C_4 Photosynthesis[1]

The CO_2-Concentrating Mechanism and Photorespiration

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Despite previous reports of no apparent photorespiration in C_4 plants based on measurements of gas exchange under 2 versus 21% O_2 at varying $[CO_2]$, photosynthesis in maize (*Zea mays*) shows a dual response to varying $[O_2]$. The maximum rate of photosynthesis in maize is dependent on O_2 (approximately 10%). This O_2 dependence is not related to stomatal conductance, because measurements were made at constant intercellular CO_2 concentration (C_i); it may be linked to respiration or pseudocyclic electron flow. At a given C_i, increasing $[O_2]$ above 10% inhibits both the rate of photosynthesis, measured under high light, and the maximum quantum yield, measured under limiting light (Φ_{CO_2}). The dual effect of O_2 is masked if measurements are made under only 2 versus 21% O_2. The inhibition of both photosynthesis and Φ_{CO_2} by O_2 (measured above 10% O_2) with decreasing C_i increases in a very similar manner, characteristically of O_2 inhibition due to photorespiration. There is a sharp increase in O_2 inhibition when the C_i decreases below 50 μbar of CO_2. Also, increasing temperature, which favors photorespiration, causes a decrease in Φ_{CO_2} under limiting CO_2 and 40% O_2. By comparing the degree of inhibition of photosynthesis in maize with that in the C_3 species wheat (*Triticum aestivum*) at varying C_i, the effectiveness of C_4 photosynthesis in concentrating CO_2 in the leaf was evaluated. Under high light, 30°C, and atmospheric levels of CO_2 (340 μbar), where there is little inhibition of photosynthesis in maize by O_2, the estimated level of CO_2 around ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in the bundle sheath compartment was 900 μbar, which is about 3 times higher than the value around Rubisco in mesophyll cells of wheat. A high $[CO_2]$ is maintained in the bundle sheath compartment in maize until C_i decreases below approximately 100 μbar. The results from these gas exchange measurements indicate that photorespiration occurs in maize but that the rate is low unless the intercellular $[CO_2]$ is severely limited by stress.

Rubisco is a bifunctional enzyme with competitive interactions between CO_2 as a substrate for RuBP carboxylase and O_2 as a substrate for RuBP oxygenase. Carboxylation of RuBP leads to photosynthesis, and oxygenation of RuBP leads to photorespiration. C_4 plants are thought to have little photorespiration due to the CO_2-concentrating mechanism of the C_4 cycle and a permeability barrier to diffusion of CO_2 out of the bundle sheath cells, where Rubisco is located exclu-

sively (Edwards and Walker, 1983; Hatch, 1987; Jenkins et al., 1989; Henderson et al., 1992). In these plants, atmospheric CO_2 is initially fixed into C_4 acids in the mesophyll cells. The C_4 acids diffuse to the bundle sheath cells, where they undergo decarboxylation, and the released CO_2 enters the C_3 pathway via RuBP carboxylase. It is well known that atmospheric levels of O_2 inhibit photosynthesis in C_3 plants but not in C_4 plants. This reversible inhibition of photosynthesis by O_2, known as the Warburg effect, is overcome by increasing $[CO_2]$ (Ogren, 1984).

Following studies published in the early 1970s, it became common practice to make comparisons between photosynthesis under atmospheric levels of O_2 (21%) and approximately 2% O_2 to assess the magnitude of apparent photorespiration, because it was found that exposure to an O_2-free atmosphere caused a decrease in stomatal conductance in some species (Akita and Moss, 1973). Little or no difference was found in the value of Γ, the rate of photosynthesis under high light, or the Φ_{CO_2} under limiting light in C_4 plants under 2 versus 21% O_2 (Edwards et al., 1985). Using these criteria, some authors concluded that photorespiration is not apparent in C_4 plants. On the other hand, switching from 2 to 21% O_2 causes a strong inhibition of photosynthesis, inhibition of the Φ_{CO_2}, and increase in Γ in C_3 plants (Chollet and Ogren, 1975; Ehleringer and Björkman, 1977; Ku and Edwards, 1978; Edwards and Walker, 1983; Edwards et al., 1985). However, the extent to which CO_2 is concentrated in the bundle sheath cells and photorespiration is suppressed during photosynthesis in C_4 plants is not known.

Some photorespiration might be expected in C_4 species, especially at low $[CO_2]$, which could limit the ability of the C_4 cycle to concentrate CO_2 in bundle sheath cells. In fact, there is considerable qualitative evidence that photorespiration occurs in C_4 plants, based on activities of photorespiratory enzymes (Ohnishi and Kanai, 1983; Ohnishi et al., 1985), experiments following incorporation of $^{14}CO_2$ and $^{18}O_2$ into metabolites formed as a consequence of photorespiration (Mahon et al., 1974; Servaites et al., 1978; Canvin, 1979; Furbank and Badger, 1982; Rumpho et al., 1984; De Veau

Abbreviations: A, CO_2 assimilation rate; C_i, intercellular CO_2 concentration; C_o, external CO_2 concentration; Φ_{CO_2}, quantum yield of CO_2 assimilation; Γ, CO_2 compensation point; RuBP, ribulose-1,5-bisphosphate; Θ_A, O_2 inhibition index for photosynthesis; $\Theta_{\Phi_{CO_2}}$, O_2 inhibition index for quantum yield of photosynthesis; VPD, water-vapor pressure deficit between leaf and atmospheric air.

and Burris, 1989), and measurement of true rates of O_2 evolution/apparent rates of CO_2 fixation under low CO_2 (Furbank and Badger, 1982). In studies with the C_4 plant maize (Zea mays), $^{14}CO_2$ and $^{18}O_2$ were incorporated into Gly and Ser of the glycolate pathway in increasing amounts with increasing O_2 (Mahon et al., 1974; Lawlor and Fock, 1978; De Veau and Burris, 1989), the Gly pool increased in the light under increasing levels of O_2 (Marek and Stewart, 1983), and under H_2O stress, where the supply of CO_2 is considered limiting because of stomatal closure, there was an increased percentage of labeling from $^{14}CO_2$ into Gly and Ser (Lawlor and Fock, 1978). Evidence for photorespiration was also found in the C_4 dicot Amaranthus graecizans, because the rate of photosynthesis, the Φ_{CO_2}, and the carboxylation efficiency in this species were progressively inhibited by increasing O_2 up to 80% at an external $[CO_2]$ of 310 μbar (Ku and Edwards, 1980).

The present study shows that O_2 has a dual effect on C_4 photosynthesis: an enhancement by moderate levels of O_2 and inhibition at higher levels of O_2, especially under low $[CO_2]$ conditions. Through analysis of the O_2 inhibition component, we evaluated the effectiveness of the CO_2-concentrating mechanism in the C_4 plant maize under various environmental conditions.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of maize (Zea mays) and wheat (Triticum aestivum) were germinated in a commercial soil containing peat moss, vermiculite, and sand (2:1:1) in pots 16 cm in diameter and 17.5 cm high. After 1 week, the seedlings were selected for uniform size. One to two maize plants and four to five wheat plants were maintained per pot. Plants were watered twice a day, once with H_2O and once with a nutrient solution (1 g L^{-1}, Peter's fertilizer; Grace-Sierra Horticulture Products Co., Milpitas, CA). In addition, maize plants were also supplemented with Fe-EDTA solution (0.29 g L^{-1}). Maize was cultivated in a growth chamber under a 16-h light (at 30°C with a VPD of 10–12 mbar of H_2O) and 8-h dark (at 18°C, VPD of 4–5 mbar) cycle. Wheat plants were cultivated in a growth chamber under a 16-h light (at 22°C with a VPD of 5–7 mbar) and 8-h dark (at 18°C with a VPD of 4–5 mbar) cycle. The PPFD on the plant canopy was 550 to 650 μmol quanta m^{-2} s^{-1}.

Gas-Exchange Measurements

A was measured on the fourth or fifth leaves from 3- to 4-week-old plants using an Analytical Development Co. IRGA (225-MK3) and a Bingham Interspace model BI-6-dp Computer Controller System or BI-2-dp Mini Cuvette Controller Manual System (Dai et al., 1992). This is operated as an open system in which a given gas mixture is passed through the sample cell (in line with the leaf enclosed in a cuvette) and the reference cell; the rate of CO_2 removal by photosynthesis was compensated for by a controlled rate of injection of CO_2 from a high CO_2 source. The leaf cuvette contained a dew point sensor for measuring humidity and a copper-constantan thermocouple for monitoring leaf temperature. A and C_i were directly calculated from gas-exchange measurements according to the method of von Caemmerer and Farquhar (1981).

The BI-2-dp manual controller was used to measure dark respiration. The leaf temperature was maintained at 30°C, and $[CO_2]$ was 300 to 345 μbar. Under different $[O_2]$ values, respiration was determined by measuring the differential in $[CO_2]$ between the sample (output from the leaf cuvette) and the reference gas. The rate of dark respiration was calculated according to the method of von Caemmerer and Farquhar (1981).

The Effect of O_2 on Photosynthesis under High Light

The effect of O_2 on photosynthesis under high light was measured at different C_i values using a computer-controlled system. With this system A and C_i were continuously displayed during the experiment. A constant C_i was maintained under varying levels of O_2 by controlling C_o and the flow rates. Usually, the C_i was controlled to within 5% of the desired level. Different O_2 and CO_2 concentrations were obtained by mixing N_2 gas, CO_2-free air (79% N_2 and 21% O_2), and 10,000 μbar of CO_2 balanced in N_2 through a BI-6-dp computerized controller. Depending on the desired C_i, the reference and span gases were prepared with a concentration difference of about 20 μbar. Measurements of photosynthesis were made under 1400 μmol quanta m^{-2} s^{-1} provided by a 1000-W metal halide lamp, 30°C leaf temperature, and a VPD of 6 to 10 mbar.

Measurement of Φ_{CO_2} under Limiting Light

The Φ_{CO_2} was measured under limiting light from the initial slope of the response of A versus absorbed PPFD (for data in Figs. 3–5). The BI-2-dp manual controller was used for mixing of gases. Depending on the photosynthetic rate, different concentrations of CO_2 were used for the high CO_2 source to compensate for CO_2 consumption during photosynthesis and to maintain C_i at the desired level. The VPD was maintained at 6 to 10 mbar by adjusting the flow rate through the cuvette containing the leaf. The light source was a lamp designed by Björkman (containing a 100-W tungsten-halogen bulb) (Walker, 1990), and the PPFD was varied using different neutral density filters or different numbers of layers of cheesecloth.

### Determining Leaf Absorption of PPFD

Light absorption by individual leaves used in the gas-exchange experiments was determined with an integrating sphere (10-cm diameter; Labsphere, North Sutton, NH). The light source was a Schott's lamp, and the detector was a Li-Cor quantum sensor, with modification of the meter to provide sensitivity over a scale of 0 to 0.3 μmol quanta m^{-2} s^{-1}. The light entering the sphere was measured with and without the leaf covering the port to determine transmittance. The light reflected from the leaf was measured by placing the leaf over a port on the opposite site of the sphere from the light source and by comparing with a reflectance calibration standard from Labsphere. The PPFDs used for reflectance and transmittance measurements were 10 and 150 μmol quanta m^{-2} s^{-1}, respectively.

RESULTS AND DISCUSSION

A Dual Effect of O_2 on Photosynthesis in Maize

As expected, there were no differences in A/C_i curves under atmospheric levels of O_2 (21%) versus 2% O_2 (results not shown). Γ, determined by the extrapolation method, was also similar between 2 versus 21% O_2 (approximately 3 μbar). These results with maize support numerous previous conclusions that photosynthesis in C_4 plants is not sensitive to atmospheric levels of O_2 (see introduction). However, when measurements were made over O_2 levels from 0 to 21%, there was a strong effect of $[O_2]$ on the rate of photosynthesis in maize either at 20 or 228 μbar C_i (Fig. 1). Photosynthesis was enhanced by O_2 (20–30%, depending on $[CO_2]$) and reached a maximum at 10% O_2, following which there was a decline in photosynthesis rate. The O_2 enhancement and O_2 inhibition are both due to effects at the biochemical rather than stomatal level, because measurements of A were made at a constant C_i.

The basis for the enhancement of photosynthesis by subatmospheric levels of O_2 in maize is not known. It may be due to an increased production of ATP for operating the C_4 cycle through pseudocyclic photophosphorylation (Huber
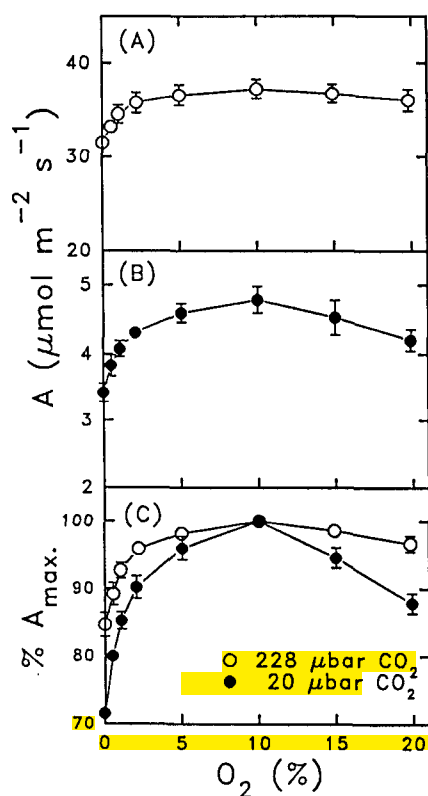

Figure 1. The responses of A in maize to $[O_2]$ at C_i of 20 μbar (B, ●) versus 228 μbar (A, O) CO_2. C, These results are shown as a percentage of the maximum value of A. The temperature was 30°C, the PPFD was 1400 μmol quanta m^{-2} s^{-1}, and the VPD was 5 ± 1 mbar. Two separate leaves of similar age were used for experiments at a given C_i. Each point is the mean of three replicates ± mean SD. Bars not seen are smaller than the size of symbols.

and Edwards, 1975) or to poising of the electron transport chain such that a proper balance of linear and cyclic electron transport is established to supply ATP for CO_2 fixation (Ziem-Hanck and Heber, 1980). Alternatively, it may be due to a requirement for mitochondrial respiration. There is some evidence that mitochondria must function (possibly to provide ATP for Suc synthesis) to achieve maximum rates of photosynthesis in C_3 plants (Kromer and Heldt, 1991). The degree of dependence of photosynthesis on $[O_2]$ (Fig. 1) may be an underestimate because some O_2 produced during photosynthesis in maize under an atmosphere of N_2 and CO_2 may be utilized in respiration (Oberhuber et al., 1993).

Further analyses were made of the O_2 inhibition of photosynthesis in maize. When one considers the O_2 inhibition of photosynthesis relative to photorespiration, it is the percentage inhibition rather than the effect of O_2 on the absolute rate of A that is most important. Expressed as a percentage of the maximum rate of A at 10% O_2, the rate of photosynthesis in maize is more sensitive to inhibition by higher O_2 levels at a C_i of 20 μbar than at a C_i of 228 μbar (Fig. 1C). The effect of a range of C_i levels on the O_2 inhibition of photosynthesis between 10 and 20% O_2 was subsequently determined; it is apparent that the degree of inhibition increased with decreasing C_i (Fig. 2A). This competitive interaction between CO_2 and O_2 suggests that the O_2 inhibition of photosynthesis in maize is due to Rubisco and photorespiration. The inhibition by O_2 is not likely due to pseudocyclic electron flow, because the Mehler reaction is thought not to proceed uncoupled and it functions no faster than the demand for ATP (Badger, 1985). It is also interesting to note that at a C_i of 228 μbar, which is in equilibrium with a C_o of 370 μbar, there was 4% inhibition of photosynthesis by increasing O_2 from 10 to 20%. This suggests that photorespiration occurs in C_4 plants such as maize under atmospheric conditions, although at a low level compared to that in C_3 plants. It is also clear that photorespiration occurs in maize, because there is an increased rate of incorporation of $^{18}O_2$ into the glycolate pathway with increasing $[O_2]$ from 2 to 40% under 350 μbar of CO_2 (De Veau and Burris, 1989). Yet, Furbank and Badger (1982) did not observe an increase in the rate of $^{18}O_2$ uptake during photosynthesis in maize with decreasing $[CO_2]$. As they explained, this could be due to maximum rates of photorespiration occurring under low CO_2 and maximum rates of pseudocyclic electron flow under high CO_2 such that the rate of O_2 uptake remains relatively constant under varying C_o. Also, there is the possibility of underestimating rates of O_2 uptake by mass spectrometric analysis if there is a degree of recycling of the $^{16}O_2$ evolved from H_2O during photosynthesis.

The degree of inhibition of photosynthesis by O_2 in maize was compared with that of the C_3 plant wheat. For maize, O_2 inhibition was calculated from measurements of photosynthesis between 10 and 20% O_2. Similar experiments were performed with wheat, in which case the maximum rate of photosynthesis, depending on the value of C_i, occurred at 1 to 2% O_2, and photosynthesis was inhibited linearly by higher $[O_2]$ values (data not shown). Thus, for wheat, O_2 inhibition of photosynthesis was calculated with increasing O_2 from 2 to 20% at varying C_i. The O_2 inhibition of photosynthesis in each species was calculated as the percentage inhibition of
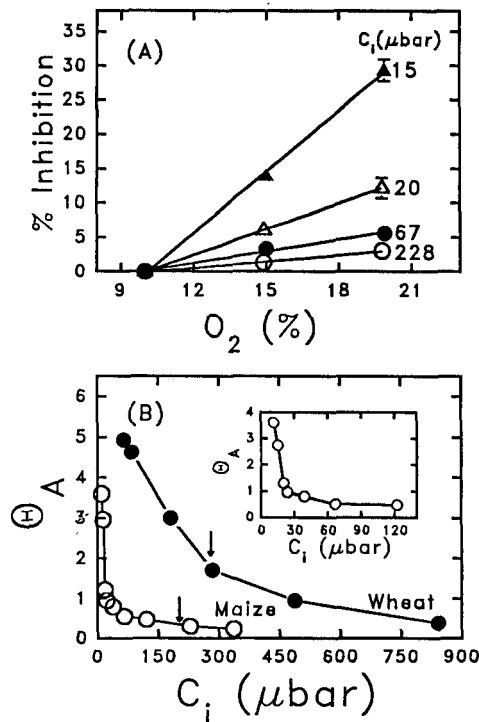
Figure 2. A, The percentage inhibition of photosynthesis by O_2 in maize at different C_i.

$$\% \text{ inhibition} = \frac{(A_{10\% \, O_2} - A_{20\% \, O_2})}{A_{10\% \, O_2}} \times 100,$$

where $A_{10\% \, O_2}$ and $A_{20\% \, O_2}$ equal the photosynthetic rate at 10 and 20% O_2, respectively. The temperature was 30°C, the PPFD was 1400 μmol quanta m^{-2} s^{-1}, and the VPD was 5 $\pm$ 1 mbar. B, The responses of Θ_A in maize and wheat to varying C_i. Θ_A was calculated (see "Results and Discussion") from the data in A plus other data (not shown) for maize and from similar experiments for wheat (data not shown). Arrows indicate the C_i values corresponding to atmospheric $[CO_2]$ of 340 μbar. Inset shows the enlarged maize response at low C_i. Different leaves, which were of similar age, were used for each experiment at a given C_i. Measurements were made from high to low O_2. Each point is mean of three replicates $\pm$ SD. Bars not seen are smaller than the size of symbols.

photosynthesis per percentage of increase in O_2 around the leaf, which is defined as Θ_A.

For maize

$$\Theta_A = \frac{(A_{10\% \, O_2} - A_{20\% \, O_2})/A_{10\% \, O_2}}{(20\% \, O_2 - 10\% \, O_2)} \times 100.$$

For wheat

$$\Theta_A = \frac{(A_{2\% \, O_2} - A_{20\% \, O_2})/A_{2\% \, O_2}}{(20\% \, O_2 - 2\% \, O_2)} \times 100.$$

Comparisons of Θ_A values show that the inhibition of photosynthesis by O_2 diminished much faster with increasing C_i in maize than in wheat (Fig. 2B). A value of 1 for Θ_A, indicating a 1% inhibition of photosynthesis per percentage

increase in O_2, occurred at a C_i of 25 μbar (C_o = 35 μbar) in maize, compared to a C_i value of 480 μbar (C_o = 605 μbar) in wheat. Under atmospheric conditions (C_o = 340 μbar, 30°C), wheat was about 5 times more sensitive to O_2, because the Θ_A value was 1.85 for wheat compared to 0.35 for maize (Fig. 2B, arrows).

O_2 Inhibition of the Maximum Φ_{CO_2} in Maize

In previous studies under atmospheric levels of CO_2, O_2 inhibited photosynthesis and Φ_{CO_2} in C_3 plants, but there was no difference in the rate of photosynthesis and the Φ_{CO_2} in C_4 plants, including maize, under 21 versus 2% O_2 (see introduction). However, the above results show that above 10% O_2 there is inhibition of the rate of photosynthesis in maize, particularly under low levels of CO_2. If, as these results suggest, photorespiration increases in maize under low CO_2, it should also be detectable from measurements of Φ_{CO_2} under limiting light.

Φ_{CO_2} was lower in maize when measured under 20% O_2 than under 10% O_2, and the degree of decrease in Φ_{CO_2} under 20% O_2 was greater under low C_i (23 μbar) than under high C_i (255 μbar) (Fig. 3). Measurements of Φ_{CO_2} were then made over a wide range of C_i levels for maize at 10 versus 20% O_2 and for wheat at 2 versus 20% O_2. In maize at 10% O_2, the quantum yield of CO_2 fixation decreased slightly at C_i values below 50 μbar, whereas at 20% O_2, there was a larger decrease in Φ_{CO_2} under low C_i (Fig. 4A). With wheat under 20% O_2, there was a much greater decrease in Φ_{CO_2} with decreasing C_i than in maize (Fig. 4B). At 2% O_2, Φ_{CO_2} was constant between 800 and 150 μbar but decreased rapidly
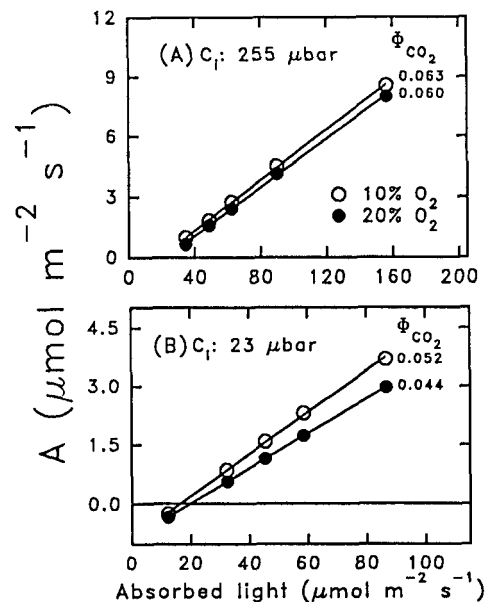
Figure 3. The responses of A in maize to absorbed light at 10% (O) versus 20% O_2 (●) and 255 (A) versus 23 μbar (B) of C_i. The temperature was 30°C. The Φ_{CO_2} was calculated from the slopes of the response curves. Separate leaves of similar age were used for each Φ_{CO_2} determination.
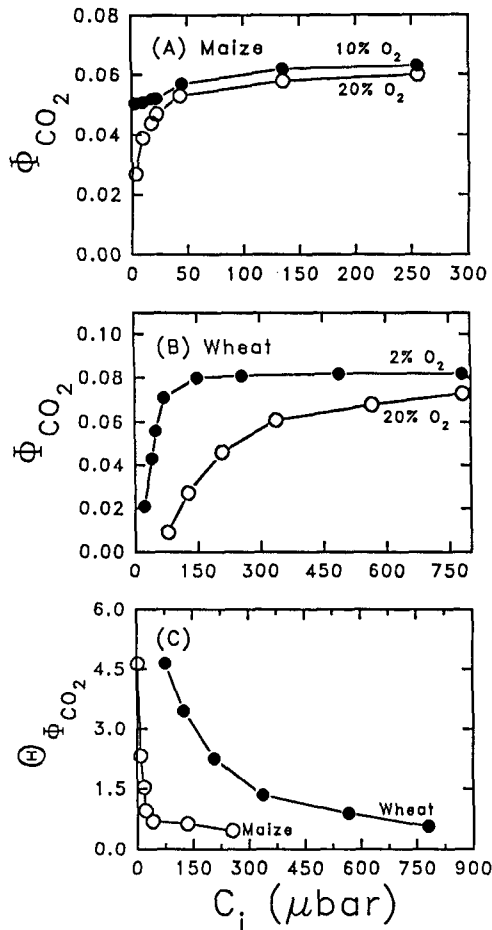
Figure 4. Φ_{CO_2} in maize under 10 and 20% O_2 (A) and wheat under 2 and 20% O_2 (B) at different values of C_i. The temperature was 30°C, and VPD was 5 ± 1 mbar. C, The response of $\Theta_{\Phi_{CO_2}}$ of maize and wheat to varying C_i. $\Theta_{\Phi_{CO_2}}$ was calculated from the data in A (maize) and B (wheat). Each value reported for Φ_{CO_2} represents an experiment with a separate leaf, using leaves of similar age. Some of the data points are averages of two replicates, which differed by less than 5%.

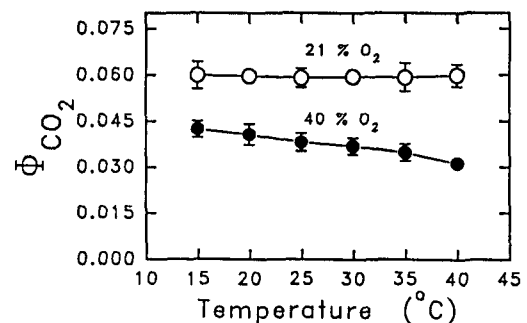at 21% O_2 and 30°C decreased under low C_i values (about 4–20 μbar).

Inhibition of the Φ_{CO_2} in Maize by Increasing Temperature under High O_2 and Low CO_2

The effect of temperature on Φ_{CO_2} in maize was determined under normal atmospheric conditions (21% O_2, C_i of 330 ± 20 μbar) versus conditions more favorable for photorespiration (40% O_2, C_i of 20 μbar) (Fig. 5). Under normal atmospheric conditions, Φ_{CO_2} remained constant over the temperature range used (15–40°C), which is in agreement with previous results with C_4 species, including maize (Ehleringer and Björkman, 1977; Ku and Edwards, 1978). However, under 40% O_2 and 20 μbar C_i, there was a linear decrease in Φ_{CO_2} with increasing leaf temperature from 15 to 40°C.

In C_3 plants under normal levels of CO_2 and O_2, there is inhibition of the Φ_{CO_2} with increasing temperature (Ehleringer and Björkman, 1977; Ku and Edwards, 1978). High temperature is known to be more favorable for photorespiration because of changes in the kinetic properties of Rubisco and the ratio of $[O_2]/[CO_2]$ with increasing temperature (Jordan and Ogren, 1984). This can explain the previously observed decrease in Φ_{CO_2} in C_3 plants with increasing temperature and the present decrease in maize under conditions that are particularly favorable for photorespiration. The results suggest that there is a temperature-dependent increase in photorespiration in maize when C_i is limiting, which is most likely under H_2O stress (Lawlor and Fock, 1978).

Estimation of the CO_2 Concentration in the Bundle Sheath Cells of Maize at Varying Levels of CO_2

If we assume that the O_2 inhibition of photosynthesis in maize, like that in wheat, is due to Rubisco and photorespiration, analyses of O_2 inhibition of photosynthesis (from Figs. 2B and 4C) can be used to predict the $[CO_2]$ in bundle sheath cells of maize at a given intercellular concentration around the mesophyll cells. The effect of increasing C_i on Θ_A of maize

below about 75 μbar CO_2 (Fig. 4B), which indicates the occurrence of photorespiration in this C_3 plant under 2% O_2 when CO_2 is also very limiting. It is also apparent from the results with wheat that a C_i of 800 μbar is not quite sufficient to suppress totally photorespiration under 20% O_2.

Using an approach similar to that for determining Θ_A, we determined the $\Theta_{\Phi_{CO_2}}$ for maize and wheat under different O_2 levels and varying C_i. $\Theta_{\Phi_{CO_2}}$, defined as the percentage inhibition of quantum yield per percentage increase in O_2, was calculated for maize (from the data in Fig. 4A) and wheat (from the data in Fig. 4B) at varying C_i (Fig. 4C). With increasing C_i from 3 to 25 μbar, $\Theta_{\Phi_{CO_2}}$ for maize decreased rapidly and then continued to decline slowly up to 250 μbar. With wheat, there was a steady decrease in $\Theta_{\Phi_{CO_2}}$ as C_i increased from 75 to approximately 800 μbar. The inhibition of Φ_{CO_2} by O_2 under low CO_2 provides further evidence for photorespiration in maize at low C_i. This is also supported by a report (Peisker and Diez, 1990) that Φ_{CO_2} in sugarcane (C_4)

Figure 5. Φ_{CO_2} in maize as a function of temperature under normal atmospheric conditions (O, 21% O_2, C_i of 330 ± 20 μbar) versus 40% O_2 and C_i of 20 μbar (●). Each value reported for Φ_{CO_2} represents an experiment with a separate leaf, using leaves of similar age. Each point is the mean ± SD of three replicates. SD bars that are not seen are smaller than the size of symbols.

and wheat measured under high light (Fig. 2B) was very similar to the effect of increasing C_i on Θ_{*CO_2} (Fig. 4C). For both maize and wheat, the C_i values indicate the $[CO_2]$ in the intercellular air space in the leaf around the mesophyll cells. However, the site of CO_2 fixation by Rubisco in the leaf is different in the two species, because the enzyme is located in the mesophyll cells in C_3 plants and in bundle sheath cells in C_4 plants. It is well known that there is a competitive interaction between O_2 and CO_2 for reaction with RuBP via Rubisco. The relative activity of carboxylase versus oxygenase is dependent on the relative concentrations of CO_2 and O_2, because $v_c/v_o = S_{rel} [CO_2]/[O_2]$ (Jordan and Ogren, 1984), where v_c is velocity of carboxylase, v_o is velocity of oxygenase, and S_{rel} is the relative specificity factor for the enzyme to function as a carboxylase versus an oxygenase. The degree of inhibition of photosynthesis by O_2 in maize or wheat depends on the relative concentration of CO_2 and O_2 at the site of Rubisco and on the value of S_{rel}. An earlier study has shown that the value of S_{rel} in maize is similar to that in C_3 plants (Jordan and Ogren, 1983). Although the $[O_2]$ may increase in bundle sheath cells of some C_4 species in which PSII activity is high (Hatch, 1987), this is not considered to occur in maize, because its bundle sheath chloroplasts are deficient in PSII activity (Edwards and Walker, 1983; Jenkins et al., 1989). If we assume that the O_2 in the atmosphere is in equilibrium with that in the bundle sheath cells in maize (Jenkins et al., 1989), for a given sensitivity of photosynthesis to O_2 the CO_2 concentration in maize bundle sheath cells would be similar to that in the mesophyll cells of wheat. Thus, the difference in O_2 sensitivity between maize and wheat at a given C_i around the mesophyll cells (Figs. 2B and 4C) should reflect differences in $[CO_2]$ at the site of Rubisco in the two species due to the CO_2-concentrating mechanism in maize.

Figure 6A is a plot of the estimated C_i for bundle sheath cells versus the C_i in the mesophyll cells of maize using the data from Figures 2B and 4C. The C_i in bundle sheath cells was predicted by assuming that at a given sensitivity of photosynthesis to O_2 (a given Θ_A or Θ_{*CO_2} value), the C_i around Rubisco in bundle sheath cells of maize will be the same as that around Rubisco in mesophyll cells in wheat. As shown in Figure 6A, there was good agreement between the two methods in estimating the C_i in bundle sheath cells. The estimated $[CO_2]$ in bundle sheath cells under normal atmospheric conditions was about 900 μbar, or 4.5-fold higher than that in the mesophyll cells of maize (C_i of 200 μbar around maize mesophyll cells at 21% O_2, 1400 μmol quanta m^{-2} s^{-1} and 30°C). If we consider that Rubisco uses free CO_2 as the carboxylation substrate, a C_i of 900 μbar in the bundle sheath cells corresponds to a concentration of 27 μM CO_2 in the aqueous phase at 30°C, which is lower than the values obtained from previous models. In an initial model, Furbank and Hatch (1987) predicted a value of 560 μM CO_2, but in a subsequent, more refined model, Jenkins et al. (1989) predicted a value for a typical C_4 plant of 70 μM (under normal air at a PPFD of 900 μmol m^{-2} s^{-1}). The value obtained in the model depends on various assumptions (e.g. pH of the cytoplasm in the bundle sheath cells, diffusive resistance to inorganic carbon across the bundle sheath cell), and differences may exist among C_4 species.
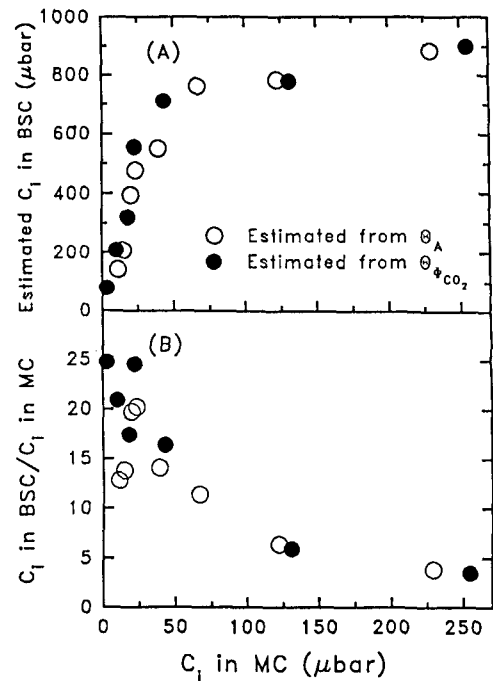
Figure 6. A, The relationship between estimated C_i in bundle sheath cells (BSC) and C_i in the mesophyll cells (MC) of maize. O, Based on measurements of Θ_A from Figure 2B. At a given C_i in maize mesophyll cells, the Θ_A value, which is dependent on the C_i at the site of Rubisco in bundle sheath cells, was compared with the corresponding value in wheat to predict the C_i in maize bundle sheath cells. ●, Based on measurements of Θ_{*CO_2} from Figure 4C. At a given C_i in maize mesophyll cells, the Θ_{*CO_2} value was compared with the corresponding value in wheat to predict the C_i in maize bundle sheath cells. B, The relationship between the ratio of the estimated C_i in bundle sheath cells/C_i in mesophyll cells versus the C_i in mesophyll cells. The ratios were calculated from the data in A.

In the present study, under atmospheric levels of CO_2, the estimated C_i in the bundle sheath compartment of maize (900 μbar) was 3.2-fold higher than the C_i around mesophyll cells where Rubisco is located in wheat (280 μbar). Based on these values, the estimated v_c/v_o ratio in maize bundle sheath cells would be about 8:1 (with S_{rel} of 70 [Jordan and Ogren, 1983, 1984], 27 μM CO_2, and 245 μM O_2 at 30°C), compared to an estimated v_c/v_o ratio of 2.5:1 for wheat mesophyll cells (with S_{rel} of 70, 8.4 μM CO_2, and 245 μM O_2). Although under atmospheric conditions of 340 μbar of CO_2, 1400 μmol quanta m^{-2} s^{-1}, and 30°C, the CO_2 level around Rubisco was about three times higher in maize than in wheat, the leaf diffusive conductance for CO_2 entry into the leaf (stomatal plus boundary layer) was lower in maize (391 mmol of H_2O m^{-2} s^{-1}) than in wheat (681 mmol of H_2O m^{-2} s^{-1}). These differences in leaf diffusive conductance and in supply of CO_2 to Rubisco allow maize to have a higher H_2O use efficiency than wheat (5.20 versus 2.14 μmol of CO_2 assimilated per mmol of H_2O transpired).

The ability of the C_4 cycle to concentrate CO_2 in the bundle sheath cells in relation to the C_i in mesophyll cells is shown in Figure 6B. The ratio of C_i in bundle sheath cells to C_i in
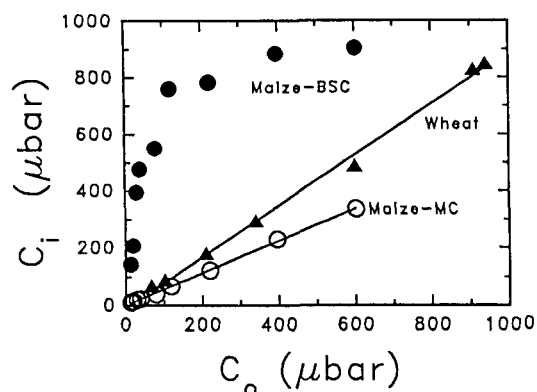
Figure 7. C$_i$ in equilibrium with wheat mesophyll cells, maize mesophyll cells (MC), and maize bundle sheath cells (BSC) with varying C$_o$. The data were calculated from the experiments of Figure 2.

mesophyll cells (i.e. fold concentration) increased exponentially from 4.5 at C$_i$ of 230 to 260 μbar to about 25 at C$_i$ below 25 μbar. This ability to concentrate CO$_2$ in the bundle sheath compartment may be particularly important when the supply of CO$_2$ to the mesophyll cells is limited by H$_2$O stress and the ensuing decreased stomatal conductance.

With decreasing C$_o$ around the leaf of wheat, there was a linear decrease in C$_i$ (Fig. 7), which is in agreement with other results for C$_3$ plants (Mott, 1990). Also, in maize, there was a linear decrease in C$_i$ around the mesophyll cells with decreasing external CO$_2$, but the slope was lower than in wheat. As C$_o$ decreased, the estimated change in C$_i$ in maize bundle sheath cells was hyperbolic, remaining high down to about 200 μbar and then decreasing rapidly below 50 μbar. It appears that at a C$_o$ of approximately 1000 μbar the C$_i$ around Rubisco in wheat would be similar to that in maize, in which case there would be no advantage in supplying CO$_2$ to Rubisco via the C$_4$ cycle. However, with decreasing C$_o$, the [CO$_2$] provided to Rubisco becomes progressively greater in maize compared to wheat, because of the CO$_2$-concentrating mechanism of C$_4$ photosynthesis.

In summary, these results provide information about photorespiration and the CO$_2$-concentrating mechanism in maize. Although maize is more effective than wheat in assimilating carbon under limiting CO$_2$, maize could have a significant level of photorespiration under stresses that restrict the supply of CO$_2$ to the photosynthetic tissue. Although O$_2$ inhibits C$_4$ photosynthesis, especially at low CO$_2$ concentrations, Γ remains low. This reflects an efficient refixation of photorespiratory CO$_2$. Because of its CO$_2$-concentrating mechanism, the degree of O$_2$ inhibition of photosynthesis and the associated photorespiration are much lower in maize than in wheat. Under atmospheric conditions, the inhibition of photosynthesis by O$_2$ in maize was about 20% of that in wheat, but as CO$_2$ decreases, maize has an even greater advantage due to the maintenance of a high level of CO$_2$ in maize bundle sheath cells (Figs. 2B and 7). The O$_2$ inhibition indices for photosynthesis and quantum yield of photosynthesis increased from 1.7 to 1.8 at a C$_i$ of 280 μbar to 4.6 to

4.8 at a C$_i$ of 75 μbar for wheat, but increased only slightly from 0.4 to between 0.5 and 0.6 under the respective C$_i$ levels for maize (Figs. 2B and 4C). Below Γ (50 μbar), there is net carbon loss in wheat, whereas in maize, there is not a strong increase in the inhibition of photosynthesis by O$_2$ and increased photorespiration until the C$_i$ around mesophyll cells decreases below 50 μbar (Figs. 2B and 4C). It has been suggested, based on geological evidence, that the major selective force for the evolution of C$_4$ photosynthesis was a decline in atmospheric levels of CO$_2$ (Ehleringer et al., 1991). Low levels of CO$_2$ in the atmosphere combined with H$_2$O stress and/or higher temperatures can limit the supply of CO$_2$ to photosynthetic tissue, which likely accounts for the adaptation of many C$_4$ plants to hot and arid conditions.

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