2 \) exchange by corn in different \( \text{O}_2 \) concentrations, temperatures, \( \text{CO}_2 \) concentrations and light intensities. Additional measurements were made with wheat to supplement the information already given in Chapter I. Major differences were observed in the pattern of \( \text{CO}_2 \) exchange by wheat and corn shoots. The relationship of these differences to photorespiration and ecology of \( \text{C}_3 \) and \( \text{C}_4 \) plants will be discussed.

MATERIALS AND METHODS

Wheat (\textit{Triticum aestivum} L. var. Spring Thatcher) and corn (\textit{Zea mays} L. var. Pioneer) plants were grown and excised as described for Experiment II in Chapter I. The chamber shown in Figure I-2 was
used for all CO₂ exchange measurements reported in this Chapter. The
methods used to control and measure the light and temperature
environment of enclosed shoots were identical to those used in
Chapter I. Also, the closed system described in Chapter I was used
to obtain the CO₂ compensation point and CO₂ concentration results
presented in Figure II-4.

A simple open system was used for the temperature and light
intensity studies reported in Figures II-5, II-6 and II-7. Outside
air was drawn into this system and passed in series through the
reference cell of the IRGA, the plant chamber, the sample cell of
the IRGA, a Fisher "Dynapump" air pump, a Matheson R-2-15-B flowmeter,
and then out of the system. The IRGA was calibrated to give zero to
full-scale deflection between 250 µl./l. and 350 µl./l. CO₂. As in
the closed system, the air flow rate through this simple open system
was 3 l./min. In this open system, the apparent rates of photosyn-
thesis were calculated from the air flow rate and the CO₂ concentration
differences between the reference and sample cells of the IRGA.

When measurements were made of the response of apparent
photosynthesis to temperature, the excised shoots were preconditioned
for an initial 30 minutes at 25° C and 32,300 lux. Then, the
temperature in the chamber was gradually reduced to about 5° C and
increased to more than 40° C. The rate of temperature change was less
than 2° C/min. The shoots were preconditioned the same way for the
light intensity studies. When the light intensity was altered,
approximately 20 minutes elapsed before apparent photosynthesis
became stable, and measurements were made only after this time.
Throughout these temperature and light intensity studies, the CO₂
concentration of the air entering the system was measured at about 15 minute intervals and was $335 \pm 10 \, \mu l./l. \, CO_2$.

A more complex open system, illustrated in Figure II-1, was used for the time course studies reported in Figures II-2 and II-3. This system was developed to provide constant CO$_2$ concentrations in a gas stream containing different O$_2$ concentrations. Pure CO$_2$ from a compressed gas tank was diluted with gas containing 1.8%, 20.8% or 100% O$_2$, balance N$_2$, by mixing these gases together using two Gallenkamp gas mixing pumps and a Matheson gas proportioner in series. An IRGA measured the CO$_2$ concentration which resulted from these dilutions. Once the desired composition of the mixed gas was established, the gas was passed in series through the reference cell of a second IRGA, the plant chamber, the sample cell of the second IRGA, a Matheson R-2-15-B flowmeter, and out of the system. The second IRGA was calibrated to give a full scale deflection for a 100 $\mu l./l. \, CO_2$ concentration difference between the reference and the sample cells. Once again, the apparent rate of photosynthesis was calculated from the observed CO$_2$ concentration difference between the reference and sample cells, and the gas flow rate, which was 1 l./min. In Figures II-2 to II-7, each point is the result of a single determination of the apparent rate of photosynthesis. As in Chapter I, the apparent rates of photosynthesis are expressed in a per unit fresh weight basis. Airflow planimeter measurements (49) determined that 1 g. fresh weight was equivalent to 0.37 dm$^2$ of corn shoots or 0.40 dm$^2$ of wheat shoots. These values can be used to approximate the apparent rates of photosynthesis on a leaf area basis.
Figure II-1

Open System Used to Generate Constant CO2 Concentrations in an Air Stream Containing Different O2 Concentrations
RESULTS AND DISCUSSION

1. The CO₂ Compensation Point

It has been reported that in air containing 21% O₂, the CO₂ compensation point of C₄ plants is close to zero μl./l. CO₂ (17, 29, 60, 64, 66). In Chapter I, it was observed that the CO₂ compensation point of wheat was always greater than 5 μl./l. CO₂ except at very low O₂ concentrations. Also, the CO₂ compensation point of wheat was increased by increasing temperatures and O₂ concentrations. Other C₃ plants also exhibit high CO₂ compensation points which respond to temperature and O₂ concentration (17, 20, 28, 81, 89). To determine whether a high CO₂ compensation point could be obtained with corn by modifying the temperature or O₂ concentration, measurements were made with corn shoots at 32,300 lux, at temperatures from 13° to 40° C and at O₂ concentrations from 1.8% to 100% O₂. In all conditions, the CO₂ compensation point of corn was found to be less than 5 μl./l. CO₂, which was indistinguishable from zero μl./l. CO₂ by techniques used. Therefore, within the limits tested, the low CO₂ compensation point of corn does not depend on the ambient temperature or O₂ concentration.

This difference between the CO₂ compensation points of C₃ and C₄ plants is important since it is indicative of a difference in the rates of CO₂ production by two types of plants during illumination. The high CO₂ compensation points of C₃ plants are evidence that substantial amounts of CO₂ are released by these plants by photorespiration, and that the relative rates of CO₂ production and release are altered by the temperature and the O₂ concentration. On the other hand, the low CO₂ compensation points of corn and other C₄
plants indicate a low rate of CO₂ release during photosynthesis by these plants. In fact, it has proven difficult to demonstrate that C₄ plants release any CO₂ at all during illumination. When the leaves of C₄ plants are exposed to light and placed in a stream of CO₂-free air, no detectable release of CO₂ into the air stream is observed (25). Irvine (47) recently carried out an experiment in which a ¹⁴C-labelled and an unlabelled corn plant were enclosed together in a sealed chamber and illuminated. After 1 hour, he was able to detect very small but measurable quantities of ¹⁴C accumulating in the initially unlabelled plant. He concluded that corn does release small amounts of CO₂ during photosynthesis. The sensitivity of this CO₂ release process to different concentrations of O₂ was not tested, so it is not known whether the CO₂ originated from photorespiration or some other source. Meidner (60, 61) was able to obtain an elevated CO₂ compensation point with corn by placing illuminated leaves under water stress. This CO₂ compensation point, however, was not responsive to changes in O₂ concentration above 2% O₂, and therefore was not due to an increased rate of photorespiration. Instead, it was attributed to a reduction in the rate of photosynthetic CO₂ fixation by the high water deficits. Corn also exhibits a high CO₂ compensation point when the light intensity is very low (9, 18). Once again, it does not appear that photorespiration is implicated in this result, which seems to be due to a simple balance between the opposing processes of CO₂ fixation and dark respiration.

The CO₂ compensation point studies therefore demonstrate a major difference between C₃ and C₄ plants. During photosynthesis, C₃ plants can release substantial quantities of CO₂ by an O₂-sensitive
photorespiratory process, while C4 plants apparently lack photorespiration.

2. Effect of O2 Concentration on the Apparent Rate of Photosynthesis

Apparent photosynthesis may be inhibited by the presence of O2 in both wheat and corn, but the characteristics of the inhibition appear to differ between the two species. This difference is illustrated by Figures II-2 and II-3 which show the time course of apparent photosynthesis of wheat and corn shoots which were exposed to atmospheres containing 300 µl/l CO2; 1.8%, 20.8% or >99% O2; balance N2. These measurements were carried out at 25°C and 32,300 lux. Figure II-2 shows that, as in Chapter I, apparent photosynthesis in wheat was inhibited by O2 concentrations above 1.8% O2. At any particular O2 concentration, however, the apparent rate of photosynthesis of wheat was constant following the initial 30 minute period of photosynthetic induction. Also, if the O2 concentration was changed from 1.8% or >99% O2 to 20.8% O2 at any time, the apparent rate of photosynthesis of wheat changed rapidly to become the same as that of shoots which had been exposed to 20.8% O2 all the time. Thus, the effect of O2 on apparent photosynthesis of wheat is constant in time and is reversible. As discussed in Chapter I, at least part of the effect of O2 on apparent photosynthesis of wheat appears to be due to the response of photorespiration to O2.

Figure II-3 shows that the apparent rate of photosynthesis of corn was the same in 20.8% O2 as in 1.8% O2. When the O2 concentration was 100%, however, CO2 assimilation by corn was greatly inhibited. Also, the degree of inhibition was not constant but increased with the time the corn shoots were exposed to >99% O2. When the corn shoots
Figure II-2

Time Course of CO₂ Exchange by Excised Wheat Shoots in Atmospheres Containing 300 µl/l. CO₂ and Different O₂ Concentrations

The Shoots were Excised at Time = 0
WHEAT

APPARENT RATE OF PHOTOSYNTHESIS (mg CO₂/hr/g fr wt)

TIME (min)

- △ 1.8% O₂
- △ 20.8% O₂
- □ > 99% O₂
Figure II-3

Time Course of CO$_2$ Exchange by Excised Corn Shoots in Atmospheres Containing 300 µl./l. CO$_2$ and Different O$_2$ Concentrations

The Shoots were Excised at Time = 0
APPARENT RATE OF PHOTOSYNTHESIS (mg CO₂/hr/g fr wt)
were returned to 20.8% O₂ after one or two hours in >99% O₂, the reversal of the inhibition was much more gradual than with wheat. Forrester et al. (29) have previously shown that high O₂ concentrations inhibit apparent photosynthesis in corn. The present results reveal that the inhibitory effect of >99% O₂ is time-dependent and only slowly reversible. These results confirm the findings of several other studies which have also indicated that photosynthesis of C₄ plants is not enhanced when the O₂ concentration is reduced from 20.8% O₂ to 2% O₂ or less (15, 29, 40).

Additional measurements were made to determine whether 20.8% O₂ inhibits apparent photosynthesis of corn under other conditions of temperature, CO₂ concentration or light intensity. Figure II-4 shows that the apparent rates of photosynthesis in 1.8% O₂ and 20.8% O₂ were similar in all conditions tested. Therefore, 20.8% O₂ does not appear to be sufficient to affect apparent photosynthesis in corn. This absence of an inhibitory effect with corn does not resemble that found with wheat in Chapter I when the inhibition was absent only at low temperatures and high CO₂ concentrations.

Since the CO₂ compensation point of corn was always less than 5 μl./l. CO₂ in these studies, photorespiration does not appear to be implicated in the response of apparent photosynthesis of corn to high O₂ concentrations. It is possible that >99% O₂ inhibited photosynthesis in corn by causing stomatal closure. There is no explanation, however, why stomata in wheat would not be similarly affected. It is perhaps more likely that the results in Figure II-3 should be interpreted in terms of an inhibitory effect of >99% O₂ on fixation itself. Because photosynthesis involves many steps, there are numerous sites
Figure II-4

Effects of O\textsubscript{2} Concentration, Temperature, CO\textsubscript{2} Concentration and Light Intensity on the Apparent Rate of Photosynthesis of Excised Corn Shoots
which could be susceptible to inhibition by O\(_2\) (13, 83). In C\(_4\) plants, it is possible that high O\(_2\) concentrations may inhibit one or several steps in the C\(_4\)-dicarboxylic acid pathway, although little evidence is now available to specify the particular site(s) of action. One enzyme of the C\(_4\)-dicarboxylic acid pathway, pyruvate, phosphate dikinase, deserves some investigation, however, since it is reversibly inactivated in the presence of O\(_2\) in vitro (1).

3. Effect of Temperature on Apparent Photosynthesis

There are marked differences in the responses of C\(_3\) and C\(_4\) plants to temperature. In air, C\(_3\) grasses generally have optimum temperatures for apparent photosynthesis and for growth which are distinctly lower than those of C\(_4\) grasses (11, 12, 21, 25, 26, 30, 31, 44, 62, 63, 67, 68). For example, Figure II-5 shows that, at 20.8\% O\(_2\) and 335 µl./l. CO\(_2\), the optimum temperature for apparent photosynthesis in wheat was about 25\(^\circ\) C, while for corn it was almost 40\(^\circ\) C. At the optima, and at all temperatures above about 18\(^\circ\) C, the apparent rate of photosynthesis of corn was greater than that of wheat. Below 18\(^\circ\) C, however, apparent photosynthesis of wheat was more rapid. These results are very similar to those of De Jager (12) who used the C\(_3\) plants Lolium perenne and Lolium multiflorum, and the C\(_4\) plant Paspalum dilatatum.

The temperature curve for wheat in Figure II-5 resembles the results of Experiment I in Chapter I but differs from the results of Experiment II. The data from Experiment II were collected over a period of 3 months while Experiment I was performed during a 3 week period and the measurements in Figure II-5 were obtained on one day. Some drift in the apparent rate of photosynthesis or in the temperature
Figure II-5

Effects of Temperature on the Apparent Rates of Photosynthesis of Excised Wheat and Corn Shoots in Air Containing 20.8% O\textsubscript{2} and 335 µl./l. CO\textsubscript{2}
response may account for the different results found in Experiment II. Since they were obtained over a short period of time, the results in Figure II-5 probably represent the best estimate of the within plant variation in apparent photosynthesis in response to changes in temperature.

Photorespiration may contribute to this difference in temperature response between C\textsubscript{3} and C\textsubscript{4} plants. In Chapter I it was observed that the optimum temperature for apparent photosynthesis in wheat decreased as the O\textsubscript{2} concentration increased. This decrease in temperature optimum was noticeable in only one case in 20.8% O\textsubscript{2}, however, and was not noted in recent studies with Marchantia polymorpha (6). Therefore, conclusive evidence is not available to indicate whether or not photorespiration is a major cause of the differences in temperature response between C\textsubscript{3} and C\textsubscript{4} plants in 20.8% O\textsubscript{2}.

Treharne and Cooper (82) have suggested that these differences may be the consequence of differences in the temperature responses of the major carboxylating enzymes of C\textsubscript{3} and C\textsubscript{4} plants. They observed that the optimum temperature for the ribulose-1, 5-diphosphate carboxylase of the C\textsubscript{3} plants Lolium perenne and Avena sativa was 20° C to 25° C. In contrast, the activity of the phosphoenolpyruvate carboxylase of the C\textsubscript{4} plants Zea mays and Cenchrus ciliaris was greatest between 30° and 35° C. In a different study, the combined activities of the phosphoenolpyruvate carboxylase and NAD-malate dehydrogenase enzymes of Bryophyllum tubiflorum were greatest at 35° C (8). Therefore the carboxylating enzymes may be a major source of the differential effect of temperature on CO\textsubscript{2} exchange by C\textsubscript{3} and C\textsubscript{4} plants.
4. Effect of CO₂ Concentration on Apparent Photosynthesis

The effect of CO₂ concentration on apparent photosynthesis of wheat was presented in Chapter I, Figures I-9 and I-13 to I-18. Corresponding results with corn are shown in Figure I-4. The effects of CO₂ concentration are generally similar except that the CO₂ compensation point of wheat is higher than that of corn at O₂ concentrations above 1.8% O₂. As a result, corn can carry out net CO₂ assimilation at much lower CO₂ concentrations than can wheat. The CO₂ concentration response of apparent photosynthesis of wheat and corn appears to resemble the results obtained in studies of the effect of substrate concentration on reaction rate in isolated enzyme systems. This similarity has led some investigators to apply methods analogous to those used in the study of enzyme kinetics to the analysis of the CO₂ concentration response of photosynthesis. In this approach, the rate of CO₂ assimilation at CO₂ saturation is termed the apparent Vmax of photosynthesis, and the CO₂ concentration at which

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**Table II-1**

Comparison of the Apparent Kinetic Constants of Photosynthetic CO₂ Assimilation of Excised Wheat and Corn Shoots

<table>
<thead>
<tr>
<th>Plant</th>
<th>Temperature (°C)</th>
<th>Light Intensity (lux)</th>
<th>O₂ Conc. (%)</th>
<th>Apparent Vmax (mg. CO₂/hr/g fr wt)</th>
<th>Apparent Km (μl CO₂/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>25</td>
<td>32,300</td>
<td>1.8</td>
<td>8.50</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>32,300</td>
<td>20.8</td>
<td>8.50</td>
<td>177</td>
</tr>
<tr>
<td>Corn</td>
<td>25</td>
<td>32,300</td>
<td>1.8</td>
<td>8.72</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>32,300</td>
<td>20.8</td>
<td>8.72</td>
<td>121</td>
</tr>
</tbody>
</table>
the rate of CO₂ assimilation is one-half the apparent Vmax is called the apparent Kₘ (32). Table II-1 and the results of Goldsworthy (32) demonstrate that at low O₂ concentrations the apparent Kₘ for CO₂ assimilation by C₃ plants is similar to that of C₄ plants. In 20.8% O₂, the apparent Kₘ of wheat was much higher than it was at 1.8% O₂, while the apparent Kₘ of corn remained low. The similar apparent Kₘ values for C₃ and C₄ plants at low O₂ concentrations indicates that under these conditions the affinity for CO₂ of the overall CO₂ assimilation systems of these two plant types is similar. In 20.8% O₂, the apparent affinity of C₃ plants for CO₂ is much lower than in 1.8% O₂ because of photorespiration and other components of the O₂ effect. As before, 20.8% O₂ did not reduce the apparent Vmax of wheat or corn.

Goldsworthy (32) has argued that the similar Kₘ values at low O₂ concentrations is evidence that the internal CO₂ fixation processes in C₃ and C₄ plants are similar in their affinity for CO₂. This conclusion could be correct if the resistances to CO₂ diffusion into leaves of C₃ and C₄ plants were equal. Unfortunately, several other studies have indicated that there is a greater resistance to CO₂ diffusion into leaves of C₄ plants than into leaves of C₃ plants (14, 44, 70). Because of this difference in CO₂ diffusion resistance, the apparent kinetic constants cannot in themselves be used to describe the characteristics of the internal CO₂ fixation systems of C₃ and C₄ plants.

Studies with isolated enzymes have indicated that phosphoenolpyruvate carboxylase has a higher affinity for CO₂ than ribulose-1, 5-diphosphate carboxylase (50, 86). These in vitro results are diffi-
cult to relate to the observations with leaves, however, since the latter are the consequence of the activities of many other enzymes as well as physical processes.

5. Effect of Light Intensity on Apparent Photosynthesis

Figure II-6 shows the response of apparent photosynthesis by wheat and corn to different light intensities. These measurements were carried out at 25° C, 335 μl./l. CO₂ and 20.8% O₂. In wheat, apparent photosynthesis was close to light saturation at light intensities above about 32,000 lux. This result is in agreement with other studies which have found that light saturation of apparent photosynthesis of C₃ plants often occurs in the range from 20,000 to 35,000 lux (10, 12, 39, 41, 67, 84).

Although the apparent rates of photosynthesis were higher for corn, the response to light intensity was similar to that of wheat at intensities below 36,000 lux. When the light intensity was increased above that value, however, an unusual response was observed. Figure II-7 shows that after a change in light intensity from 32,000 lux to 100,000 lux there was a temporary increase in the apparent rate of photosynthesis by corn followed by a gradual decline in the rate. This decline continued after the rate became less than that observed at the preceding lower light intensity. The temporary increase in the apparent rate of photosynthesis after the increase in light intensity may have been due to the accompanying increase in temperature from 25° C to 31.5° C. The inhibitory effect of high intensity light in corn was not reversible since the apparent rate of photosynthesis remained below the initial rate when the light intensity was restored to 32,300 lux. The occurrence of this high light intensity
Figure II-6

Effects of Light Intensity on the Apparent Rates of Photosynthesis of Excised Wheat and Corn Shoots in Air Containing 20.8% O₂ and 335 µl./l. CO₂
Effects of Exposure to High Light Intensity on the Apparent Rate of Photosynthesis of Excised Corn Shoots
response of corn was verified with five different samples of corn shoots.

The behavior seems to be a peculiarity of the plants used, since the author has not encountered other descriptions of high light intensity inhibition of corn photosynthesis. It is possible that the photosynthetic apparatus of these corn shoots was susceptible to damage by high light intensity because the plants were grown at a relatively low light intensity, 21,600 lux. In the C₄ plant sugar-cane, however, leaves which develop at low light intensities are still capable of photosynthesis at full efficiency in full sunlight (85). Since the optimum temperature for photosynthesis of these corn shoots was about 40° C, it seems unlikely that the inhibition of photosynthesis was due to a high temperature lesion. Therefore, there is no convenient explanation for this high light intensity effect on the basis of the available evidence. All other experiments with corn shoots were performed at 32,300 lux or lower, where there was no noticeable inhibition of apparent photosynthesis by light.

The high light intensity results with corn were unexpected since most studies with corn and other C₄ plants have shown that the apparent rate of photosynthesis by individual leaves continues to increase, although not linearly, up to light intensities of 60,000 lux or more (10, 12, 21, 22, 35, 39, 41, 42, 67, 74, 84, 85). The dotted line on Figure II-6 illustrates this typical response. Thus, most investigations have indicated that C₄ plants are much more efficient than C₃ plants in utilizing high intensity light for photosynthesis. This difference in efficiency may be related to different ATP requirements for the photosynthesis of C₃ and C₄ plants. Two moles of
ATP are required for the pyruvate, phosphate, dikinase reaction of the C₄-dicarboxylic acid pathway (10, 37). If this pathway operates in series with the reductive pentose phosphate pathway in C₄ plants (36, 52), then these plants will have a higher ATP requirement for photosynthesis than do C₃ plants which possess only the second pathway. Thus, C₄ plants may require a high rate of photosynthetic phosphorylation during CO₂ fixation. In accord with this, Chen et al. (10) have recently demonstrated that chloroplasts of the C₄ plant Cynodon dactylon have a higher affinity for inorganic phosphate and ADP than chloroplasts of the C₃ plants which have been tested.

The leaf anatomy of C₄ plants may also contribute to their efficiency at utilizing high intensity light. The high chlorophyll content of the bundle sheath of C₄ plants may require higher light intensities for saturation of photosynthesis than are necessary with leaves of C₃ plants in which the chlorophyll is more evenly distributed (5).

6. General Discussion

It is clear from the preceding sections that there are pronounced differences in the CO₂ exchange characteristics of wheat and corn. The CO₂ compensation point of corn was ordinarily indistinguishable from zero µl./l. CO₂ and was not dependent on the O₂ concentration and temperature as was the CO₂ compensation point of wheat. Apparent photosynthesis of corn was not affected by 20.8% O₂, but >99% O₂ inhibited CO₂ assimilation, and the degree of inhibition increased with time and was only slowly reversible. The inhibition of apparent photosynthesis of wheat by 20.8% O₂ or by >99% O₂ was constant in time and was rapidly reversible. In 20.8% O₂, corn was
more efficient at carrying out CO₂ assimilation at low CO₂ concentrations and high temperatures than was wheat, and there is evidence from other studies that corn is more efficient at utilizing high intensity light for photosynthesis. On the other hand, apparent photosynthesis was more rapid with wheat than with corn at low temperatures.

Photorespiration appears to contribute greatly to these differences between wheat and corn. At 1.8% O₂, where photorespiration is virtually suppressed, wheat resembled corn in that it possessed a low CO₂ compensation point, a high apparent rate of photosynthesis, and possibly an elevated optimum temperature for apparent photosynthesis (Chapter I). It would be an oversimplification, however, to conclude that differences in photorespiration are the source of all the differences in CO₂ exchange between wheat and corn. It is evident from the preceding sections that many of the differences in CO₂ exchange can be related to differences in photosynthetic carbon metabolism or in leaf anatomy. For example, in Section II-3, it was suggested that the differential effect of temperature on apparent photosynthesis by wheat and corn may arise from differences in the temperature response of the major enzymes responsible for CO₂ fixation in the two species.

Although corn and other C₄ plants do not release appreciable quantities of CO₂ during photosynthesis, they do seem to contain some components which may be involved in photorespiration by C₃ plants. It has been suggested that photorespiratory CO₂ production by C₃ plants may arise from the oxidation of glycolic acid (18, 19, 61, 65, 87, 88, 89, 90). Glycolic acid oxidase occurs in corn (69, 75, 76), sugar cane
and *Amaranthus edulis*, although the activity is low in these C₄ species (78). Glycolic acid has been shown to accumulate when corn is treated with an inhibitor of glycolic acid oxidase (87). In addition, CO₂ release is stimulated when glycolic acid is fed to corn leaves (18). Peroxisomes, which may be associated with photorespiratory metabolism (53, 77, 78), have been detected in C₄ species (78). Finally, mass spectrometric measurements have indicated that large quantities of O₂ are assimilated by illuminated corn plants (48). This last result, however, may not be due to photorespiration, but may have another cause, such as the activity of phenol oxidase which is abundant in chloroplasts of the C₄ plant sugar cane (33).

It is possible that CO₂ may be produced in significant quantities during photosynthesis in corn, but it may be prevented from leaving the plant because of an efficient internal CO₂ recycling mechanism (24). Phosphoenolpyruvate carboxylase has a high affinity for CO₂ (86) and it is located in the mesophyll surrounding the parenchyma bundle sheath (4, 72). If CO₂ is produced by the photorespiration of compounds in the parenchyma bundle sheath of C₄ plants, it may be refixed into C₄ dicarboxylic acids in the mesophyll before it can escape from the leaf. Therefore, although the CO₂ compensation point of C₄ plants is ordinarily indistinguishable from zero, it does not necessarily follow that C₄ plants lack the internal capability of photorespiratory CO₂ production during photosynthesis. If photorespiration does occur in corn, however, it does not cause a carbon deficit.

The general geographical distribution of C₃ and C₄ plants is correlated with their CO₂ exchange properties, photosynthetic
metabolism and leaf anatomy. C₃ grasses, such as members of the Festucaceae, Aveneae and Agrosteae, are abundant in temperate regions. The C₄ Andropogoneae, Eragrosteae and Paniceae, however, are widespread in the tropics (34). In the dicotyledonous C₄ groups, the Amaranthaceae are thought to be of tropical origin (58). Also, species of Atriplex which possess the characteristic C₄-type of leaf anatomy are native to the hot and arid regions of Australia and the United States (55). C₄ plants, therefore, thrive in locations which have warm temperatures, high light intensities and often seasonal dry periods (55).

The physiological characteristics of C₄ plants, particularly their ability to utilize high intensity light and high temperatures for rapid photosynthesis, would seem to be well suited for a warm well-illuminated environment. Their ability to assimilate CO₂ at low CO₂ concentrations may also be advantageous in closely packed stands on calm days when the CO₂ concentration within the canopy may decrease appreciably. There is also evidence that C₄ plants are efficient in their use of water. Results of Schantz and Piemeisel (71) indicate that C₄ plants use about one-half as much water during the production of 1 g. of dry matter as do C₃ plants. Since C₄ plants transpire at lower rates than C₃ plants (14, 44), this difference in water requirement does not simply reflect differences in CO₂ assimilation rates between the two types of plants.

In temperate conditions, C₄ characteristics may not be favoured. In Figure II-5 it was seen that, at temperatures below 18°C, the apparent rate of photosynthesis of wheat was higher than that of corn. Also, chlorophyll accumulation is retarded in corn leaves if
the day temperatures are below 15° C (59). No information is available on whether this effect of low temperature occurs in other C4 species.

These correlations between some physiological characteristics and the geographical distribution of C3 and C4 plants are not absolute. The Bambuseae, for example, have C3 characteristics (17), but are abundant and grow rapidly in the tropics. So do many other plants which are not C4. Factors other than the apparent rate of photosynthesis can control the abundance of a species in a locality. A plant which exhibits low apparent rates of photosynthesis per unit leaf area may still dominate if it produces a large leaf area.

Many C4 plants are common weeds (7). In fact, of the ten species listed by Holm (45) as the most serious weed pests throughout the world, seven belong to C4 genera and the others have not been tested. Certainly, the discovery of a selective herbicide for C4 species would be of great value for weed control. Black et al. (7) have suggested that the high competitive ability of many C4 species may be based on the CO2 exchange characteristics outlined in the preceding sections.

There appear to be additional differences between C3 and C4 plants which may be related to some characteristics already discussed. Compared with C3 species, C4 plants are composed of carbon which is enriched in 13C (3,73). This difference could arise from the difference in the photosynthetic carboxylation pathway or the difference in apparent photorespiratory activity between the two types of plants. Carbonic anhydrase activity is low in C4 plants (27) and this may be associated with the C4-dicarboxylic acid pathway. The acidic compounds produced by photosynthetic CO2 fixation in C4 plants may be responsible for the slightly greater acidity of sap expressed
from C₄ plants compared with sap obtained from C₃ plants (46). Also, translocation of recent photosynthate may be more rapid in C₄ plants than in C₃ plants (43). Whether or not these differences as well as the others noted in this Chapter exist between all C₃ and C₄ plants remains to be proven. Future investigations may discover additional differences between these two plant groups and should prove helpful in relating their physiological properties to their distribution and behavior in nature.
LITERATURE CITED


Chapter III

PHOTORESPiration AND THE POST-ILLUMINATION CO₂ BURST IN
WHEAT AND AMARANTHUS EDULIS

INTRODUCTION

During the first minute of darkness following a period of illumination, plants often exhibit high rates of CO₂ production into air containing 21% O₂. This post-illumination CO₂ burst is eliminated when the O₂ concentration is reduced to 2% and it is enhanced when the O₂ concentration is raised to 100% (2, 3, 7, 8, 10, 12, 24). The size of the burst also increases with increasing light intensity in the period preceding darkness (3, 6, 7, 8, 14, 22, 23, 24). Mainly because of the sensitivity of the burst to O₂ concentration, it has been suggested that the burst is a brief extension of photorespiration into the dark period (5, 6, 7, 8, 12, 19, 22, 23, 24). In accord with this interpretation is the observation that, with the exception of Amaranthus edulis, those plants which photorespire have post-illumination CO₂ bursts, and those plants which apparently lack photorespiration also lack bursts (13, 23).

Amaranthus edulis, on the other hand, exhibits a distinct post-illumination CO₂ burst even though it does not release CO₂ in the light (2, 9). Also, Heath and Orchard (14) have demonstrated that the post-illumination CO₂ burst and the CO₂ compensation point of wheat leaves respond differently to light intensity and temperature. They concluded from this that the burst was not associated with photorespiration.
The interpretation of post-illumination CO$_2$ exchange transients of plants is complex since changes in CO$_2$ diffusion resistance, and in the rates of photosynthesis, photorespiration, and dark respiration can be involved. Stomata close in the dark (25, 26), and transpiration is reduced (10, 25), indicating that the gas diffusion resistance of leaves is increased during the post-illumination period. These changes, however, are relatively slow, (25, 26), compared with the kinetics of the burst. Also, since the burst occurs in liverworts (7, 8), it cannot be the result of stomatal response. Photosynthetic CO$_2$ fixation may continue briefly after illumination, for example falling to zero within 30 seconds after darkening *Scenedesmus* (1). Dark respiration may be inhibited in the light and increase after the onset of darkness (4, 15, 16, 17, 18, 20, 21).

In an effort to clarify the relationship between photorespiration and the post-illumination CO$_2$ burst, the kinetics of the burst and the effects of different O$_2$ and CO$_2$ concentrations on the two processes were examined. The effect of O$_2$ concentrations on the burst of *Amaranthus edulis* was also investigated.

MATERIALS AND METHODS

Wheat plants were grown in the same way as described for Experiment I in Chapter I. *Amaranthus edulis* Speg. plants were grown in similar conditions except that soil, not vermiculite, was used. At the start of each experiment, 2.0 g. fresh weight of 9 to 11 day-old wheat shoots, or single, fully expanded *Amaranthus edulis* leaves from 25 to 28 day-old plants, were excised in the usual manner and enclosed
in the transparent chamber shown in Figure I-2.

After enclosure, the plant material was conditioned for an initial 30 min. period at 32,300 lux (3000 ft.-c.) and 25± 1° C in air. Thereafter it was exposed to a series of light-dark cycles, each cycle consisting of a minimum of 15 min. of illumination at 32,300 lux followed by 5± 0.5 min. of darkness. Darkening was carried out in less than 0.5 sec. by covering the chamber with an opaque black cloth and turning off the light. During illumination, the air temperature in the chamber was 25± 1° C, and in the dark it fell to 24± 1° C. An air flow rate of 1.0 l./min. was maintained while plant material was in the chamber.

The gas flow system used for these experiments was the same as that shown in Figure II-1. Measurement of the temperature within the plant chamber, the CO₂ concentration in the air stream, and control of the O₂ and the CO₂ concentration of the air entering the plant chamber were all carried out as described in Chapters I and II.

RESULTS AND DISCUSSION

1. Kinetics of the Post-illumination CO₂ Burst

The solid line in Figure III-1 illustrates the data obtained when illuminated wheat shoots were darkened. The air entering the plant chamber contained 100 μl./l. CO₂, 20.8% O₂, balance N₂. During illumination, CO₂ assimilation reduced the CO₂ concentration of the air leaving the chamber to 74 μl./l. When the shoots were darkened, there was a 3 sec. delay before the CO₂ concentration readings increased sharply. This lag can be attributed to the time required for CO₂ concentration changes in the chamber to reach the CO₂ analyzer.
Figure III-1

Effects of Darkening on CO₂ Exchange by Excised Wheat Shoots in 20.8% O₂
Within 12 sec. of darkening the shoots, the CO₂ analyzer readings exceeded 100 µl./l. CO₂, and a peak of 121.4 µl./l. CO₂ was reached after 27 sec. After the peak, the readings declined and reached 111 µl./l. CO₂ at 120 sec. Wheat, therefore, exhibits a post-illumination CO₂ burst under these conditions.

The burst as it occurred within the plant chamber, however, must have differed in kinetics and magnitude from these observations. Part of this difference may be ascribed to characteristics of the CO₂ analyzer which operates by detecting the absorption of infra-red light across a 100 ml. sample cylinder. During the burst, the rapid variations of the CO₂ concentration of the air stream generate CO₂ concentration gradients in the sample cylinder and distort the results. This effect could be eliminated by calculating the CO₂ concentration which must have been entering the CO₂ analyzer to produce the observed results. The method of this calculation is described in Appendix I. The dashed line in Figure III-1 shows that when the results were corrected this way, the initial CO₂ concentration increase was more abrupt, and the peak of the burst was about 4 sec. earlier and slightly higher than the measured result. The difference between the observed and corrected results, however, was not large, and all subsequent results in this chapter were left uncorrected.

An additional source of error arose from the separation of the site of the burst from the site of its measurement. Before a CO₂ exchange event which had occurred within the plant chamber was detected by the CO₂ analyzer, it was modified according to the air flow characteristics of the plant chamber and the tubing connecting the chamber to the IRGA. As the result of this modification, the burst
was extended in time, and the peak of the burst was delayed and reduced in size. This was confirmed by comparing the results, shown in Figure III-2, of pulse (less than 0.5 sec.) injections of 0.1 ml. CO₂ in air into the air stream either at the entrance to the CO₂ analyzer or into the middle of the empty plant chamber. The complexity of the air flow characteristics of the experimental system prevented the exact assessment of their effects on the kinetics of the burst. It is clear, however, that the true peak of the burst occurred earlier than 20 sec. after the initial CO₂ analyzer response, and that the peak was higher and more abrupt than that recorded by the CO₂ analyzer.

Bulley (3) recently used a highly simplified open system to measure the post-illumination CO₂ burst of soybean leaves. He estimated that the true peak of the burst occurred between 7 and 12 sec. after the initial CO₂ analyzer response, and calculated that it was about 150% as high as the recorded peak. In view of the above considerations, similar kinetics would not seem to be unreasonable for the post-illumination CO₂ burst of wheat. The peak of the burst, therefore, occurs very soon after illumination is stopped, and it is highly susceptible to modification by measuring techniques.

Other experiments by Bulley (3) have indicated that the burst is not the result of processes commencing at the onset of darkness. When the light intensity was reduced, but not extinguished, a dip in the rate of CO₂ assimilation occurred, and this dip corresponded kinetically to the post-illumination CO₂ burst. It was concluded that the burst was the resultant of two CO₂ exchange processes which differed in rate of response to changes in light intensity.
Response of the IRGA to Pulse Injections of CO₂ into the Air Stream in the Plant Chamber and at the Entrance to the IRGA Sample Cylinder
A second broad peak of CO₂ production usually occurring between 2 and 10 minutes after darkening was observed in this study and has frequently been noted before (7, 12, 13, 22, 23, 24). This peak was evident in both 20.8% O₂ and 1.8% O₂ and is therefore not related to photorespiration. It may be the result of a post-illumination overshoot of dark respiration. Comparable peaks of post-illumination CO₂ production are exhibited by plants lacking photorespirations such as corn (13, 23) and *Amaranthus edulis* (2), as well as by *Chlorella* (7, 8).

2. Effect of O₂ and CO₂ Concentrations on the Post-illumination CO₂ Burst

Figures III-3 to III-7 show the recorded post-illumination CO₂ exchange transients of wheat shoots in atmospheres containing 20.8% or 1.8% O₂ and CO₂ concentrations between 100 µl./l. and 300 µl./l. Each point on these curves is the average of 5 determinations. There was no burst in 1.8% O₂ at any of the CO₂ concentrations used. In 20.8% O₂, there was a pronounced burst in 100 µl./l. CO₂, and the peak height of the burst decreased as the CO₂ concentration increased. Two minutes after the shoots were darkened, there was no significant difference in the rates of CO₂ production between the 20.8% O₂ and 1.8% O₂ treatments.

It is apparent from the preceding discussion of burst kinetics that the observed peak height is affected by the experimental system, so comparisons based on this characteristic of the burst are hazardous. Since the burst in wheat is dependent on O₂, an appropriate index of the size of burst is the overall difference in post-illumination CO₂ exchange between the 20.8% O₂ and 1.8% O₂ treatments. Using this index, Figure III-8 summarizes these results on the effect of CO₂
Figure III-3

Post-illumination CO$_2$ Exchange by Excised Wheat Shoots in 1.8% O$_2$ and 20.8% O$_2$. The CO$_2$ Concentration of the Air Entering the Plant Chamber was 100 µl/l. CO$_2$
Figure III-4

Post-illumination CO$_2$ Exchange by Excised Wheat Shoots in 1.8% O$_2$ and 20.8% O$_2$. The CO$_2$ Concentration of the Air Entering the Plant Chamber was 200 μl./l. CO$_2$
Figure III-5

Post-illumination CO₂ Exchange by Excised Wheat Shoots in 1.8% O₂ and 20.8% O₂. The CO₂ Concentration of the Air Entering the Plant Chamber was 300 μl./l. CO₂.
Figure III-6

Post-illumination CO₂ Exchange by Excised Wheat Shoots in 1.8% O₂. The CO₂ Concentration of the Air Entering the Plant Chamber was 400 μl./l. CO₂
Figure III-7

Post-illumination CO₂ Exchange by Excised Wheat Shoots in 1.8% O₂ and 20.8% O₂. The CO₂ Concentration of the Air Entering the Plant Chamber was 500 μl/l. CO₂.
Figure III-8

Effects of CO₂ Concentration on the Size of the Post-illumination CO₂ Burst and the Magnitude of the Depression of Apparent Photosynthesis by 20.8% O₂
concentration on the size of the post-illumination CO₂ burst. The burst was greatest at 100 μl./l. CO₂ and 200 μl./l. CO₂ and it decreased at higher CO₂ concentrations. As will be shown in Chapter IV, the size of the burst was sufficiently large that it is improbable that the burst originated from a CO₂ free-exchange pool.

In Chapter I it was concluded that the results of studies on the inhibitory effect of O₂ on apparent photosynthesis could not be used to indicate the rate of photorespiration at high CO₂ concentrations. If the post-illumination CO₂ burst is a manifestation of photorespiration, however, then the CO₂ concentration response of photorespiration must resemble that of the burst size shown in Figure III-8. The effect of CO₂ concentration on the burst can be correlated with its effect on the inhibition of apparent photosynthesis by 20.8% O₂. Figure III-8 shows that the burst size and the magnitude of the depression of apparent photosynthesis by 20.8% O₂ respond similarly to CO₂ concentration.

Fock et al. (11) have recently studied the effect of CO₂ concentration on the post-illumination CO₂ burst of the liverwort Conocephalum conicum and have also noted this similarity. In their study, 50% O₂ and 75% O₂ were used and the size of the burst was greatest at about 300 μl./l. CO₂ and declined at lower and higher CO₂ concentrations. If Figure I-13 is reexamined, it can be seen that the depression of apparent photosynthesis by 60% O₂ or 80% O₂ is greatest between 225 μl./l. CO₂ and 300 μl./l. CO₂ at 25° C. Thus, the results with Conocephalum appear to be in general agreement with the present observations.

The correlation between the effect of O₂ on apparent
photosynthesis and on the burst at different CO₂ concentrations can be illustrated in another way. In Chapter I, it was seen that the percent inhibition of apparent photosynthesis by O₂ was:

\[ \% I_p = \frac{P_1 - P_2}{P_1} \times 100 \]

As before, \( P_1 \) and \( P_2 \) are the apparent rates of photosynthesis at a low and a higher O₂ concentration respectively. An analogous expression can be developed for the post-illumination CO₂ burst. Let \( P_b \) be the average rate of CO₂ production during the burst, in mg. CO₂/hr./g. fr. wt., as calculated from the burst size and duration. Once again, the burst size is the overall difference in post-illumination CO₂ exchange between the 20.8% and 1.8% O₂ treatments. The duration of the burst was taken to be the length of time after darkening the shoots that the CO₂ concentration readings in 20.8% O₂ and 1.8% O₂ were different at the 5% level of significance.

Then, if the effects of 20.8% O₂ on apparent photosynthesis and on the burst are due to the same cause or causes, \((P_2 + P_b)\) may be substituted into the above formula in place of \( P_1 \). That is:

\[ \% I_b = \frac{(P_2 + P_b) - P_2}{P_2 + P_b} \times 100 = \frac{P_b}{P_2 + P_b} \times 100 \]

and the response of this function to CO₂ concentration should resemble that of \( \% I_p \). The results presented in Figure III-9 show the similarity of the CO₂ concentration responses of \( \% I_p \) and \( \% I_b \). This is further evidence linking the effects of O₂ on apparent photosynthesis with its effects on the post-illumination CO₂ burst.

In addition to the data already presented on kinetics and the
Figure III-9

Effects of CO₂ Concentration on the Per Cent Inhibition of Apparent Photosynthesis by 20.8% O₂ and on the Analogous Burst Function % B
effects of \( O_2 \) and \( CO_2 \) concentrations on the burst, other results are consistent with the hypothesis that the burst is related to photorespiration. The size of the burst and the depression of apparent photosynthesis by 20.8% \( O_2 \) are affected similarly by light intensity (3). Also, the effect of wavelength of light on the apparent rates of photosynthesis in 21% \( O_2 \) or 2% \( O_2 \) is the same as its effect on the size of the burst (3). These effects of preceding light conditions are further evidence that the burst is linked to processes occurring during the light.

Heath and Orchard (14), on the other hand, concluded that the burst was not related to photorespiration. This conclusion was founded on the assumption that at the \( CO_2 \) compensation point, the rates of \( CO_2 \) fixation and production are unaffected by light intensity. Recent studies (3), however, indicate that at the \( CO_2 \) compensation point, increases in light intensity result in increases in \( CO_2 \) fixation. Therefore, their assumption was not valid, and their reasoning cannot be used to contradict the view that the post-illumination \( CO_2 \) burst in wheat and similar plants is an extension of photorespiration into the dark period.

3. Effect of \( O_2 \) Concentration on the Post-illumination \( CO_2 \) Burst of *Amaranthus edulis*

Figure III-10 shows the results of consecutive determinations of post-illumination \( CO_2 \) exchange by an *Amaranthus edulis* leaf in 20.8% \( O_2 \) or 1.8% \( O_2 \). These data are representative of the results of 16 determinations at 300 \( \mu l./l. \) \( CO_2 \) and 32,300 lux. Although the rates of \( CO_2 \) exchange were found to be more variable from one determination to the next than with wheat, several important features are
Figure III-10

The Post-illumination CO$_2$ Burst of *Amaranthus edulis* in 1.8\% O$_2$ and 20.8\% O$_2$
nevertheless apparent. Unlike wheat, *Amaranthus edulis* exhibited a substantial peak in post-illumination CO₂ production in 1.8% O₂ as well as in 20.8% O₂. The height of the peak in 1.8% O₂ was similar to the height of the peak in 20.8% O₂, although there were often differences in the kinetics of CO₂ exchange subsequent to the peak. The results of Björkman (2) appear to be similar.

Since the peak is not sensitive to O₂, the burst of *Amaranthus edulis* cannot be related to conventional photorespiration or to the burst in wheat. It is possible that the burst of *Amaranthus edulis* is the result of an overshoot of dark respiration which is recovering from inhibition during the light (2), or to some other cause associated with C₄ metabolism. There is insufficient evidence to specify the cause of the differences in CO₂ exchange often observed after the peak of the burst of *Amaranthus edulis*.
LITERATURE CITED


Chapter IV

ESTIMATION OF THE CO₂ FREE-EXCHANGE POOL SIZE
IN WHEAT AND CORN LEAVES

INTRODUCTION

One consideration in the study of photosynthesis, photorespiration and respiration is that CO₂ exchange by other processes should be insignificant or accounted for.

There is some evidence that plants may contain quantities of carbon which can exchange freely with atmospheric CO₂. Many studies have shown that CO₂ is released by plants when they are treated with acid (1, 10, 13, 17, 18, 19). In addition, CO₂ is reversibly absorbed by leaves when they are placed in a vacuum and then exposed to CO₂ (13). Illumination and chlorophyll are not required (19, 13), and leaves which are dried and then rewetted also absorb CO₂ (14). The quantity of CO₂ involved is substantial. For example, 0.64 to 1.19 ml of CO₂ per g. fresh weight were absorbed by leaves of 13 species at 15° and 1 Atm. CO₂ (14). The relationship of these observations to a possible CO₂ free-exchange pool under ordinary conditions is not known.

A number of processes could contribute to the formation of a CO₂ free-exchange pool. CO₂ is absorbed by water, partly by physical solution and partly by chemical hydration (4, 6, 11):

\[ \text{CO}_2 \text{(gas)} \rightleftharpoons \text{CO}_2 \text{(solution)} \]

\[ \text{H}_2\text{CO}_3 \rightleftharpoons \text{HCO}_3^- + \text{H}^+ \rightarrow \text{CO}_3^{2-} + 2\text{H}^+ \]

At alkaline pH, the formation of HCO₃⁻ and CO₃²⁻ are favored, and the
reaction:

\[ \text{CO}_2 + \text{OH}^{-} \rightarrow \text{HCO}_3^{-} \]

also becomes important (9, 11). In plant cells, the presence of the enzyme carbonic anhydrase (E.C. 4.2.1.1.) (3, 5) or the anions of many acids, e.g. phosphate and acetate (4, 12), may accelerate the otherwise slow hydration steps. The solution of \( \text{CO}_2 \) in water is enhanced in the presence of alkaline earth carbonic acid esters, and with amines to form carbamates (11).

The following experiments were designed to estimate the size of the \( \text{CO}_2 \) free-exchange pools in wheat and corn leaves.

**MATERIALS AND METHODS**

Wheat (Triticum aestivum L.) and corn (Zea mays L.) plants were grown in the same conditions described for wheat in Chapter I. About 2 g. fresh weight of ten to fourteen day-old shoots were excised and transferred to a 20 x 3 x 0.5 cm plexiglass chamber shown in Figure IV-1 and placed in the dark. The temperature in the chamber was measured by a thermistor placed behind some of the leaves. A Beckman IR 215 infra-red \( \text{CO}_2 \) analyzer, used differentially in the open system, measured the \( \text{CO}_2 \) concentration. The air flow rate was 1.6 l./min. for all measurements.

The acid-labile \( \text{CO}_2 \) was measured after the addition of 20 ml. of 5 M HCl in ethanol to 1 g. fresh weight of plant material. The \( \text{CO}_2 \) evolved was trapped in 1 M NaOH and then released again into a closed system by the addition of excess 5 M aqueous HCl.

Two techniques were used to measure the amount of freely exchanged \( \text{CO}_2 \). The first, \(^{12}\text{CO}_2\) exchange, involved the comparison of
Figure IV-1

Plant Chamber Used for Measurements of the CO$_2$ Free-Exchange Pool Size with Excised Wheat and Corn Shoots
dark respiration rates measured in an open gas flow system with those observed in a closed system. The apparatus used is represented in Figure IV-2A. Air from compressed gas tanks was introduced into the open system, and the respiration rate was calculated from the increase in CO₂ concentration after the air stream had crossed the chamber. CO₂ concentrations used with the open system, with relative frequency of about 1:2:1, were 130 μL/l, 325 μL/l, and 390 μL/l. In the 0.29 l. closed systems, the respiration rate was calculated from the time required for the CO₂ concentration to increase from 100 to 400 μL/l. Open and closed system measurements were alternated.

The CO₂ free-exchange pool, if present, should enlarge response to the 300 μL/l. increase in CO₂ concentration in the closed system. The exchange pool should not enlarge appreciably in the open system where the CO₂ concentration varied by less than 5 μL/l.

Since some of the CO₂ produced by respiration in the closed system contributes to the increase in exchange pool size, the observed rate of respiration should be less in the closed system than in the open system. The change in pool size after the CO₂ concentration had increased from 100 μL/l. to 400 μL/l. was calculated from:

$\text{Pool CO}_2 \text{ Increase (μL/g.)} = \frac{R_0 \Delta t - V \Delta [CO_2]}{W}$

where $R_0$ represents the respiration rate in the open system (μL CO₂/min), $\Delta [CO_2]$ was the CO₂ concentration increase (300 μL/l.) in the closed system during $\Delta t$ minutes; $V$ was the closed system volume (l.); and $W$ was the fresh weight of plant material (g.).

For the second technique, $^{14}$CO₂ exchange, the CO₂ free-
Figure IV-2

Gas Flow System Used for $^{14}$CO$_2$ Exchange and $^{12}$CO$_2$ Exchange Measurements with Excised Wheat and Corn Shoots
A $^{12}$CO$_2$ EXCHANGE

OUTLET INLET

- TELETHERMOMETER
- PUMP
- FLOW METER
- PLANT CHAMBER
- MgClO$_4$ DRIER
- SAMPLE REFERENCE
- IRGA

B $^{14}$CO$_2$ EXCHANGE

- PUMP
- FLOW METER
- RECORDER
- RATEMETER
- GEIGER TUBE
- PLANT CHAMBER
- MgClO$_4$ DRIER
- RELEASE FLASK
- RECORDER
- IRGA
exchange pool size was estimated by measuring the incorporation of $^{14}\text{CO}_2$ into the pool in the dark. A 0.23 l. closed system, depicted in Figure IV-2B, was used. The entire system was initially flushed with CO$_2$ free-air to reduce the $^{12}\text{CO}_2$ concentration below 100 $\mu$l./l.

One $\mu$l. of $^{14}\text{CO}_2$ was generated in isolation by the addition of excess 1 M H$_2$SO$_4$ to NaH$^{14}\text{CO}_3$ in the release flask. The $^{14}\text{CO}_2$ was then let into the rest of the system. A Geiger tube, which was connected to a Nuclear Chicago "Labitron" ratemeter and a chart recorder, was placed at the entry to the plant chamber to detect the $^{14}\text{C}$ activity in the air stream. The initial observation of the $^{14}\text{C}$ activity was made within 10 seconds of the introduction of the $^{14}\text{CO}_2$, and subsequent measurements were made at 6 second intervals until the CO$_2$ concentration had reached 400 $\mu$l./l.

A consistent decrease in the $^{14}\text{C}$ activity of the air stream was observed when plants were not included in the system. Since no leakage of $^{12}\text{CO}_2$ was evident under these conditions, this loss was presumably due to absorption of $^{14}\text{CO}_2$ on the walls of the system. Because of this, an equivalent number of measurements were made with and without plants.

It was also necessary to correct for the dark fixation of $^{14}\text{CO}_2$. When the CO$_2$ concentration reached 400 $\mu$l./l., the plants were removed from the chamber, immersed in liquid nitrogen and ground to a powder. One $\mu$l. per g. fresh weight of 1 M acetate buffer, pH 4.0, was added to remove "unbound" CO$_2$ and the mixture was dried in a dessiccator. Fixed $^{14}\text{C}$ was measured by spreading the dried material on planchets, counting with a Geiger counter, and correcting for self-absorption and counter efficiency.
On the basis of this information, the total size of the CO₂-free-exchange pool was calculated from:

$$\text{Pool CO}_2 (\mu l./g.) = \frac{14C_o - (14C_p + 14C_f)}{14C_o} \times \frac{V[CO_2]}{W}$$

where $14C_p$ and $14C_o$ were the gas phase $14C$ activities in the presence or absence of plants; $14C_f$ was the amount of $14C$ fixed; $V$ was the closed system volume (l.); $[CO_2]$ was the final CO₂ concentration (400 µl/l.); and $W$ was the fresh weight (g.) of plant material.

The $12CO_2$ and $14CO_2$ exchange techniques are similar in that they are both based on gas exchange measurements. They differ because $12CO_2$ exchange requires the formation of a chemical equilibrium while $14CO_2$ exchange involves an isotopic equilibrium. Also the $12CO_2$ exchange technique can only detect changes in exchange pool size, while the $14CO_2$ exchange technique allows the estimation of the total pool size. The two methods were equally difficult.

To test the sensitivity of both the $12CO_2$ and $14CO_2$ technique, the absorption of $12CO_2$ or $14CO_2$ by a wick of Whatman No. 1 filter paper containing solution of 0.2 M phosphate buffers, pH 6.0 and pH 7.75, was used. In the case of the $12CO_2$ absorption measurements, the phosphate buffers had previously been stored for seven days over 1 M NaOH in a closed desiccator and were therefore CO₂-free. At the start of the $12CO_2$ absorption measurements, the phosphate buffers were in equilibrium with normal air. These absorption measurements were terminated after 25 minutes, since that was the average duration of experiments with plants. Figures IV-3A and IV-3B indicate the apparatus used for the absorption measurements.
Figure IV-3

Gas Flow System Used for $^{12}\text{CO}_2$ Exchange and $^{14}\text{CO}_2$ Exchange Measurements with 0.2 M Phosphate Buffers
A ¹²CO₂ EXCHANGE CONTROL

- PUMP
- FLOW METER
- IRGA
- RECORDER
- CHAMBER

B ¹⁴CO₂ EXCHANGE CONTROL

- PUMP
- FLOW METER
- GEIGER TUBE
- RELEASE FLASK
- RATEMETER
- RECORDER
- CHAMBER
RESULTS AND DISCUSSION

Between 40 and 55 μl. of CO₂ per g. fresh weight were released after wheat and corn shoots were acidified with 5 M HCl. Similar results were recently reported by Yemm (19). Smith (13) found that over 300 μl. of CO₂ per g. fresh weight were released if plants were boiled in 4.4 M HCl for 1 hour. If a substantial portion of this acid-labile CO₂ were ordinarily in free and rapid exchange with the atmosphere, it could prove to be a complicating factor in studies of CO₂ exchange by plants.

Measurements of the absorption of CO₂ by 0.2 M phosphate buffers are given in Table IV-1. The calculated values were derived from the solubility of CO₂ in pure water (7), the apparent pK of carbonic acid (2) and the Henderson-Hasselbach equation (16)*. At pH 7.75, the CO₂ absorption measured by both techniques was slightly less than the calculated value. Failure to come to equilibrium, or differences in the solubility of CO₂ or the pK of carbonic acid in 0.2 M phosphate may account for this underestimation. Nevertheless, it is apparent that small CO₂ free-exchange pools can be detected, and their approximate sizes determined, by both the ¹²CO₂ and ¹⁴CO₂ exchange techniques.

From Table IV-2 it can be seen that the CO₂ free-exchange pools in both wheat and corn were found to be very small or non-existent by both techniques used. During these experiments, no significant effect of CO₂ concentration on the rate of dark respiration was observed in the open system. Although the measurements were

*Appendix II
Table IV-1

Magnitude of CO₂ Free-exchange Pools in 0.2 M Phosphate Buffers

<table>
<thead>
<tr>
<th>Method</th>
<th>Temperature (°C)</th>
<th>CO₂ Concentration (µl./l.)</th>
<th>pH</th>
<th>CO₂ Free-exchange Pool Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Measured (µl. CO₂/ml. Buffer)</td>
</tr>
<tr>
<td>¹²CO₂ Exchange</td>
<td>27</td>
<td>382</td>
<td>6.0</td>
<td>1.62*</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>358</td>
<td>7.75</td>
<td>5.90±0.76**</td>
</tr>
<tr>
<td>¹⁴CO₂ Exchange</td>
<td>20</td>
<td>300</td>
<td>6.0</td>
<td>-0.85±3.12**</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>300</td>
<td>7.75</td>
<td>4.96±1.19**</td>
</tr>
</tbody>
</table>

* Average of 2 measurements.

** 95% confidence limits of the mean.
Table IV-2

Magnitude of CO₂ Free-exchange Pools in Wheat and Corn Shoots

<table>
<thead>
<tr>
<th>Method</th>
<th>Plant</th>
<th>No. of Measurements</th>
<th>Average Temperature (°C)</th>
<th>CO₂ Free-exchange Pool Size (μl CO₂/g. fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>¹²CO₂ exchange</td>
<td>wheat</td>
<td>27</td>
<td>21.4</td>
<td>1.69 ± 2.48*</td>
</tr>
<tr>
<td></td>
<td>corn</td>
<td>12</td>
<td>22.9</td>
<td>0.14 ± 2.99*</td>
</tr>
<tr>
<td>¹⁴CO₂ exchange</td>
<td>wheat</td>
<td>6</td>
<td>20.5</td>
<td>-0.78 ± 1.52*</td>
</tr>
<tr>
<td></td>
<td>corn</td>
<td>6</td>
<td>21.0</td>
<td>-0.04 ± 2.90*</td>
</tr>
</tbody>
</table>

* 95% confidence limits of the mean.
made with the shoots in darkness, it seems reasonable to expect that the pool would not be larger during photosynthetic utilization of CO₂. If this is correct, the size of exchange pool is too small for it to be an important factor in gas exchange transients such as the post-illumination CO₂ burst (Chapter III, 15), or as a source of CO₂ released by acid. Nor could a CO₂ free-exchange pool of such small size significantly effect the CO₂ response of photosynthesis or act as an important CO₂ reservoir for photosynthesis.

The pH of juice expressed from wheat leaves is about 6.0, and that from corn leaves, consistently lower, about 5.5 (8). The formation of HCO₃⁻ from the hydration of CO₂ is low in this pH range. This is a further indication that a CO₂ free-exchange pool, if it is dependant on the carbon dioxide-water equilibrium, ought to be small.
LITERATURE CITED


CONCLUSIONS

1. The inhibitory effect of atmospheric O\textsubscript{2} on apparent photosynthesis of wheat is at least partly due to photorespiration and is increased by:
   
increasing O\textsubscript{2} concentration,
   increasing temperature and
   decreasing CO\textsubscript{2} concentrations.

Moderate to very high light intensities do not affect the per cent inhibition of apparent photosynthesis of wheat by O\textsubscript{2}. The effects of varying more than one of these factors are additive.

2. At temperatures below 30\degree C in saturating CO\textsubscript{2} concentrations, apparent photosynthesis of wheat is not inhibited by 20.8% O\textsubscript{2}. During part of the growing season in temperate conditions, the O\textsubscript{2} present in the air may not cause a significant decrease in photosynthetic productivity in wheat.

3. The inhibitory effect of atmospheric O\textsubscript{2} on photosynthesis of corn is not due to photorespiration. It differs from the effect of O\textsubscript{2} on wheat in that inhibition of photosynthesis occurs only at O\textsubscript{2} concentrations greater than 20.8% O\textsubscript{2}, and the degree of inhibition is not constant but increases with time of exposure to >99% O\textsubscript{2}.

4. The CO\textsubscript{2} exchange characteristics of wheat and corn also differ with respect to their CO\textsubscript{2} compensation concentrations and the effects of temperature, CO\textsubscript{2} concentration and light intensity on apparent photosynthesis. These differences are correlated with and may be the result of differences in photorespiration, photosynthetic carbon metabolism and leaf anatomy.
5. The post-illumination CO₂ burst of wheat is an extension of photorespiration into the dark period following illumination.

6. The CO₂ concentration response of the post-illumination burst of wheat indicates that photorespiration decreases with increasing CO₂ concentration in the same way that the size of the depression of apparent photosynthesis by 20.8% O₂ decreases with increasing CO₂ concentration.

7. The post-illumination CO₂ burst of Amaranthus edulis is not the result of an extension of photorespiration into the dark period following illumination, but it has some other cause.

8. The quantity of carbon within wheat and corn leaves which can exchange fully with atmospheric CO₂ is too small to cause the post-illumination CO₂ burst or to significantly affect the CO₂ concentration response of apparent photosynthesis.
Appendix I

CALCULATION OF THE CO₂ CONCENTRATION IN THE AIR ENTERING
THE IRGA DURING THE POST-ILLUMINATION CO₂ BURST

This calculation was developed to avoid the error in CO₂ concentration measurement caused by rapid fluctuations in CO₂ concentration in the air stream passing through the IRGA sample cylinder. For this calculation to be used, the initial CO₂ concentration in all parts of the sample cylinder must be known. In the present case, the CO₂ concentration was initially similar throughout the sample cylinder and was equivalent to the IRGA reading.

Let the air stream entering the IRGA sample cylinder be divided into a series of 10 ml. elements. At the air flow rate of 1.0 l./min., 0.6 sec. are required for each element to enter the sample cylinder. Let C be the CO₂ concentration of an element just entering the sample cylinder. It is assumed that as the element passes through the sample cylinder, the relative response, r, of the IRGA to C is the same as the IRGA response to a pulse injection of CO₂ into the air stream entering the sample cylinder which was given in Figure III-2. Therefore the IRGA will detect the CO₂ contained in the element according to rC.

In Figure III-2, it can be seen that an element will exert an appreciable influence (1% or more of maximum response) on the IRGA up to 9.6 sec. after it began to enter the sample cylinder. Therefore, at any one time 9.6/0.6 = 16 elements will contribute to the IRGA reading. From this reasoning, the following equation was developed to relate the IRGA reading to the CO₂ present in the elements which
entered the sample cylinder during the preceding 9.6 sec.:

\[ 0.9223 \text{(IRGA)} = \frac{r_1 c_n + r_2 c_{n-1} + \cdots + r_{16} c_{n-15}}{10} \]

IRGA \text{\(n\)} represents the CO\textsubscript{2} concentration detected by the IRGA. In this equation, the factor 0.9223 can be considered to be a correction for uneven flow of air through the sample cylinder. When the pulse injection data of Figure III-2 is integrated with respect to time, the integral is almost 8% less than it would be if the air flow rate was equal in all parts of the sample cylinder.

The above equation can be rearranged to give directly the CO\textsubscript{2} concentration in the element which just entered the IRGA. Since \( r_1 = 1.0 \):

\[ c_n = 9.223 \text{(IRGA}_{n}) - (r_2 c_{n-1} + \cdots + r_{16} c_{n-15}) \]

This equation is then used in a serial calculation to give \( c_n \) at 0.6 sec. intervals during the post-illumination CO\textsubscript{2} burst.
Appendix II

CALCULATION OF THE ABSORPTION OF CO₂
BY 0.2 M PHOSPHATE BUFFERS

The absorption of CO₂ by pH 6.0 phosphate buffer at 27° C will be used as an example. Ideal gas laws are assumed throughout, and the pK of carbonic acid will be taken as 6.37¹. The concentration of CO₃⁻ is very small when the pH is less than 8, and may be neglected for these calculations.

The Bunsen solubility coefficient, which is the volume of CO₂, reduced to 0° C, dissolved in unit volume of water at t° C with a CO₂ pressure of 1 atm., is 0.718 for 27° C². This solubility coefficient includes all aqueous species of carbon as "dissolved CO₂". However, under the conditions in which the Bunsen coefficient is measured, the concentrations of species other than true dissolved CO₂ are not appreciable.

Therefore, when the partial pressure of CO₂ is 0.000382 atm., the concentration of dissolved CO₂ is:

$$\text{CO}_2 = 0.718 \times 0.000382 \times 300 = 0.30 \text{ μl. CO}_2/\text{ml. H}_2\text{O}$$

From the Henderson-Hasselback equation³:

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\[ \text{HCO}_3^- = \text{CO}_2 \times \text{antilog} (\text{pH} - \text{pK}) \]

Therefore, "Total CO\(_2\)" = \(\text{CO}_2 + \text{HCO}_3^-\)

= \(\text{CO}_2 + \text{CO}_2 \times \text{antilog} (\text{pH} - \text{pK})\)

= \(0.30 + 0.30 \times \text{antilog} (6.0 - 6.37)\)

= \(1.01 \mu\text{l. CO}_2/\text{ml. H}_2\text{O}\)

The absorption of CO\(_2\) by 0.2 M phosphate buffers at pH 7.75 and at other temperatures were calculated similarly and reported in Table IV-1.