Effect of Oxygen on Photosynthesis, Photorespiration and Respiration in Detached Leaves. II. Corn and other Monocotyledons[1]

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Summary. The effect of O_2 on the CO_2 exchange of detached leaves of corn (*Zea mays*), wheat (*Triticum vulgare*), oats (*Avena sativa*), barley (*Hordeum vulgare*), timothy (*Phleum pratense*) and cat-tail (*Typha angustifolia*) was measured with a Clark oxygen electrode and infrared carbon dioxide analysers in both open and closed systems.

Corn leaves did not produce CO_2 in the light at any O_2 concentration, as was shown by the zero CO_2 compensation point and the absence of a CO_2 burst in the first minute of darkness. The rate of photosynthesis was inhibited by O_2 and the inhibition was not completely reversible. On the other hand, the steady rate of respiration after a few minutes in the dark was not affected by O_2.

These results were interpreted as indicating the absence of any measurable respiration during photosynthesis. Twelve different varieties of corn studied all responded to O_2 in the same way.

The other 5 monocotyledons studied did produce CO_2 in the light. Moreover, the CO_2 compensation point increased linearly with O_2 indicating a stimulation of photorespiration.

The implications of the lack of photorespiration in studies of primary productivity are discussed.

In previous communications from this laboratory it was shown that part of the inhibition of apparent photosynthesis by O_2 in tobacco and soybean leaves was due to a stimulation of photorespiration which differed from dark respiration (1, 3, 6). However, in addition to stimulating photorespiration, O_2 also had a second effect, which was attributed to a direct inhibition of photosynthesis.

This second effect can be studied directly in a plant which does not produce CO_2 in the light (i.e. which has no photorespiration). Corn is such a plant since, in air, young corn leaves lack both a measurable CO_2 compensation point and a measurable CO_2 burst i.e. the initial high rate of CO_2 production during the first minute of darkness following a light period (2, 6). This means that either there is no CO_2 produced by leaves of this species in the light, or the CO_2 produced is re-utilized before it can escape into the atmosphere. If this latter explanation is correct, then increasing the O_2 concentration from 1 to 100 % would increase the rate of CO_2 production in the light and decrease the rate of photosynthesis, as shown in the preceding paper for soybean (1). This should result in an increased CO_2 compensation point and an increase in the magnitude of the CO_2 burst (assuming that this burst represents the overshoot of photorespiration in the dark).

On the other hand, if the first explanation is true (i.e. that there is no CO_2 produced in the light), no increase in the CO_2 compensation point would be observed even with increasing O_2 concentration. If this is true, any effects of O_2 on the gas exchange in the light can be attributed to a direct effect on photosynthesis.

The present investigation was designed to answer this question by studying the effect of O_2 on the CO_2 exchange in several varieties of corn and also in a number of other species of monocotyledons.

Materials and Methods

Corn. Twelve varieties of *Zea mays* L. were used: Co 170; Co 171; Co 172; Co 173; Co 109; Co 106; Co 52; CMS 106; $W_{33}G$; $W_{34}G$; Golden Bantam; Golden Sunshine.

Seeds were soaked over night in aerated tap water and then planted in pots of vermiculite. The plants were grown in a growth chamber as described in the preceding paper for soybean (1). Leaves were used 2 weeks from the date of planting.

Other Monocotyledons. Seeds of barley, *Hordeum vulgare* L., wheat, *Triticum vulgare* L., oats, *Avena sativa* L., and timothy, *Phleum pratense* L., were soaked overnight in aerated tap water and then planted in pots of vermiculite. Plants were grown in a greenhouse and watered daily with tap water. Leaves used for the experiments were detached from plants 3 to 4 weeks from the date of planting.

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Cat-tail, *Typha angustifolia* L., rhizomes with shoots 15 to 20 cm long were dug on May 12, 1963, from a marshy area on the shore of the St. Lawrence River. These were transferred to the greenhouse and potted in soil which was kept wet. Leaves were used 10 days after transplanting.

Two methods were used to measure CO_2 exchange. A closed system apparatus, incorporating a Beckman Infrared CO_2 analyser (IRCA) and a Clark oxygen electrode, was used to determine CO_2 compensation points and the CO_2 burst. An open system, which included a second IRCA, was used to determine the steady rates of CO_2 absorption in the light and production in the dark. These 2 systems are described in detail in the preceding paper (1).

Experimental Results

Experiment 1. The object of this experiment was to study the CO_2 exchange of corn leaves (var. Golden Bantam) at various O_2 and CO_2 concentrations and at various light intensities.

Three samples, each consisting of 12 leaves (total fr wt of each sample approximately 4 g), were studied separately. The procedure was the same as that outlined for soybean in the previous paper (1). For the open system measurements, the average CO_2 concentration of the gas stream entering the plant chamber for samples 1 and 2 was 281 ppm, and for sample 3 was 135 ppm.

CO_2 Compensation Point. The CO_2 compensation points were determined in the closed system at each of several O_2 concentrations varying between 1 and 100 % O_2. Figure 1 shows a typical tracing obtained from one such measurement. When the light was turned on, the CO_2 concentration in the system was reduced to zero. When the light was turned off, there was no CO_2 produced for at least 1 minute, after which time the concentration increased steadily until the light was turned on again. Similar tracings were obtained at all O_2 concentrations studied. Thus, the CO_2 compensation point for corn leaves was zero even at 100 % O_2.

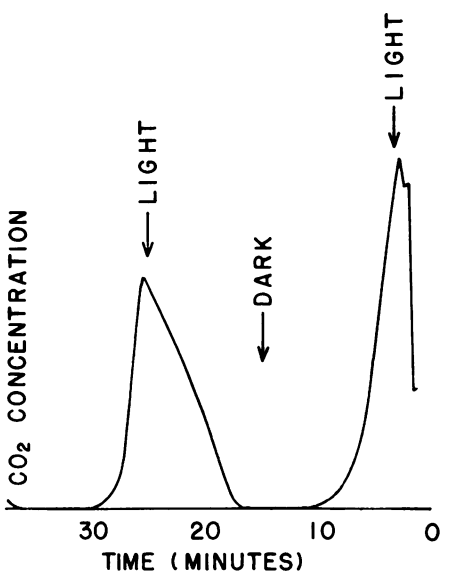

FIG. 1. A typical tracing from a CO_2 compensation point determination of detached corn leaves. Graph to be read from right to left.

CO_2 Burst. Figure 2 shows the rates of CO_2 production for leaf sample 3 during the first 5 minutes of darkness following 10-minute light periods at 1000 ft-c and at 1, 21, or 100 % O_2. There was no CO_2 produced for the first few seconds at any O_2 concentration. The duration of this period in which there was no CO_2 production varied inversely with O_2 concentration: 60 seconds at 100 %, 80 seconds at 21 % and 100 seconds at 1 % O_2. After these time periods, the rate of CO_2 production gradually increased to a more or less steady value at 300 seconds. The final rates obtained after 300 seconds in the dark were about the same at 21 and 100 % O_2 but somewhat lower at 1 %. Thus, there was no CO_2 burst immediately after the light was turned off.

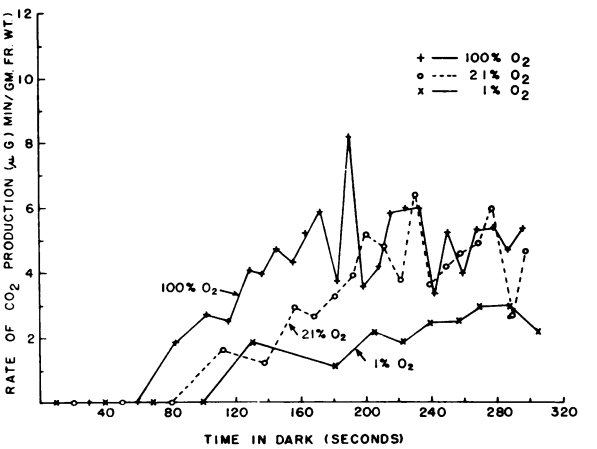

FIG. 2. Effect of O_2 on the CO_2 burst in detached corn leaves after 10 minutes of previous illumination at 1000 ft-c and at 1, 21, or 100 % O_2.

Photosynthesis and Respiration. Since there was neither a CO_2 compensation point nor a CO_2 burst even at 100 % O_2, it is concluded that leaves of this variety of corn do not produce CO_2 in the light. Photosynthesis, then, can be studied without the complication of photorespiration.

Figure 3 shows the effect of O_2 on the steady rate of photosynthesis at 600 and 1000 ft-c, and also on the steady rate of respiration in the dark. Dark respiration was not affected by O_2, but photosynthesis was inhibited. The points are numbered in chronological order. The rate of photosynthesis obtained initially at 21 % O_2 (point 1) was always higher than the rate obtained at 21 % O_2 after high O_2 treatments (point II). Therefore, the inhibition was only partially reversible. Similar results were obtained for samples 2 and 3. Thus, under the conditions of

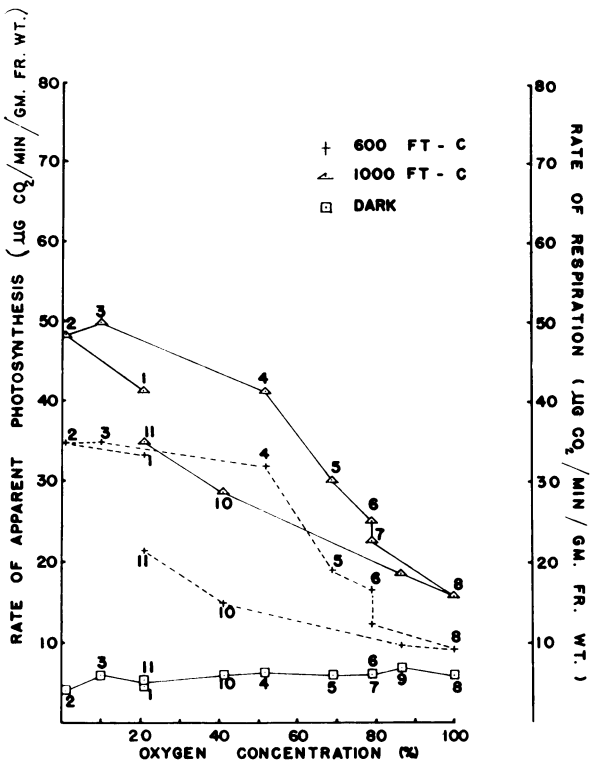

FIG. 3. Effect of O_2 on the CO_2 exchange in detached corn leaves. Points are numbered in chronological order.

this experiment, corn leaves lack photorespiration, and photosynthesis per se is inhibited by O_2.

Experiment 2. It was shown in experiment 1 that photorespiration is absent from 2-week-old corn leaves of the variety Golden Bantam. The object of experiment 2 was to determine whether this lack of photorespiration is restricted to the variety Golden Bantam, is characteristic of the species, *Zea mays*, or of monocotyledons in general. This was done by studying the effect of O_2 on the CO_2 compensation points of 12 different varieties of corn and of 5 other species of monocotyledons: wheat, oats, barely, timothy, and cat-tail.

Samples of experimental material, each of several comparable leaves, were placed in the closed system and subjected to a 10-minute light, 10-minute dark cycle at each of several successive O_2 concentrations ranging from 1 to 100 %. The first and the last cycle was always at 21 % O_2 in order to check whether the leaves were responding the same way at the beginning as at the end of a run. Light intensity was always 1000 ft-c.

For all 12 varieties of corn and at all O_2 concentrations studied, tracings similar to that shown in figure 1 were obtained. Therefore, all 12 varieties tested lack a mechanism for photorespiration.

For each of the other 5 species of monocotyledons, the CO_2 compensation point increased linearly with O_2 as was found previously for tobacco (6) and soybean (1). The linear regressions for these points are shown in table I.

Table I. *Effect of O_2 on the CO_2 Compensation Points of 5 Species of Monocotyledons*

Species	(a) Slope of linear* regression	(b) CO_2 compensation point at zero O_2 (ppm)
Wheat	1.78	0.3
Oats	1.50	4.2
Timothy	1.57	2.1
Barley	1.77	3.6
Cat-tail	1.69	8.8
Corn	0	0

* $y = ax + b$ where y = CO_2 compensation point (ppm), x = O_2 concentration (%), a = slope, b = y intercept.

Values which were less than 5 ppm were not significantly different from zero. Thus, the graphs for all species except cat-tail extrapolated to zero at zero O_2. The somewhat higher value obtained with cat-tail at 1 % O_2 probably reflects the higher percentage of nongreen tissues in the leaves of this species.

In conclusion, the lack of photorespiration seems to be an attribute of the species, *Zea mays*, whereas all other species of monocotyledons studied possess a mechanism for photorespiration which resembles that of soybean and tobacco in its response to O_2.

Discussion

It was shown previously that the depressing effect of O_2 on the apparent photosynthesis in leaves of tobacco and soybean has 2 components. One was due to a stimulation of photorespiration; the other was assumed to be a direct inhibition of photosynthesis (1, 6).

In experiment 1, photorespiration was shown to be absent from 2-week-old corn leaves. This meant that photosynthesis in these leaves could be studied directly without the complication of photorespiration. When this was done, photosynthesis per se was shown to be inhibited by O_2. However, this inhibition was only partially reversible within the time limits of the experiment. The reason for this irreversibility is not known but it may be the result of a photooxidation.

The close relationship between the CO_2 burst and photorespiration was suggested by the similarity of the effects of O_2 and light intensity on these 2 phenomena in tobacco and soybean (1, 3, 4, 5, 6), and by the absence of both a CO_2 burst and photorespiration in young corn leaves at 21 % O_2 (4). This close relationship was strenghtened by the results of experiment 1. Here, it was shown that young corn leaves lacked both a CO_2 burst and a CO_2 compensation point over a wide range of O_2 concentrations. This also lends support to the hypothesis that dark respiration is inhibited in the light.

The absence of photorespiration seems to be a unique characteristic of *Zea mays*. As shown in experiment 2, all varieties of corn tested lacked photorespiration, whereas all of the other species of monocotyledons studied were similar to tobacco and soybean. There are 2 reports that sugarcane is similar to corn in that it also lacks a CO_2 compensation point (Constance E. Hartt and Israel Zelitch, personal communications).

It is interesting to note that both corn and sugarcane are extremely high yield crop plants, and to speculate that the explanation of this high productivity may rest in the characteristic lack of photorespiration by these 2 species. That photorespiration (or its absence) might affect the magnitude of primary productivity is demonstrated in table II. Thus,

Table II. *A Comparison of the Primary Productivity of Corn and Soybean*

Data derived for corn from experiment 1 of this paper and for soybean from the preceding paper (1). Net carbon gained in 24 hours if the light intensity were 1000 ft-c for 16 hours (μg c/g fr wt).

% O_2	1	21	100
Corn	12,000	10,100	3600
Soybean	7100	4600	750
Ratio $\frac{\text{corn}}{\text{soybean}}$	1.7	2.2	4.8

if the rates of CO_2 exchange obtained under the experimental conditions presented in this and in the previous paper remained constant for 24 hours, corn leaves would assimilate about twice as much carbon per g fresh weight as the soybean leaves at 1 and 21 % O_2 and about 5 times as much at 100 % O_2.

Further information about the effect of O_2 on photosynthesis could be obtained by comparing the $^{14}CO_2$-labeling patterns of the products of photosynthesis in leaves of corn and soybean. The results of such an investigation will be reported elsewhere.

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