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C_4 Photosynthesis[1]

The Effects of Leaf Development on the CO_2-Concentrating Mechanism and Photorespiration in Maize

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The effect of O_2 on photosynthesis was determined in maize (*Zea mays*) leaves at different developmental stages. The optimum level of O_2 for maximum photosynthetic rates was lower in young and senescing tissues (2–5 kPa) than in mature tissue (9 kPa). Inhibition of photosynthesis by suboptimal levels of O_2 may be due to a requirement for functional mitochondria or to cyclic/pseudocyclic photophosphorylation in chloroplasts; inhibition by supraoptimal levels of O_2 is considered to be due to photorespiration. Analysis of a range of developmental stages (along the leaf blade and at different leaf ages and positions) showed that the degree of inhibition of photosynthesis by supraoptimal levels of O_2 increased rapidly once the ribulose-1,5-bisphosphate carboxylase/oxygenase and chlorophyll contents were below a critical level and was similar to that of C_3 plants. Tissue having a high sensitivity of photosynthesis to O_2 may be less effective in concentrating CO_2 in the bundle sheath cells due either to limited function of the C_4 cycle or to higher bundle sheath conductance to CO_2. An analysis based on the kinetic properties of ribulose-1,5-bisphosphate carboxylase/oxygenase was used to predict the maximum CO_2 level concentrated in bundle sheath cells at a given degree of inhibition of photosynthesis by supraoptimal levels of O_2.

In C_3 plants, O_2 inhibits photosynthesis by competing with CO_2 for reaction with RuBP in Rubisco catalysis, resulting in photorespiration (Ogren, 1984; Andrews and Lorimer, 1987). In C_4 plants the function of the specialized reactions of C_4 photosynthesis is to concentrate CO_2 in bundle sheath cells, where Rubisco is exclusively located. The resulting elevated ratio of CO_2 to O_2 suppresses the RuBP oxygenase reaction and reduces photorespiration, which accounts for many of the special physiological features typical of C_4 plants (e.g. high light-saturated photosynthetic rate, low Γ, limited O_2 inhibition of photosynthesis, and negligible apparent photorespiration) (Edwards and Walker, 1983; Hatch, 1987; Dai et al., 1993).

Under atmospheric levels of CO_2 (approximately 34 Pa) and high light, the $[CO_2]$ inside bundle sheath cells of the C_4 leaf is estimated from modeling to be approximately 200 Pa (Jenkins et al., 1989). From analyses of the O_2 inhibition of photosynthesis in mature leaves of maize (*Zea mays*; C_4) versus wheat (C_3), the calculated $[CO_2]$ inside bundle sheath cells during C_4 photosynthesis under atmospheric CO_2 and high light was suggested to be approximately 90 Pa, with a C_i around maize mesophyll cells of 20 Pa (Dai et al., 1993). Under such conditions there is little evidence for photorespiration in maize (measurements on the midsection of the fourth or fifth leaf from 3- to 4-week old plants), but as the C_i around maize mesophyll cells is decreased below 5 Pa by lowering the ambient CO_2 level, the degree of inhibition of photosynthesis by O_2 levels above the optimum increases dramatically (Dai et al., 1993). The mature leaves of maize exhibit an optimum of 9 kPa O_2 for maximum photosynthesis. In the present study, the effect of varying partial pressures of O_2 and CO_2 on photosynthesis was examined at different stages of leaf development in maize. The results show that very young and senescing tissues have substantial photorespiration under normal atmospheric conditions and require a lower O_2 partial pressure to reach maximum photosynthesis than mature tissue.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of maize (*Zea mays*) were germinated in a commercial soil containing peat moss, vermiculite, and sand in a 2:1:1 ratio in pots 16 cm in diameter and 17.5 cm high (usually four seeds per pot). After germination, one to two seedlings were retained per pot. Plants were watered twice a day and supplemented every 2 or 3 d with a nutrient solution (1 g L^{-1}, Peter's fertilizer, Grace-Sierra Horticultural Products Co., Milpitas, CA). In addition, the plants were supplemented with Fe-EDTA solution (0.29 g L^{-1}) once a week. Plants were cultivated in a growth chamber under a cycle of 16 h of light (at 30°C with a VPD of

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Abbreviations: **A**, CO_2 assimilation rate; C_i, intercellular CO_2 partial pressure; C_o, atmospheric CO_2 partial pressure; F'_m, maximal yield of fluorescence from a saturating flash of white light under steady-state photosynthesis; F_s, steady-state fluorescence under given environmental conditions; Γ, CO_2 compensation point; ϕ_{CO2} quantum yield of CO_2 assimilation; ϕ_{PSII}, quantum yield of PSII; R_d, rate of respiration in dark; RuBP, ribulose-1,5-bisphosphate; S_{rel}, relative specificity factor for Rubisco; Θ_A, O_2 inhibition index for photosynthesis (percentage inhibition of photosynthesis per kPa increase in O_2); VPD, water-vapor pressure difference between the leaf and atmospheric air.

1000–1200 Pa water) and 8 h of dark (at 18°C, VPD of 400–500 Pa). The PPFD on the plant canopy was 550 to 650 μmol quanta m^{-2} s^{-1}.

Gas-Exchange Measurements

A was measured on a section of the second to the sixth leaf of intact plants with an Analytical Development Co. (Hertfordshire, UK) IRGA (225-MK3) and a Bingham Interspace (Hyde Park, UT) model BI-6-dp Computer Controller System or BI-2-dp Mini Cuvette Manual Controller System (Dai et al., 1992, 1993). For each measurement, a 3-cm longitudinal section of a single intact leaf was sealed in the gas-exchange cuvette. This was operated as an open system where 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 (VPD maintained at 500 ± 100 Pa) and a copper-constantan thermocouple for monitoring leaf temperature (maintained at 30°C). **A** and C_i were directly calculated from gas-exchange measurements according to von Caemmerer and Farquhar (1981). For experiments on $\mathbf{A}/C_i$ curves, photosynthesis was determined under 19.5 kPa O_2 and varying [CO_2], using the BI-2-dp Manual Controller for gas mixing. Rates of respiration were determined by measuring the differential in [CO_2] between the sample (output from the leaf cuvette) and the reference gas.

The Effect of O_2 on Photosynthesis under High Light

The effect of O_2 on photosynthesis under high light (1300 μmol quanta m^{-2} s^{-1} provided by a 1000-W metal halide lamp) was measured at two given C_i partial pressures (approximately 16 versus 3 Pa) using a computer-controlled system. With this system, **A** and C_i were continuously displayed during the experiment. A given C_i level was maintained under varying levels of O_2 by controlling C_o and the flow rates. The range in external CO_2 partial pressures used to maintain C_i at approximately 16 Pa was normally between 32.5 and 35.5 Pa (i.e. near normal atmospheric levels of CO_2). Different O_2 and CO_2 partial pressures were obtained by mixing N_2 gas, CO_2-free air (73.3 kPa N_2 and 19.5 kPa O_2), and 500 Pa CO_2 balanced in 73.3 kPa N_2 and 19.5 kPa O_2 through a BI-6-dp computerized controller. Depending on the desired C_i, the reference and span gases were prepared with a partial pressure difference of about 2 Pa.

The O_2 inhibition of photosynthesis above the optimum partial pressure of O_2 was calculated as the percentage inhibition of photosynthesis per kPa increase in O_2 around the leaf, which gives a value for Θ_A (similar to Dai et al., 1993). For C_4 plants, such as maize, this was determined from data collected between 9.3 and 18.6 kPa O_2.

$$\Theta_A = \frac{(\mathbf{A}_{9.3\ \text{kPa}\ O_2} - \mathbf{A}_{18.6\ \text{kPa}\ O_2})/\mathbf{A}_{9.3\ \text{kPa}\ O_2}}{(18.6\ \text{kPa}\ O_2 - 9.3\ \text{kPa}\ O_2)} \times 100.$$

Photosynthetic Γ

Γ was determined at 30°C, 19.5 kPa O_2, and a PPFD of 1300 μmol m^{-2} s^{-1} by measuring **A** in response to low CO_2 partial pressure (0–5 Pa) and extrapolating the initial CO_2 response curve through the x axis (Ku et al., 1990).

Simultaneous Measurement of ϕ_{PSII} and ϕ_{CO_2}

For determination of ϕ_{PSII} fluorescence measurements were made with a PAM fluorometer (H. Walz, Effeltrich, Germany; model 101) simultaneously with the gas-exchange measurements (Krall and Edwards, 1990) at 700 to 1000 μmol quanta m^{-2} s^{-1} and 30°C. The probe was positioned at an angle (about 45°) above the cuvette and slightly to the side so as not to interfere with the incident light. During the experiment, F_s, the steady-state fluorescence under given environmental conditions, was monitored continuously and, for periodic determination of F'_m, saturating pulses (800 ms duration) of white light (about 9000 μmol quanta m^{-2} s^{-1}) were applied automatically at 300-s intervals by a PAM 103 trigger control unit. The data were collected with a Data Acquisition and Control Interface Board (Keithley Metrabyte, Taunton, MA). Locally designed software was used to record F'_m and F_s, to display the fluorescence signal for each saturating pulse of light, and to calculate values of ϕ_{PSII}, the quantum yield of PSII-dependent electron transport (calculated as $[F'_m - F_s]/F'_m$ in accordance with the method of Genty et al. [1989]). For determination of $\mathbf{A}/C_i$ responses, measurement began with the ambient level of CO_2 and then the level of CO_2 was varied in descending order of partial pressure. The quantum yield for photosynthesis, ϕ_{CO_2}, was calculated by dividing the apparent photosynthetic rate by the absorbed quanta.

Determining Leaf Absorptance of PPFD

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

Rubisco Activity and Chl Content Measurements

Samples were taken from leaves of three to five plants following measurement of photosynthesis or from leaf tissue at the same stage of development. Before harvesting

leaf material, the area of the leaf to be used was marked (following measurement of photosynthesis) and traced on paper and the area was measured with a portable area meter (LI-3000, Li-Cor). Leaf samples were collected in the light, excised, and divided in half. One-half was immersed and stored in liquid nitrogen until measurements were made on Rubisco activity on a Chl basis. The other half was immediately used for determining Chl on a leaf area basis. This was done by cutting the leaf sample into small strips and incubating them in a test tube containing 10 mL of 95% ethanol for Chl extraction. The samples were kept in the dark at room temperature until all the Chl was completely extracted (usually 2 d). Then, 10 to 20 μL of clear supernatant was removed and diluted with ethanol to 1 mL and the Chl was determined spectrophotometrically (Wintermans and De Mots, 1965). The measured Rubisco activity per unit Chl and Chl per unit leaf area were used to calculate Rubisco activity per leaf area.

For determination of Rubisco activity at the in vivo state of activation, approximately 0.5 g leaf tissue was ground under liquid nitrogen in a mortar and pestle with 10 volumes (w/v) of grinding buffer (100 mM Bicine, pH 8.0, 25 mM $MgCl_2$, 10 μM leupeptin, 1 mM PMSF, 1 mM EDTA-Na_2, 0.5% β-mercaptoethanol, and 12.5% glycerol) and 5% (w/w) insoluble PVP. The inclusion of glycerol, protease inhibitor, and reducing agents protected against loss of catalytic activity for up to 30 min at room temperature. Following centrifugation of the homogenate for 5 min at 14,000*g*, the supernatant was stored on ice. Rubisco activity was assayed by incorporation of [^{14}C]bicarbonate into acid-stable product. The reaction mixture contained 50 mM Tris-HCl at pH 8.0, 30 mg $MgCl_2$, 5 mM DTT, and 20 mM $NaH^{14}CO_3$. Assays (total volume of 200 μL) were performed at 30°C in glass scintillation vials. Twenty-five microliters of enzyme extract was added to the reaction mixture. The reaction was started by injection of 25 μL of 5 mM RuBP and stopped after 3 min by adding 100 μL 5 N HCl (assays over 3 min were not completely linear; activities obtained were always higher than the photosynthetic rates measured on the same tissue). The reaction mixture was allowed to dry by evaporation. The resulting residue was resuspended in 0.1 mL of deionized water, followed by addition of 10 mL of Bio-safe II biodegradable scintillation fluid (Research Products International Corp., Mount Prospect, IL). The sample was counted in a Beckman LS-7000 liquid scintillation counter; following corrections for background counts and counting efficiency, the mol of CO_2 fixed was determined as a measure of Rubisco activity.

RESULTS

Development of a Single Leaf

Experiments were first conducted on a 3-cm longitudinal section of the fifth leaf of maize as it progressed through development. Five days after the fifth leaf emerged, a 3-cm section near the base of the leaf was marked for measurement. This section had developed for approximately 2 d under direct light (d 2 of exposure). Subsequent measurements of photosynthesis were made on the same section as it progressed through development, e.g. d 8 means development for 8 d under direct light. By d 2 the tissue had almost reached its maximum longitudinal expansion, so measurements could essentially be made on the same 3-cm longitudinal section at subsequent stages of development. Days 2, 8, and 16 were taken as a reasonable representation of young, mature, and senescent tissues, respectively, based on changes in photosynthesis rate and Chl content per unit leaf area (Fig. 1; Table I).

Under normal CO_2 (C_i = 16 Pa, obtained with external CO_2 near atmospheric levels) and low CO_2 (C_i = 3 Pa) the rates in mature tissue (d 8) were much higher than in young (d 2) or senescent (d 16) tissue over a range of O_2 levels from 0 to 18.6 kPa (Fig. 1). However, at all three stages of development there was an optimum level of O_2 for photosynthesis, where either lower or higher levels of O_2 caused reduction in the rate. There were differences in the partial pressure of O_2 that gave maximum rates of

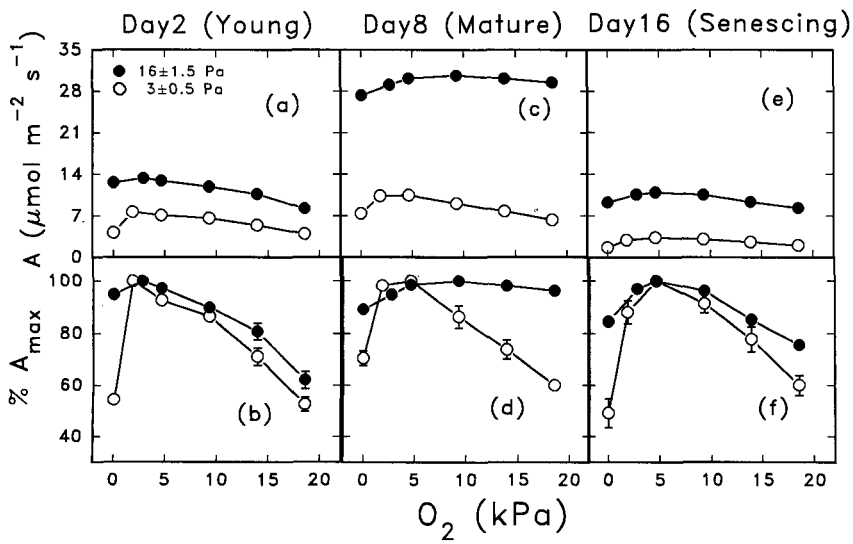

Figure 1. The responses of **A** to [O_2] of a section of the fifth leaf of maize as it progressed through development (d 2, 8, and 16 after emergence) under C_i of 3 Pa (○) versus 16 Pa (●). Results in the lower panels are shown as a percentage of the maximum value of **A** for each C_i. The temperature was 30°C, PPFD was 1300 μmol quanta m^{-2} s^{-1}, and VPD was 500 ± 100 Pa. Each point is the mean ± SD of three replicates. Bars not seen are smaller than the size of the symbols.

Table I. *Chl content, photosynthesis rates at 9.3 and 18.6 kPa O_2, Θ_A, R_d rates, and Γ during development of the fifth leaf of maize*

Data presented are means of three replicate measurements (each replicate from a different plant; values in parentheses are SD) except for R_d, which is for one measurement.

Day[a]	Chl Content	Photosynthesis[b]		Θ_A	R_d[c]	Γ
		9.3 kPa O_2	18.6 kPa O_2			
	mg m^{-2}	*μmol CO_2 m^{-2} s^{-1}*		*% inhibition kPa^{-1} O_2*	*μmol CO_2 m^{-2} s^{-1}*	*Pa*
2	231	12.0 (±0.2)	8.3 (±0.2)	3.32 (±0.30)	−1.10	0.30 (±0.03)
8	513	30.7 (±0.2)	29.5 (±0.3)	0.42 (±0.10)	−1.05	0.12 (±0.02)
16	249	10.6 (±0.3)	8.3 (±0.2)	2.43 (±0.03)	−1.02	0.24 (±0.03)

[a] Day 2, 8, and 16 represent days of exposure of the developing fifth leaf section to direct light (see text). [b] Photosynthesis rates were measured at 30°C, PPFD of 1300 μmol quanta m^{-2} s^{-1}, 500 ± 100 Pa VPD, and 34 Pa CO_2. Data are from Figure 1. [c] R_d was measured at 30°C.

photosynthesis; these were approximately 2 kPa O_2 or less in young tissue and 5 kPa O_2 in senescing tissue, under both normal and low CO_2. For mature tissue, the optimum level of O_2 for maximum photosynthesis was approximately 9 kPa for normal CO_2 and 4 kPa for low CO_2. A striking developmental difference in response to O_2 was the strong inhibition of photosynthesis by supraoptimal levels of O_2 in the young and senescing tissues compared to that in the mature tissue under 16 Pa CO_2. Under low C_i, photosynthesis at all three developmental stages was strongly inhibited by supraoptimal levels of O_2.

A summary of the effects of leaf age on Chl content, photosynthetic rate, Θ_A, and Γ at three different developmental stages is shown in Table I. The Chl content on d 2 versus d 16 of emergence was 231 and 249 mg m^{-2}, respectively, whereas it was 513 mg m^{-2} on d 8. The degree of inhibition of photosynthesis by O_2 above the optimum level was calculated as Θ_A between 9.3 and 18.6 kPa. The young and senescing leaf tissues (d 2 and 16, respectively) had much higher Θ_A values than the mature tissue (d 8) (Table I), approaching the values for mature C_3 leaves under similar conditions (Dai et al., 1993). However, the values of Γ at these three stages were similar and remained low (0.12–0.3 Pa) although photosynthetic rates were quite different (Table I).

To follow the developmental changes more closely, the O_2 inhibition index was determined at 2-d intervals during the progressive development of the fifth leaf under two CO_2 levels (Fig. 2). Under a C_i of 16 Pa, the value of Θ_A decreased dramatically from a high value of 3.0 on d 2 to a minimum value of 0.5 on d 8, and then progressively increased up to d 16 to a value of 1.6. The value for the mature leaves is similar to that reported earlier (Dai et al., 1993). Thus, young and senescing tissues had much higher inhibition of photosynthesis by supraoptimal levels of O_2 than did the mature tissue. Compared with the higher CO_2 (C_i of 16 Pa), under low CO_2 (3 Pa) the Θ_A values were much higher, but the pattern of change during development was similar.

A/C_i response curves showed that photosynthesis in young and senescing tissues of the fifth leaf of maize (2- and 16-d measurements, respectively) reached maximum rates of photosynthesis at 6 to 8 Pa CO_2, as did the mature leaf tissue, although the rates in mature tissue were much higher (Fig. 3a). ϕ_{CO_2} was determined from measured rates of CO_2 uptake, and simultaneously ϕ_{PSII} was measured by fluorescence analysis. The ratio of ϕ_{PSII}/ϕ_{CO_2}, a relative measure of PSII activity per CO_2 fixed (Krall and Edwards, 1990), was much higher in young and senescing tissues than in mature tissue at any given C_i (Fig. 3b). As C_i decreased, the ϕ_{PSII}/ϕ_{CO_2} ratio in mature tissue stayed constant until approximately 2.5 Pa CO_2, below which there was a sharp rise in the ratio; in young and senescing tissues there was a gradual rise in the ϕ_{PSII}/ϕ_{CO_2} ratio as the C_i dropped below approximately 10 Pa.

The above experiments were conducted on a section of the fifth leaf as it proceeded through development. Another inquiry was made on the degree of sensitivity of photosynthesis to supraoptimal levels of O_2 at the tip, middle, and basal sections of a fully expanded fifth leaf. The Chl contents were 345, 510, and 505 mg m^{-2} and the rates of photosynthesis under atmospheric levels of CO_2 were 18.3, 29.6, and 19.9 μmol CO_2 m^{-2} s^{-1} for the basal, middle, and tip sections of leaves, respectively. These results show a developmental trend in photosynthetic activity; however, the Chl content and photosynthetic rate do not vary as much along the blade of a fully expanded leaf

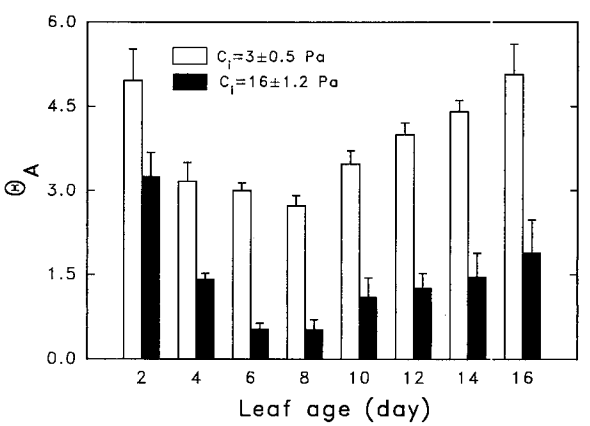

Figure 2. Θ_A of a section of the fifth leaf of maize as it developed from d 2 after emergence up to d 16. Θ_A was calculated from the data of Figure 1 (based on the response between 9.3 and 18.6 kPa O_2; see Table I) plus data for other days (not shown) for maize. The temperature was 30°C, PPFD was 1300 μmol quanta m^{-2} s^{-1}, and VPD was 500 ± 100 Pa. Each point is the mean + SD of three replicates.

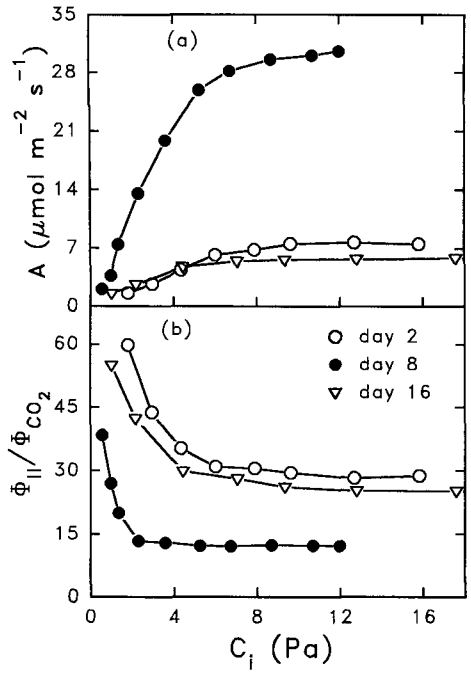

Figure 3. The response of **A** (a) and the ratio of ϕ_{PSII}/ϕ_{CO_2} (b), measured on a section of the fifth leaf of maize during its development (d 2, 8, and 16 after emergence), to changes in C_i. The temperature was 30°C, PPFD was 1000 μmol quanta m^{-2} s^{-1} or 700 μmol quanta m^{-2} s^{-1} when C_i was below 3 Pa, O_2 was 19.5 kPa, and VPD was 500 ± 100 Pa. Lower PPFDs were used in these experiments to increase the accuracy of measuring fluorescence under saturating pulses of light.

as during the course of development of a section of tissue that is followed from time of emergence through senescence (Fig. 1; Table I). From measurements of the O_2 inhibition of photosynthesis, the basal and tip sections of the fully expanded blade had higher values of Θ_A than the middle section when comparisons were made at either 3 or 16.5 Pa CO_2 (Fig. 4). As before, the values of Θ_A were much higher under the lower level of CO_2. However, differences in O_2 inhibition of photosynthesis measured along a fully expanded leaf blade (Fig. 4) are not nearly as large as those shown previously for tissue as it proceeds from a very young stage to a stage where senescence is more apparent (Fig. 2).

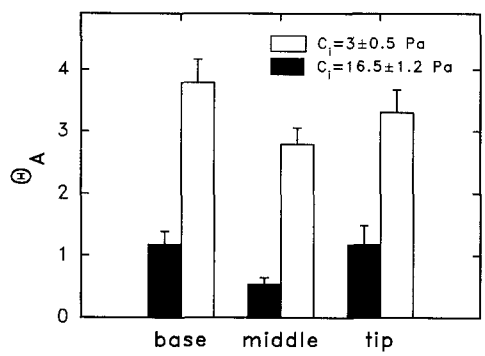

Figure 4. Θ_A measured at the base (70–75 cm from the tip of the leaf), middle (40–45 cm from the tip), and tip (10–15 cm from the tip) of the fifth leaf of maize after it reached full expansion. The leaf was approximately 80 cm long and growth of the sheath had terminated. The temperature was 30°C, PPFD was 1300 μmol quanta m^{-2} s^{-1}, VPD was 500 ± 100 Pa, and the C_i was 3 versus 16.5 Pa CO_2. Each point is the mean + SD of three replications.

Leaf Position

To further understand the effects of O_2 on C_4 photosynthesis, leaves at different positions on the plant (from the second to the sixth leaf) were studied. Measurements were made on the midsection of each leaf as it became fully expanded. Compared to leaf numbers 4 and 6, the rate of photosynthesis in leaf number 2 was substantially lower (Fig. 5). Under C_i of 2.6 versus 16 Pa, the response of photosynthesis to varying $[O_2]$ was very similar between leaf numbers 4 and 6 (Fig. 5, a and b). When photosynthesis rates at C_i of 16 Pa were plotted as a percentage of the maximum rate (Fig. 5, c and d), leaf numbers 4 and 6 had maximum rates of photosynthesis at approximately 9 kPa O_2, whereas leaf number 2 had a maximum rate of photosynthesis at approximately 5 kPa O_2. Under low C_i the optimum level of O_2 for maximum photosynthesis was 2.5 to 5 Pa for all three leaf positions. From these types of experiments, the Θ_A values were determined for leaves 2 through 6 (Fig. 6). Under low C_i the value of Θ_A decreased progressively with increasing leaf position, whereas under higher C_i the value of Θ_A decreased from leaf positions 2 to 4 and remained constant thereafter.

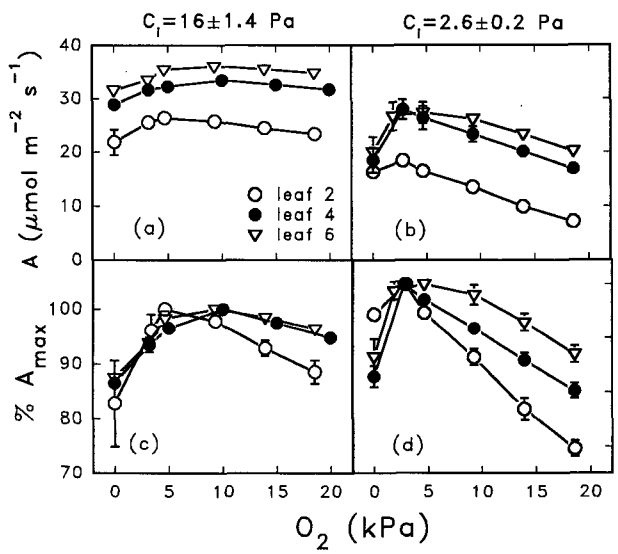

Figure 5. The responses of **A** in maize to $[O_2]$ for leaf positions 2 (○), 4 (●), and 6 (▼) (counting from the bottom of the plant) at C_i of 2.6 versus 16 Pa. Measurements were made on the midsection of mature leaves. Results in the lower panels are shown as a percentage of the maximum value of **A**. The temperature was 30°C, PPFD was 1300 μmol quanta m^{-2} s^{-1}, and VPD was 500 ± 100 Pa. Each point is the mean ± SD of three replicates. Bars not seen are smaller than the size of the symbols.

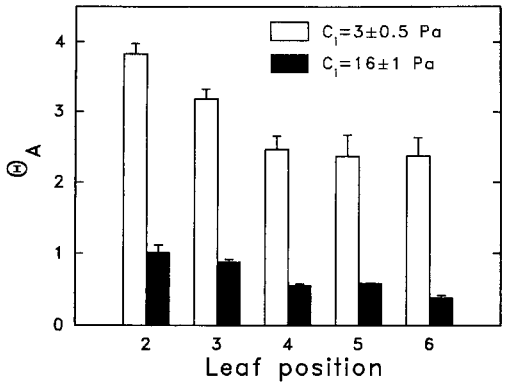

Figure 6. Θ_A of mature maize leaves at different leaf positions. Θ_A was calculated from the data from Figure 5 (between 9.3 and 18.6 kPa O_2) plus data for other leaf positions (not shown). The temperature was 30°C, PPFD was 1300 μmol quanta m^{-2} s^{-1}, and VPD was 500 ± 100 Pa. Each point is the mean + SD of three replicates. Bars not seen are smaller than the size of the symbols.

Value of Θ_A versus Rubisco and Chl Content

The relationships of photosynthesis rate, Rubisco activity, and Chl content on a leaf area basis to Θ_A in maize were evaluated with respect to leaf development (Fig. 7). Comparisons were made including data on development of an individual leaf (experiments in Table I and Fig. 4) and leaf position (experiments in Fig. 6). These data show that tissues having high photosynthetic rates, Chl content, and Rubisco activity have low Θ_A values, whereas high Θ_A values occur only in tissues where these parameters are low (Fig. 7).

Model of Θ_A versus CO_2 Concentration

The nature of the O_2 inhibition of photosynthesis due to the process of photorespiration at supraoptimal partial pressures of O_2 at a given partial pressure of CO_2 will depend on the kinetic properties of Rubisco, specifically the K_c for CO_2 and the K_o for O_2 and S_{rel}. Figure 8 shows modeled values of Θ_A at different concentrations of CO_2 based on the kinetic properties of Rubisco reported by Jordan and Ogren (1984). This model, adopted from Sage and Sharkey (1987), was used to estimate the levels of CO_2 that may exist in bundle sheath cells of maize at a given value of Θ_A.

DISCUSSION

In previous studies on developmental changes in photosynthesis and photorespiration in maize, measurements were made on single leaves and on leaves at different positions on the plant. These studies show that significant changes occur in the expression of the C_4 syndrome and photosynthetic capacity during development (Williams and Kennedy, 1978; Crespo et al., 1979; Miranda et al., 1981; Thiagarajah et al., 1981; Sheen and Bogorad, 1985; Aoyagi and Bassham, 1986; Langdale et al., 1988a; Nelson and Langdale, 1989, 1992; Ngernprasirtsiri et al., 1989). Although there are suggestions of developmental changes in photorespiration in maize, no clear view has emerged (Williams and Kennedy, 1977; Crespo et al., 1979; Perchorowicz and Gibbs, 1980; Langdale et al., 1988b; De Veau and Burris, 1989). Based on the reported lack of effect of 21% versus 2% O_2 on rates of C_4 photosynthesis under varying C_i, it was concluded that photorespiration is not apparent (see Edwards et al., 1985). However, it is now clear that previous reports of insensitivity of C_4 photosynthesis to O_2 are due to lack of recognition of the biphasic response of photosynthesis to varying O_2 between 0 and 19.5 kPa, and they are inconsistent with other reports of measurable levels of photorespiration in C_4 plants (see Dai et al., 1993).

In the present study we examined the O_2 sensitivity of photosynthesis in maize considering the influences of both development of a single leaf and leaf position. Analyses were not made on leaves prior to their emergence, when they are curled and screened by other leaves, since it is the exposed tissue that largely determines photosynthetic rate in the plant. The progression in development was examined in a given section of the base of the leaf following its emergence. Measurements were also made along the blade

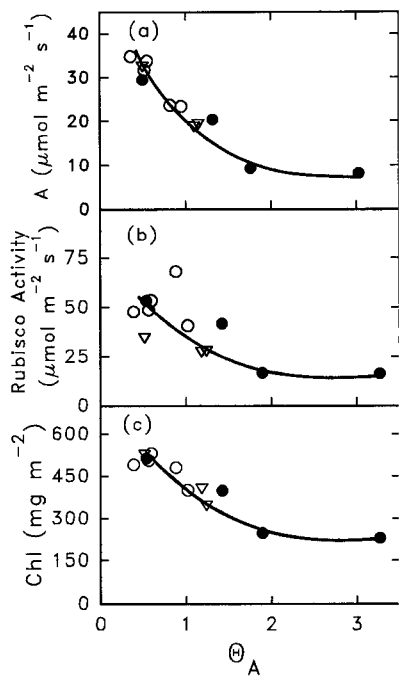

Figure 7. The relationship between A and Θ_A (a), the Rubisco activity and Θ_A (b), and Chl content and Θ_A (c) from measurements on various maize leaves during development. Measurements were made under a C_i of approximately 16 Pa CO_2 (C_o near atmospheric levels), 1300 μmol quanta m^{-2} s^{-1}, and 500 ± 100 Pa VPD. Data on photosynthesis rate and Θ_A are from results on plant material shown in Figures 2, 4, and 6. Chl content and Rubisco activity were determined from leaf sections harvested from three to five different plants using leaves at developmental stages similar to those used in gas-exchange measurements (this included some samples taken following measurement of photosynthesis). ●, Leaf age (measurements made on a section of leaf as it develops); ○, different leaf positions; ▼, measurements on the base, middle, and tip of the mature leaf.

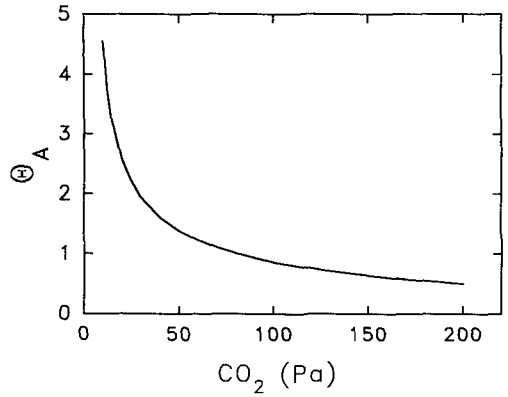

Figure 8. Modeled values of Θ_A versus partial pressure of CO_2 based on the kinetic properties of Rubisco. Θ_A was calculated adopting the model of Sage and Sharkey (1987) assuming photosynthesis is limited by enzyme where:

$$\Theta_A = \frac{\left(1 - \frac{A_{18.6kPa}}{A_{9.3kPa}}\right)}{18.6\ \text{kPa} - 9.3\ \text{kPa}}(100) \cong 100 - \frac{\dfrac{1 - 0.5(\phi_1)}{C + K_c\left(1 + \frac{O_1}{K_O}\right)}}{\dfrac{1 - 0.5(\phi_2)}{C + K_c\left(1 + \frac{O_2}{K_O}\right)}}(100)$$

$$9.3\ \text{kPa}$$

where C is the CO_2 concentration and ϕ_1 and ϕ_2 are the ratios of oxygenase/carboxylase (v_o/v_c) activities calculated at O_2 concentrations O_1 and O_2, respectively, based on the equation

$$\phi = \frac{v_O}{v_c} = \frac{1}{S_{rel}}\frac{O}{C}.$$

The kinetic constants of Jordan and Ogren (1984) were used for Rubisco with S_{rel} = 76 at 30°C, K_o = 600 μM O_2, and K_c = 14 μM CO_2. Inputs were 9.3 kPa O_2, 18.6 kPa O_2, variable Pa of CO_2, and the ratio of oxygenation to carboxylation (ϕ). Partial pressures of gases were converted to a micromolar basis at 30°C for calculation of percentage inhibition of photosynthesis where C = concentration of CO_2, and O_1 and O_2 equal concentrations of oxygen at 18.6 and 9.3 kPa, respectively, since values of kinetic constants are on a concentration basis (see Sage and Sharkey, 1987). The O_2 partial pressure in maize bundle sheath was assumed to be in equilibrium with that of the atmosphere (see Dai et al., 1993).

of a fully expanded leaf. With respect to leaf position, measurements were made in the middle section of the blade as each leaf reached maturity.

Leaf Development and O_2 Requirement for Maximum Rates of Photosynthesis

The O_2 partial pressure giving maximum rates of photosynthesis in maize varied with development, being highest in mature tissue having high rates of photosynthesis and lower in young or senescing tissues having low rates of photosynthesis (Figs. 1 and 5). The basis for the change in optimum O_2 partial pressures for maximum photosynthetic rate with development is not known, but it may be associated with inhibition of photosynthesis by supraoptimal levels of O_2 becoming a dominant factor in very young and senescing tissues of maize. In C_3 plants where O_2 causes strong inhibition of photosynthesis, the O_2 partial pressure giving maximum rates of photosynthesis is also low (our unpublished data). Inhibition of photosynthesis by low O_2 in maize was observed at all developmental stages examined including young, mature, and senescing tissues of the fifth leaf and leaves at different positions (leaves 2, 4, and 6). The greatest degree of reduction in the rate of photosynthesis in the absence of O_2 in the atmosphere occurred when the partial pressure of CO_2 was low (Figs. 1 and 5). However, this may be only an apparent difference, since more O_2 will be produced in the leaf photosynthetically under normal CO_2 than under low CO_2, so that the true O_2 requirement for maximum rates of photosynthesis under normal CO_2 may be somewhat masked. The reason for the inhibition of photosynthesis by low O_2 is uncertain. Maximum rates of C_4 photosynthesis may require respiration (e.g. to provide ATP for Suc synthesis), which may be limited under low O_2. Alternatively, cyclic and/or pseudocyclic electron flow may be required to provide additional ATP for the C_4 cycle, and low O_2 may limit the production of ATP by these means (see Dai et al., 1993).

An O_2 requirement for maximum rates of photosynthesis has been reported in other photosynthetic organisms. An enhancement of photosynthesis by O_2 was observed in three photosynthetic microorganisms (in *Anacystis nidulans* by Miyachi and Okabe [1976]; in a cryptomonad, *Chroomonas* sp. by Suzuki and Ikawa [1984]; and in the diatom *Nitzschia rattneri* by Suzuki and Ikawa [1993]), and was suggested to be linked to photophosphorylation. In terrestrial C_3 plants there are cases of enhancement of photosynthesis by O_2 for which several possible explanations have been given (see Dietz et al., 1985; Sharkey and Vassey, 1989; Harley and Sharkey, 1991; Kromer and Heldt, 1991).

Leaf Development and Photorespiration

Θ_A, the percent inhibition of photosynthesis per kPa increase in O_2 above the optimum partial pressure, can be used as an indicator of the degree of photorespiration in different tissues of maize. Another potential indicator of a change in photorespiration is measurement of the ϕ_{PSII}/ϕ_{CO_2} ratio. A higher ϕ_{PSII}/ϕ_{CO_2} ratio, which is indicative of a higher PSII activity per CO_2 fixed, can occur as a result of higher photorespiration (e.g. as observed in C_3 plants with decreasing CO_2; see Edwards and Baker [1993]; Oberhuber and Edwards [1993]).

First, there is evidence that mature maize leaves, prior to the onset of senescence, have low levels of photorespiration. In a fully expanded leaf, prior to substantial loss of Chl and senescence, the values of Θ_A obtained from measurements along the leaf were low, indicative of low photorespiration (Fig. 4). Previous measurements of the ϕ_{PSII}/ϕ_{CO_2} ratio along the developed second leaf of maize also indicated that photorespiration is limited, since there was only a slight increase in the ratio from the tip to the base (Edwards and Baker, 1993). Thus, although there is evidence for substantial photorespiration in maize in the extremes of development where Chl deficiency is apparent (as shown in this study with both young and senescing leaf

tissues and discussed below), it should be emphasized that a fully expanded blade, prior to senescence, has low photorespiration.

In young and senescing maize leaf tissues, supraoptimal partial pressures of O_2 caused greater inhibition of photosynthesis than in mature tissue, indicative of higher levels of photorespiration in the former (such comparisons need to be made on a percentage basis, since it is the relative effect that varying O_2 has on the photosynthetic rate that is important in considering the magnitude of photorespiration [Ku and Edwards, 1977]). Under atmospheric levels of CO_2, mature leaves of maize have a Θ_A value of approximately 0.4, which indicates low levels of photorespiration, compared to Θ_A values of approximately 2 for the C_3 plant wheat (Dai et al., 1993; Figs. 2 and 4 of present study). However, under atmospheric CO_2 conditions very young, emerging leaf tissue and senescing tissue of the fifth leaf of maize have Θ_A values (Fig. 2) similar to that of wheat, indicating substantial photorespiration. Also, at a given C_i value, the ratio of ϕ_{PSII}/ϕ_{CO_2} was higher in very young and senescing tissues than in mature tissue of maize (Fig. 3). A higher level either of photorespiration or of dark-type respiration relative to A could result in an increase in the ϕ_{PSII}/ϕ_{CO_2} ratio. Although the rates of dark respiration were similar in young, mature, and senescing tissues, the actual rates of respiration in the light are not known, so no correction was applied in calculating ϕ_{CO_2}. If dark respiration is low under the light intensities used (700–1000 PPFD; see Brooks and Farquhar [1985]), the higher ϕ_{PSII}/ϕ_{CO_2} ratios would be fully accounted for by higher rates of photorespiration.

As far as development of a single leaf is concerned, the capacity for photosynthesis and status of photorespiration can be viewed in five stages: curled leaf prior to emergence, very young exposed tissue, mature tissue, early senescence, and late senescence (Scheme 1).

Curled Leaf Prior to Emergence

The basal section (0–2.5 cm) of the third leaf of maize (while curled up and surrounded by the first and second leaves) has high levels of photorespiration (Perchorowicz and Gibbs, 1980). The light/dark ratio of the release of $^{14}CO_2$ into CO_2-free air measured following assimilation of $^{14}CO_2$ in this tissue was 1.3 compared to 2.0 in the C_3 plant pea. The basal tissue has very poor capacity for C_4 photosynthesis in that there was little turnover of C_4 acids, and the NADP-malic enzyme activity was particularly low.

Very Young Exposed Tissue

Results of the present study indicate that young exposed tissues have substantial photorespiration based on high O_2 inhibition of photosynthesis and high ϕ_{PSII}/ϕ_{CO_2} ratios, but the values of Γ (Table I) and the light/dark ratios for $^{14}CO_2$ release (Williams and Kennedy, 1977) are low. These results indicate that there is little loss of photorespired CO_2 from the leaf, presumably because of efficient refixation of this photorespired CO_2.

Mature Leaf Tissue

Mature tissue has high capacity for photosynthesis coinciding with high Chl content and Rubisco activity, and low photorespiration based on the low ϕ_A values and low ϕ_{PSII}/ϕ_{CO_2} ratios (also see De Veau and Burris, 1989; Dai et al., 1993).

Senescing Leaf Tissue

The present study shows that when leaf tissue has lost about 50% of the Chl during senescence, photorespiration is apparent based on increased O_2 sensitivity, but Γ again remains low, indicative of efficient recycling of photorespired CO_2. In later stages of senescence, loss of photorespired CO_2 may occur. Senescent leaf tips of maize, having approximately 20% of the Chl content and one-third of the rate of photosynthesis of mature leaves, have a Γ of 22 μL/L and a light/dark ratio for release of prefixed $^{14}CO_2$ of 3.3 in CO_2-free air, which is typical of C_3 plants (Williams and Kennedy, 1977).

With respect to leaf position, there was a progressive decrease in the value of the O_2 inhibition index from lower to upper leaves (from values of 0.9–0.4 under normal CO_2 with measurements made on the midsection of leaves as they matured; Fig. 6). These results suggest that the lower leaves have higher levels of photorespiration than the upper leaves.

Leaf Age and Effectiveness of the CO_2-Concentrating Mechanism

A model for Θ_A versus CO_2 concentration around Rubisco (Fig. 8) suggests that the level of CO_2 in bundle

Curled leaf, shaded by surrounding tissue ↓	● NADP-malic enzyme very low ● C_4 cycle turnover very low ● Loss of photorespired CO_2 ● Photosynthesis rate very low
Emerging, exposed tissue ↓	● C_4 enzymes increase ● O_2 inhibition of photosynthesis high ● Efficient refixation of photorespired CO_2, Low Γ ● Photosynthesis rate low
Mature tissue ↓	● C_4 enzymes high ● CO_2 concentrated in bundle sheath ● O_2 inhibition of photosynthesis low ● Low photorespiration, Low Γ ● Photosynthesis rate high
Early senescence ↓	● O_2 inhibition of photosynthesis increases ● Efficient refixation of photorespired CO_2, Low Γ ● Photosynthesis rate declines
Late senescence	● C_4 enzymes decline ● O_2 inhibition of photosynthesis high ● Loss of photorespired CO_2, High Γ ● Photosynthesis rate low

Scheme 1. Proposed developmental stages of photosynthesis and photorespiration in a maize leaf.

sheath cells of mature leaves of maize under high light may reach 100 to 200 Pa (Θ_A = 0.5–0.8), which is in the range for C_4 photosynthesis predicted by Jenkins et al. (1989) by a separate method, whereas that of very young and senescing tissue may be as low as 25 Pa (e.g. Θ_A = 2.0) (Figs. 2, 4, and 6). Thus, it may be possible to estimate the capacity of C_4 photosynthesis to concentrate CO_2 in the bundle sheath of maize leaves from measurements of the photosynthetic rate at 9.3 and 18.6 kPa O_2 and the calculation of Θ_A. Although it is clear that the value of Θ_A should decrease as C_i in the bundle sheath increases, the relationship shown in Figure 8 is an approximation because it depends on a number of factors. The kinetic values (K_c, K_o, S_{rel}) used for Rubisco in the model (Fig. 8) are for the spinach enzyme, which has a S_{rel} value similar to that of maize (Jordan and Ogren, 1983, 1984), but the exact in vivo kinetic properties of the enzyme for maize are not known. Θ_A is calculated for a given C_i value with varying O_2. However, there may be some rise in the level of CO_2 in bundle sheath cells with an increase in O_2 due to decreased use of CO_2 by the carboxylase and increased production of CO_2 by photorespiration. O_2 insensitivity of photosynthesis in C_4 plants under low atmospheric levels of CO_2 is proposed to occur through the effects of low bundle sheath conductance on the levels of CO_2 and O_2 in bundle sheath cells when changing the external O_2 partial pressure (Berry and Farquhar, 1978; Brown and Byrd, 1993). Yet, the present study clearly shows that there is measurable O_2 inhibition of photosynthesis in maize once analyses are made at supraoptimal levels of O_2. Whether there is a significant rise in the CO_2 level in maize bundle sheath cells when increasing O_2 from 9.3 to 18.6 kPa under normal atmospheric levels of CO_2 in the present study is uncertain; with young and senescing tissue in particular this rise in the CO_2 level may be minimal if the higher Θ_A values are reflecting a high bundle sheath conductance. A significant rise in the concentration of CO_2 in bundle sheath cells with increasing O_2 would decrease the O_2 sensitivity of photosynthesis and cause an overestimation of the CO_2 level in bundle sheath cells based on the response shown in Figure 8. Considering this, the C_i values of Figure 8 would represent the upper limits of the predicted levels of CO_2 in the bundle sheath cells of maize leaves based on O_2 sensitivity.

The Basis for O_2 Inhibition of Photosynthesis and Occurrence of Photorespiration

There are a number of possible explanations for why young and senescing tissues of maize may be inefficient in concentrating CO_2 in bundle sheath cells, resulting in higher levels of photorespiration. It could be explained (a) by bundle sheath cells having lower levels of CO_2 under any circumstance where the rate of photosynthesis is limiting, (b) by lack of differential compartmentation of certain photosynthetic enzymes between mesophyll and bundle sheath cells (allowing partial function of C_3 photosynthesis in the mesophyll), (c) by limited function of the C_4 cycle, and/or (d) by leaky bundle sheath cells.

There is no support for the first explanation because under normal atmospheric CO_2 levels the percentage inhibition of photosynthesis by O_2 in maize is low under both low-light intensity, which yields a low photosynthetic rate, and high-light intensity, which yields a high photosynthetic rate (Dai et al., 1993). These results suggest that in mature tissue a high level of CO_2 is maintained in the bundle sheath under both limiting and high-light intensities (i.e. low light is limiting for assimilatory power but not in provision of high CO_2 to Rubisco).

With respect to enzyme compartmentation, if at some stage of development part of the Rubisco of the maize leaf were localized in the mesophyll cells, this could cause an increased photorespiration and increased inhibition of photosynthesis at supraoptimal O_2. However, only when maize seedlings are grown in the dark is the gene for the Rubisco large subunit transcribed in both mesophyll and bundle cells, and Rubisco protein appears in plastids of both cell types. When maize leaves develop in the light, there is an immediate, proper compartmentation of mRNAs and respective proteins for several photosynthetic enzymes including Rubisco and NADP malic enzyme in bundle sheath cells and PEP carboxylase in mesophyll cells (Sheen and Bogorad, 1985; Langdale et al., 1988b; Ngernprasirtsiri et al., 1989). This is consistent with the primary initial products of $^{14}CO_2$ fixation being C_4 dicarboxylic acids at various developmental stages (Williams and Kennedy, 1977; Perchorowicz and Gibbs, 1980).

If function of the C_4 cycle were rate limiting for photosynthesis due to low activities of C_4 pathway enzymes, this could result in low levels of CO_2 in bundle sheath cells and increased photorespiratory activity. Evidence for this has been obtained only in the young, curled leaf tissue prior to emergence (Perchorowicz and Gibbs, 1980).

A possible explanation for higher levels of photorespiration in very young and senescing tissues is a higher bundle sheath cell conductance to CO_2. From the analysis of bundle sheath conductance on isolated cells from mature C_4 leaves and from modeling CO_2 assimilation, leakage of CO_2 from bundle sheath cells is suggested to constitute about 10% of C_4 acid flux under atmospheric conditions (Jenkins et al., 1989). The results from carbon isotope discrimination suggested approximately 20% leakage of CO_2 from the bundle sheath among 11 C_4 species, including maize, which would require 25% overcycling of the C_4 pathway to provide CO_2 to Rubisco in bundle sheath cells (Henderson et al., 1992). Obviously, higher rates of leakage in very young or senescing tissues of maize could result in lower CO_2 levels in the bundle sheath and an increase in photorespiration.

Regarding why the first leaves to develop in maize may have more photorespiration, it is clear that differences in O_2 sensitivity in maize with respect to leaf position are not due to lack of differential compartmentation of enzymes or to a degree of C_3 photosynthesis in these leaves. Leaves 1 through 3 of maize all have a normal, complete differential compartmentation of certain key enzymes between mesophyll and bundle sheath cells (Rubisco, PEP carboxylase) such that mesophyll cells are not capable of performing C_3 photosynthesis (Langdale et al., 1988b). A lower capacity to concentrate CO_2 in bundle sheath cells likely accounts for

lower leaves having a higher O_2 inhibition of photosynthesis. It is clear that newly matured lower leaves of maize have a lower rate of photosynthesis than subsequent leaves, but the basis for this is uncertain (Thiagarajah et al., 1981).

In conclusion, younger and senescing tissues and lower-position leaves of maize are suggested to have a lower capacity to concentrate CO_2 in bundle sheath cells during C_4 photosynthesis, which is likely due to a higher bundle sheath conductance. This results in higher photorespiration as reflected in higher Θ_A values and higher ϕ_{PSII}/ϕ_{CO_2} ratios. When the Chl and Rubisco content are 50% or less than that in mature leaves, the degree of photorespiration could approach that of C_3 plants, based on measures of O_2 inhibition of photosynthesis under atmospheric levels of CO_2. However, even in very young and early senescing tissues there is efficient refixation of photorespired CO_2 in maize, as indicated by the low Γ values. Healthy leaves with reasonably high Chl and Rubisco content would have little O_2 inhibition of photosynthesis and little photorespiration under normal conditions. The exact basis for the changes in CO_2-concentrating capacity and control of photorespiration during C_4 leaf development awaits further investigation.

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