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Photorespiratory Phenomena in Maize

OXYGEN UPTAKE, ISOTOPE DISCRIMINATION, AND CARBON DIOXIDE EFFLUX[1]

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R. J. VOLK AND W. A. JACKSON
Department of Soil Science, North Carolina State University, Raleigh, North Carolina 27607

ABSTRACT

Concurrent O_2 evolution, O_2 uptake, and CO_2 uptake by illuminated maize (*Zea mays*) leaves were measured using $^{13}CO_2$ and $^{18}O_2$. Considerable O_2 uptake occurred during active photosynthesis. At CO_2 compensation, O_2 uptake increased. Associated with this increase was a decrease in O_2 release such that a stoichiometric exchange of O_2 occurred. The rate of O_2 exchange at CO_2 compensation was directly related to O_2 concentration in the atmosphere at least up to 8% (v/v).

When illuminated maize leaves were exposed to saturating CO_2 concentrations containing approximately equal amounts of $^{12}CO_2$ and $^{13}CO_2$, the latter was taken up more rapidly, thus depressing the atom % ^{13}C in the atmosphere. Moreover, upon exposure to CO_2 containing 96 atom % ^{13}C, there occurred a directly measurable efflux of $^{12}CO_2$ from the leaves for at least 15 minutes. During this period an equimolar evolution of $^{16}O_2$ and uptake of $^{13}CO_2$ was observed. Thereafter, although the rate of $^{16}O_2$ evolution remained unchanged, the rate of $^{13}CO_2$ uptake declined markedly, suggesting continual ^{13}C enrichment of the photorespiratory substrate.

It is concluded that a finite photorespiratory process occurs in maize and that the CO_2 generated thereby is efficiently recycled. Recycling maintains the internal CO_2 concentration at a level difficult to detect by most photorespiratory assays.

Maize lacks most of the common external indices of photorespiration. Its CO_2 compensation concentration (Γ[2]) approaches zero at temperatures less than 30 C (28, 29, 42) and is unaffected by O_2 concentration (7, 10); it releases little or no CO_2 to CO_2-free air (8, 30) or even to CO_2-free oxygen (48); it fails to exhibit a CO_2 burst upon darkening (6, 10, 42); its net photosynthetic rate is not stimulated by lowering the ambient O_2 concentration from 21 to 2% (3, 5, 7, 14, 18); and it does not depress the specific radioactivity of ^{14}C during photosynthetic CO_2 fixation (15, 45). The absence of these external photorespiratory indices can be interpreted in two ways: maize either lacks photorespiration altogether, or it recycles photorespiratory CO_2 with considerable efficiency.

Several lines of evidence support the latter interpretation. Maize leaves do contain peroxisomes (41), the organelle commonly associated with photorespiration. In addition, illuminated maize leaves can synthesize the primary photorespiratory substrate, glycolate (46), and can metabolize it both in light and darkness (7, 22, 31, 37, 47). Finally, appreciable O_2 is taken up by maize leaves when illuminated at Γ (19).

In the present report we shall examine the O_2 exchange phenomenon (19) in greater detail and shall submit two additional types of evidence which indicate that maize does indeed have an active photorespiratory process.

MATERIALS AND METHODS

General Experimental Procedure. *Zea mays* varieties NC 222 × NC 83 and Golden Cross Bantam were grown in the greenhouse and field, respectively. In most experiments two or three tips (17 cm long) of mature leaves were excised under water and enclosed in a 940-cm^3 Plexiglas leaf chamber (43). After flushing the chamber with N_2 for 7 to 10 min, we injected predetermined amounts of argon (an internal standard), $^{18}O_2$, and $^{13}CO_2$. The contained gases were circulated at 50 cm^3 sec^{-1}, and periodically 0.25-cm^3 samples were introduced directly into a mass spectrometer for analysis. Illumination, provided by a 750-w projection lamp, was filtered through 10 cm of water and measured with a Weston model 756 illumination meter. The equipment provided air temperature control at ±0.5 C of the values indicated in the figure legends.

The light intensity, 500 to 600 ft-c, used in these experiments provided optimal conditions for measuring steady state rates. At higher intensities, CO_2 was depleted too rapidly for precise measurement. Meidner (28) has reported that the Γ of maize leaves was 4 μl/liter or less at 600 ft-c provided the temperature did not exceed 35 C. Since the air temperatures in our experiments ranged from 28 to 34 C, we consider the results reported herein to be typical of maize exhibiting low Γ values.

In some experiments the oxygen data have been corrected to include a minimal estimate of $^{16}O_2$ recycling (19, 20). These data have been labeled "O_2 uptake" and "O_2 release" without reference to the isotopic species measured. They are, of course, still minimal estimates of actual O_2 uptake and O_2 release (19, 20).

Direct Measurement of CO_2 Efflux. The primary objective of the final experiment (Figs. 7 and 8; Table I) was to expose an illuminated maize leaf to highly enriched $^{13}CO_2$ (96 atom % ^{13}C) and to measure at 30-sec intervals thereafter the quantity of $^{13}CO_2$ and of $^{12}CO_2$ in the chamber. To accomplish this, we constructed a rapid sampling device (Fig. 1) and used it in conjunction with a small chamber (153 cm^3) bearing side flask A (56 cm^3). The latter could be quickly incorporated into or isolated from the system by turning a four-way stopcock 90°.

Prior to the experiment a set of sample tubes (Fig. 1) was filled with boiled, distilled, N_2-saturated water. A maize leaf

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[2] Abbreviation: Γ: compensation point.

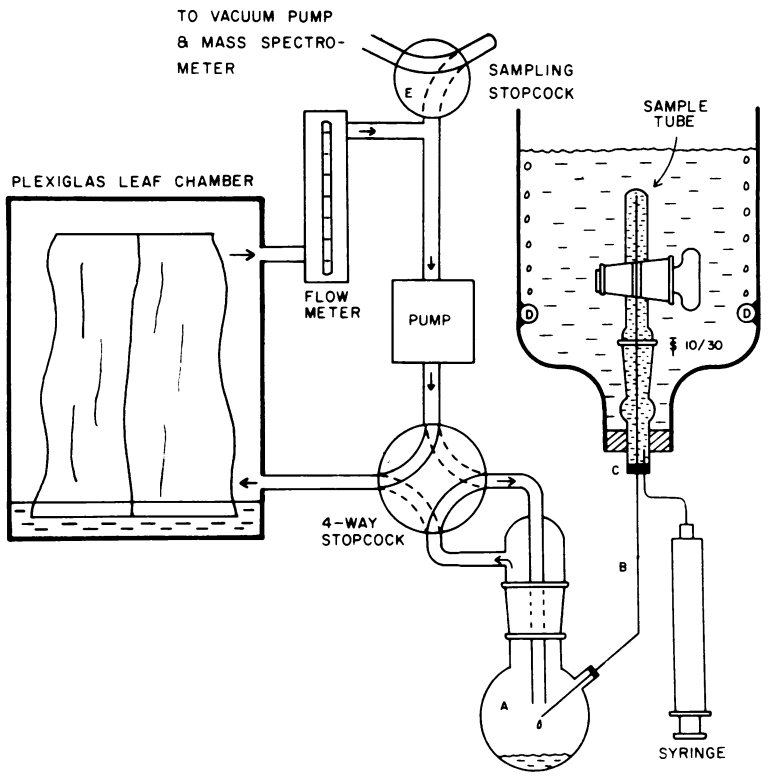

FIG. 1. Photosynthesis chamber and rapid sampling device. See text for details.

segment was enclosed in the chamber, and its net photosynthetic capacity was determined at 500 ft-c. After flushing of the apparatus with N_2, appropriate amounts of $^{18}O_2$ and $^{13}CO_2$ were injected into the chamber and side flask, respectively. Rapid exposure of the leaf to $^{13}CO_2$ was accomplished by incorporating the side flask into the system.

One set of samples was taken at 11- to 15-min intervals, introduced directly into the mass spectrometer through stopcock E, and analyzed at once. Concurrently, a second set of samples was taken at 30- to 120-sec intervals and stored in sample tubes for subsequent analysis. The latter set was taken by the following procedure. Side flask A was connected to a rapid sampling device via a length of flexible, stainless steel capillary tubing, B. This tubing extended through serum cap C and through the stopcock of a water-filled sample tube. To take a sample, water was withdrawn rapidly from the sample tube with the syringe, the water being replaced by gas from the side flask. After a sample was taken, the capillary tubing was partially withdrawn, the stopcock was closed, and the sample tube was replaced with another. As each sample tube was removed, the pressure was quickly equalized by water entering the side flask via capillary B. This prevented contamination of the following sample by gas which might have remained in the capillary. To avoid contamination of the sample with O_2, we continuously flushed the water surrounding the sample tube with N_2 through manifold D. Likewise, the sample tubes were stored under N_2-flushed water until analyzed.

RESULTS

Oxygen Uptake by Maize. The data in Figure 2 are typical of those of a number of experiments in which illuminated maize leaves were exposed to 97 atom % $^{18}O_2$ at oxygen concentrations ranging from 2 to 8% (v/v) and air temperatures from 28 to 34 C. Significant O_2 uptake during active CO_2 fixation was characteristic of maize leaves. Moreover, when photosynthesis had depleted CO_2 to Γ, the rate of O_2 uptake was accelerated. Concurrently, the rate of O_2 evolution declined. As a consequence, there occurred at Γ an equimolar exchange of oxygen.

Since in high Γ plants photorespiration is stimulated by O_2, it was of interest to examine the effects of increasing O_2 concentration on O_2 exchange by maize. To accomplish this, we sequentially exposed illuminated maize leaves at Γ to O_2 concentrations ranging from 0.14 to 7.82% (v/v). At each concentration, O_2 uptake and release were measured for 1.5 to 2.5 hr to provide a valid estimate of the steady state rate. This is illustrated in Figure 3, where 0.46% O_2 was used. The slopes of the linear regression lines were calculated by the least squares method. These slopes are plotted as a function of oxygen concentration in Figure 4 to portray the over-all increase in O_2 exchange as ambient O_2 increased to 8% (v/v).

Relative Uptake of $^{13}CO_2$ and $^{12}CO_2$ by Maize. The pattern of $^{12}CO_2$ and $^{13}CO_2$ uptake characteristic of illuminated maize at high CO_2 concentrations is illustrated in Figure 5. Based on their relative molar concentrations, $^{13}CO_2$ was taken up considerably faster than $^{12}CO_2$. This caused a continual decline in the atom % ^{13}C from 58 to less than 51 during the 5-hr photosynthetic period. An experiment of long duration was selected to document the intransient nature of this phenomenon.

To show more clearly the approach of each isotopic species to its compensation concentration, we have replotted the data beyond 5 hr on an expanded scale in Figure 6. It is apparent that $^{13}CO_2$ approached its compensation point more rapidly than did $^{12}CO_2$. Six hours after CO_2 injection the $^{13}CO_2$ concentration was essentially zero, within the limits of detection by the mass spectrometer. At this same time the $^{12}CO_2$ concentration was finite, measurable, and still declining. The precipitous drop in atom % ^{13}C is a natural consequence of the more rapid approach of $^{13}CO_2$ to its compensation concentration.

Carbon Dioxide Evolution by Illuminated Maize. After pretreatment with $^{12}CO_2$, an illuminated maize leaf was exposed to

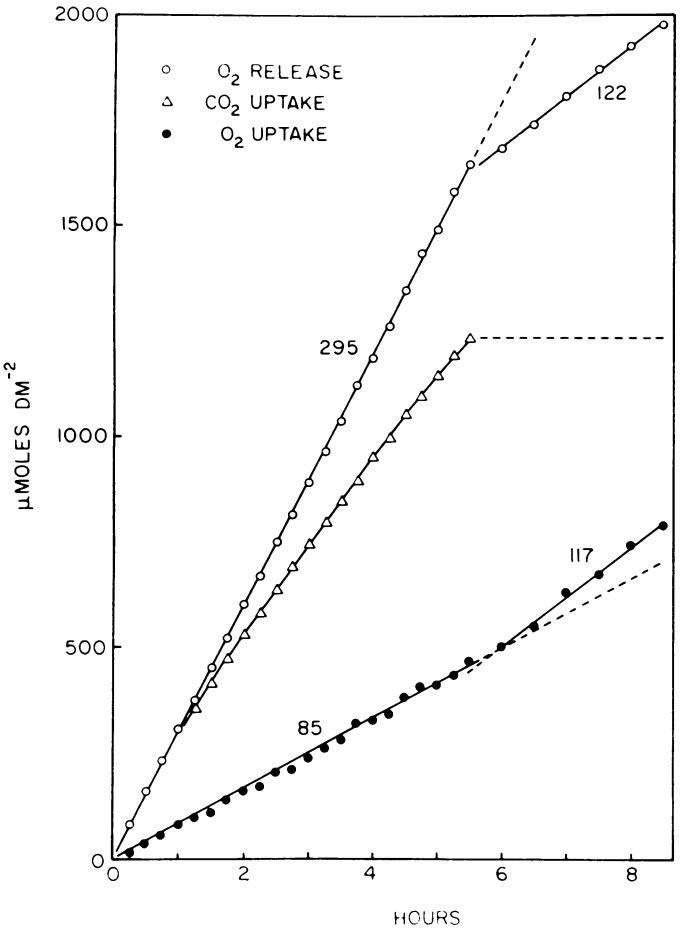

FIG. 2. Oxygen uptake and release during active CO_2 uptake and at CO_2 compensation by excised leaves of maize, NC 222 × NC 83, at 34 C air temperature and 600 ft-c. Initial CO_2 and O_2 concentrations were 1.9 and 5.0% (v/v), respectively. Rates of O_2 exchange are presented in µmoles dm^{-2} hr^{-1} adjacent to each regression line.

CO_2 containing 96 atom % ^{13}C. This saturated the photosynthetic system with $^{13}CO_2$, thus making possible the measurement of $^{12}CO_2$ exiting from the leaf during active photosynthesis. For a period of 15 min the $^{12}CO_2$ content of the chamber increased slowly, while photosynthesis rapidly depleted $^{13}CO_2$ (Fig. 7). The rates were 15 and 262 µmoles dm^{-2} hr^{-1}, respectively. It is of interest to note that $^{12}CO_2$ was evolved from the maize leaf against a $^{12}CO_2$ concentration over twice that in normal air, 2.8 µmoles $^{12}CO_2$ per chamber.

The over-all patterns of $^{13}CO_2$ uptake and $^{12}CO_2$ release are presented in Figure 8. For clarity, only four of the 16 data points taken during the first 15 min have been plotted. After the initial period of $^{12}CO_2$ release, there occurred an extended period during which both $^{12}CO_2$ and $^{13}CO_2$ were taken up by the leaf. The decline in atom % ^{13}C reflects the more rapid uptake of $^{13}CO_2$ than $^{12}CO_2$, relative to their molar concentrations.

In this experiment, $^{18}O_2$ was present both during exposure of the maize leaf to $^{13}CO_2$ and during the following period at Γ. The rates of O_2 exchange are listed in Table I along with those for CO_2. Little change occurred in $^{16}O_2$ evolution until Γ was reached; it then decreased markedly from 257 to 54 µmoles dm^{-2} hr^{-1}. Likewise, $^{18}O_2$ uptake remained constant as long as the CO_2 supply lasted. Thereafter it increased from 31 to 50 µmoles dm^{-2} hr^{-1}.

Of special interest is the high rate of $^{13}CO_2$ uptake during the first 15 min after exposure to the isotope. This rate, 262 µmoles dm^{-2} hr^{-1}, was considerably higher than the steady rate of net CO_2 fixation during phase I, 191 µmoles dm^{-2} hr^{-1}. Moreover, it exhibited a stoichiometric relationship to the rate of $^{16}O_2$ evolution. Although the latter remained constant, the rate of $^{13}CO_2$ uptake declined by 45% during the photosynthetic period. Since the decline was not offset by a comparable increase in $^{12}CO_2$ uptake, the $^{12}CO_2 + {}^{13}CO_2$ uptake/$^{16}O_2$ release quotient dropped from 0.96 to 0.63.

DISCUSSION

Illuminated maize leaves took up considerable O_2 both during active photosynthesis and at Γ (Fig. 2). In our experience the O_2 uptake rate at Γ has always exceeded that in the presence of abundant CO_2. Moreover, the uptake of O_2 at Γ was

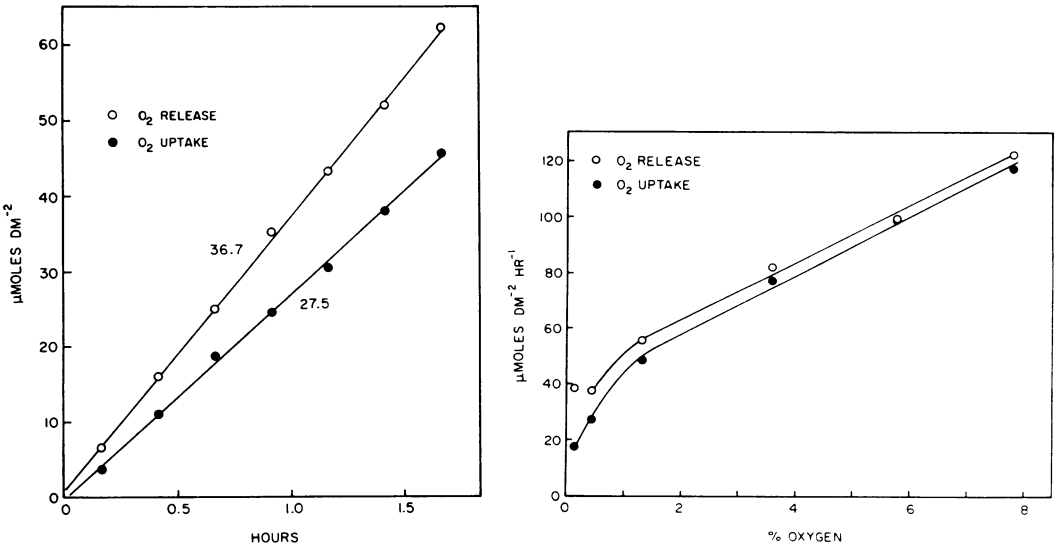

FIG. 3. Oxygen uptake and release at CO_2 compensation by excised leaves of maize, NC 222 × NC 83, at 34 C air temperature and 600 ft-c. Initial O_2 concentration was 0.46% (v/v). Rates of O_2 exchange in µmoles dm^{-2} hr^{-1} are presented adjacent to each regression line.

FIG. 4. Effect of O_2 concentration on O_2 uptake and O_2 evolution at CO_2 compensation by excised leaves of maize, NC 222 × NC 83, at 34 C air temperature and 600 ft-c.

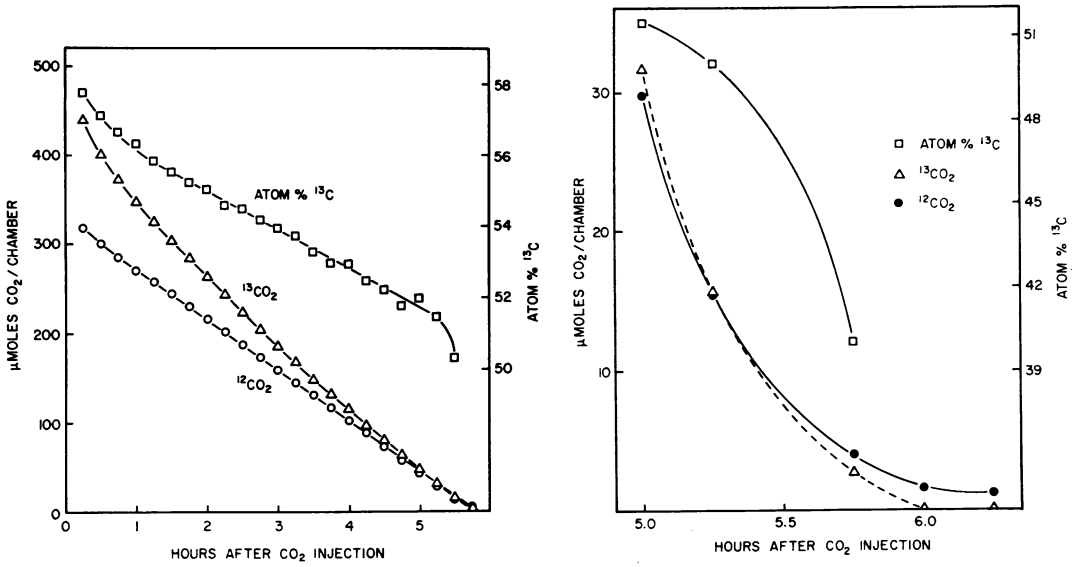

FIG. 5. Uptake of $^{13}CO_2$ and $^{12}CO_2$ by excised leaves of maize, NC 222 × NC 83, at 34 C air temperature and 600 ft-c. Initial $^{13}CO_2$, ^{12}CO and O_2 concentrations were 1.1, 0.8, and 5.0% (v/v), respectively.

FIG. 6. The approach of $^{12}CO_2$ and $^{13}CO_2$ to their compensation concentrations. Experimental conditions are as in Figure 5.

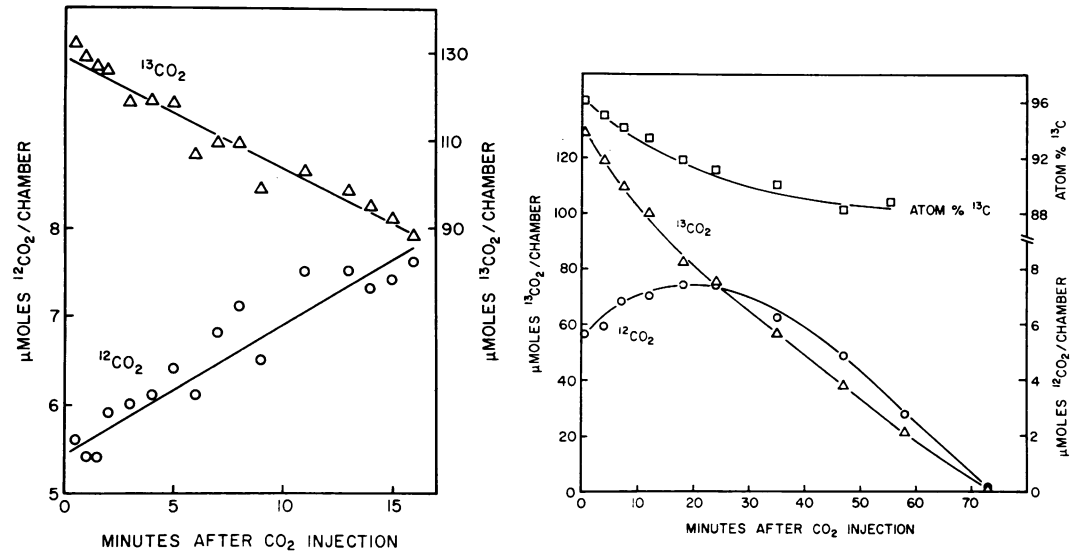

FIG. 7. Changes in concentration of $^{12}CO_2$ and $^{13}CO_2$ in a closed system during the first 15 min after exposure of an illuminated (500 ft-c) leaf of maize, Golden Cross Bantam, to 1.64% CO_2 containing 96 atom % ^{13}C. Air temperature was 28 C and initial O_2 concentration was 8.2% (v/v).

FIG. 8. Changes in concentration of $^{12}CO_2$ and $^{13}CO_2$ in a closed system containing an excised leaf of maize, Golden Cross Bantam, illuminated at 500 ft-c and exposed initially to 1.64% CO_2 and 8.2% O_2 (v/v) in a 28 C environment.

dependent on O_2 concentration at least up to 8% (Fig. 4). The biphasic nature of the dependency of O_2 exchange on ambient O_2 concentration (Fig. 4) suggests the possibility of two separate processes involving O_2 uptake, one of which is saturated at about 2% O_2 whereas the other is linearly related to O_2 concentration at least to 8% O_2.

The O_2 exchange phenomena in maize are similar to those reported previously for *Phaseolus vulgaris* and *Glycine max* (32, 35, 36), both of which are high Γ species. As in maize, O_2 uptake was stimulated upon transition to Γ and was directly related to O_2 concentration. It is of considerable interest that in *G. max* at least half of the O_2 exchange at Γ was associated with a concurrent release and refixation of CO_2 (32). This conclusion was derived from experiments in which O_2 exchange was measured first at Γ and then during a period in which the circulating gas stream was scrubbed with alkali to remove CO_2. Comparable experiments with maize have not been conducted. Hence, it is not yet possible to estimate what fraction of the O_2 exchange of maize is associated with CO_2 recycling.

It is conceivable that O_2 uptake by illuminated maize may be attributed in part to oxidation of photosynthetically reduced ferredoxin or other intermediates at the reducing side of photosystem I (20) without concomitant photorespiratory CO_2 generation. This can be considered a Mehler reaction (26, 27) in which O_2 substitutes for NADP as the terminal electron acceptor during photophosphorylation (11, 23). Such a reaction occurs in fragmented spinach chloroplasts in which an equimolar exchange of O_2 is accompanied by ATP synthesis (24, 33). Since the photophosphorylation occurs during transfer of electrons from H_2O to O_2, this process has been called

Table I. *Oxygen and Carbon Dioxide Exchange by Illuminated Maize*

Rates of $^{12}CO_2$ and $^{13}CO_2$ uptake and release (negative sign), $^{16}O_2$ release, and $^{18}O_2$ uptake by an excised leaf (0.57 dm²) of maize, Golden Cross Bantam, during pretreatment with $^{12}CO_2$ (phase I), after exposure to CO_2 containing 96 atom % ^{13}C (phase II), and at CO_2 compensation (phase III). Illumination was 500 ft-c and air temperature was 28 C.

Phase	Time after $^{13}CO_2$ Injection	Average % CO_2, v/v	Average % O_2, v/v	$^{12}CO_2$ Uptake	$^{13}CO_2$ Uptake	$^{16}O_2$ Release	$^{18}O_2$ Uptake
	min			*μmoles dm⁻² hr⁻¹*			
I	−60–0	1.36	2.0	191	[1]	245	[1]
II	0–15	1.64	8.4	−15.0	262	257	...
	15–43	1.14	9.1	−5.8 to 12.3	196	257	31
	43–69	0.80	9.8	19.2	142	257	31
III	69–155		10.2	...	...	54	50

[1] Isotope not present.

"oxygen-linked noncyclic photophosphorylation" (1). Its importance may lie in providing sufficient ATP, in addition to that produced during NADP synthesis, to convert CO_2 to the level of carbohydrate (13, 16, 36).

Heber (13) has proposed that the relative transfer of electrons to NADP and O_2 during photosynthesis is regulated by the NADPH/NADP ratio. As this ratio is increased, by a shortage of either ATP or CO_2, additional ATP is synthesized by electron transfer to O_2. Thus, electron transfer to O_2 likely occurs only when NADP is limiting. The higher affinity of reduced ferredoxin for NADP than for O_2 (1) lends credence to this concept.

If the product of O_2 uptake by maize is H_2O_2, as in the Mehler reaction (26), then a mechanism must exist for H_2O_2 removal. The process of photorespiration may play a dominant role in this mechanism. Coombs and Whittingham (4) have suggested that the H_2O_2 produced by transfer of electrons from reduced ferredoxin to O_2 oxidizes a two carbon precursor of glycolate. Alternatively, Ogren and Bowes (34) have proposed that O_2 concurrently inhibits photosynthesis and stimulates photorespiration by oxidation of ribulose diphosphate to 3-phosphoglycerate and phosphoglycolate (see Ref. 12). Both proposals indicate that the production of glycolate, a photorespiratory substrate, is directly linked to O_2 uptake by the chloroplast. Subsequent metabolism of glycolate to serine, via glyoxylate and glycine, is considered to be the source of photorespiratory CO_2 (22). To the extent that these reactions occur in maize, O_2 uptake may indeed reflect photorespiratory activity. If so, the lack of external indices of photorespiration must be attributed to a very efficient recycling of photorespiratory CO_2. This likely occurs in part through the unique cellular arrangement (17, 38) characteristic of maize and other low Γ plants. The presence of high concentrations of phosphopyruvate carboxylase in the mesophyll cells of maize (2, 39, 40) and the strong affinity of this enzyme for CO_2 (9, 25, 44) suggest that these cells are able to maintain their internal CO_2 concentration near zero. Under these conditions, most methods for detecting photorespiration would indicate its absence, for they depend on the maintenance of an internal CO_2 level significantly above zero. A low internal CO_2 level could account for the nearly equimolar uptake of $^{14}CO_2$ and $^{12}CO_2$ by maize exposed to low (atmospheric) CO_2 levels (15, 45). Under these conditions the diffusion of each CO_2 species would be directly proportional to its atmospheric concentration if its internal concentration were essentially nil.

Accepting this as a working hypothesis, we reasoned that if the photosynthetic capacity of maize were saturated by exposure to a high level of CO_2 containing appreciable ^{13}C, photorespiration, if active, should significantly increase the internal $^{12}CO_2/^{13}CO_2$ ratio relative to that in the atmosphere. The resultant decrease in the $^{12}CO_2$ concentration gradient would depress $^{12}CO_2$ diffusion into the leaf compared to $^{13}CO_2$, thereby lowering the atom % ^{13}C of the atmospheric CO_2 during photosynthesis. This did in fact occur (Fig. 5), and the change in atom % ^{13}C diminished with time, probably reflecting increasing enrichment of the photorespiratory substrate with ^{13}C. However, the continued decline in atom % ^{13}C throughout the 5-hr period and the slower approach of $^{12}CO_2$ to its compensation concentration (Fig. 6) both indicate that a considerable fraction of the photorespiratory substrate was derived from endogenous sources. An alternate explanation, the existence of a large pool of glycolate, seems unlikely, since glycolate accumulates in higher plants only in the presence of an inhibitor of glycolic acid oxidase (46).

In experiments of the type reported in Figure 5, both $^{12}CO_2$ and $^{13}CO_2$ diffused into the leaf because both were present in high concentration. Assuming that the slower rate of $^{12}CO_2$ uptake truly reflects an active photorespiratory process, we hypothesized that an actual $^{12}CO_2$ efflux from the leaf might be measured during active photosynthesis if a leaf were exposed rapidly to nearly pure $^{13}CO_2$. Under these conditions the photosynthetic capacity would be saturated with $^{13}CO_2$, and photorespiration would increase the internal $^{12}CO_2$ concentration. To the extent that this concentration exceeded that in the atmosphere, $^{12}CO_2$ diffusion from the leaf would occur.

Carbon dioxide containing 96 atom % ^{13}C was utilized in the experiment reported in Figures 7 and 8. Although the $^{12}CO_2$ concentration was over twice that found in air, $^{12}CO_2$ diffused from the leaf at a measurable rate for 15 min. After this time the $^{12}CO_2/^{13}CO_2$ ratio had so increased that both species diffused into the leaf. Enrichment of the photorespiratory substrate with ^{13}C likely accelerated this occurrence.

The physical evolution of CO_2 from an illuminated maize leaf during photosynthesis provides conclusive evidence that a photorespiratory process does exist in this species. It may reflect CO_2 release via one or several pathways, such as the glycolate pathway, the citric acid cycle, or the decarboxylation of malate, a possible CO_2 carrier from mesophyll to bundle sheath cells (6, 21). A valid estimate of the over-all rate of CO_2 release cannot yet be made, for the extent of internal recycling is unknown. Thus the value reported in Table I, 15 μmoles of CO_2 dm^{-2} hr^{-1}, must be considered to be minimal under the conditions employed. Perhaps the rate of $^{18}O_2$ uptake measured during the latter part of phase II, 30 μmoles dm^{-2} hr^{-1}, provides a more realistic estimate of photorespiratory activity. However, it should be emphasized that a stoichiometry between O_2 uptake and CO_2 generation has not yet been demonstrated for illuminated maize.

Additional insight can be gained by an examination of the oxygen exchange data (Table I). The constancy of $^{16}O_2$ evolution attests to the constancy of noncyclic electron transport, *i.e.*, the generation of reductant. In contrast, the over-all uptake of CO_2 from the atmosphere declined significantly during the course of the experiment. That the biochemical capacity of the leaf was sufficient to utilize all the reductant for CO_2 fixation is evident from the stoichiometry of $^{16}O_2$ release and $^{13}CO_2$ uptake during the first 15 min of phase II. Thereafter, it appeared that internal recycling of $^{13}CO_2$ depressed $^{13}CO_2$ uptake from the atmosphere. Note that the increase in $^{12}CO_2$

uptake was much less than the decline in $^{13}CO_2$ uptake. Thus the total uptake of CO_2 from the atmosphere exhibited a substantial decline, 91 μmoles dm^{-2} hr^{-1}, providing yet another estimate of photorespiratory CO_2 production and recycling.

In summary, the oxygen-dependent O_2 uptake by maize, the discrimination against $^{12}CO_2$, and the demonstration of an actual $^{12}CO_2$ efflux during active photosynthesis lead us to conclude that there occurs in maize a finite photorespiratory process which is ordinarily masked by the high efficiency with which photorespiratory CO_2 is recycled.

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