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Differential Oxygen Response of Photosynthesis in Soybean and *Panicum milioides*

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ABSTRACT

The carbon dioxide compensation concentration of *Panicum milioides* was less than that of soybean over the range of 15 to 35 C. In soybean (*Glycine max* [L.] Merr. cv. Wayne), the compensation concentration was directly proportional to O_2 concentration. In *P. milioides*, the compensation concentration was near zero up to 10% O_2 and then increased linearly with higher O_2, although the slope of the response was less than that in soybean. Leaf extracts of *P. milioides* contained 3-fold higher phosphoenolpyruvate carboxylase activity than soybean leaf extracts. Oxygen inhibition of photosynthesis and carboxylation efficiency was less in *P. milioides* than that observed in soybean. The affinity of *P. millioides* ribulose-1,5-di-P carboxylase for CO_2 appeared to be slightly greater than that of soybean. The affinity of both enzymes for O_2 was similar. The reduced response of the compensation concentration and photosynthesis to O_2 in *P. milioides* may be explained by photosynthetic phosphoenolpyruvate carboxylase fixation and by an apparent increased affinity of ribulose-1,5-di-P carboxylase for CO_2.

Two distinctions between C_3 and C_4 photosynthesis are O_2 sensitivity and the CO_2 compensation concentration – CO_2 concentration where the rates of photosynthesis and respiration are equal. Photosynthesis at 300 μl/l CO_2 in C_3 plants is about 45% higher in 2% O_2 than in 21% O_2, whereas C_4 photosynthesis is only slightly stimulated in 2% O_2 (9). The CO_2 compensation concentration of C_3 plants at 25 C and 21% O_2 is about 40 μl/l CO_2, and that of C_4 plants is about zero μl/l CO_2 (9). Recently, plant species with intermediate O_2 sensitivity of photosynthesis (3) and intermediate Γ[2] (3, 12) have been described.

Previous reports from this laboratory (1, 2, 13, 15, 16) have established the concept that O_2 sensitivity of C_3 photosynthesis and Γ in C_3 plants are determined by the kinetic properties of RuDP carboxylase with respect to its two mutually competitive substrates, CO_2 and O_2. In C_4 plants, O_2 sensitivity of photosynthesis and Γ are thought to be reduced to near zero because the C_4 cycle acts to concentrate CO_2 in the bundle sheath, the site of RuDP carboxylase, allowing CO_2 to compete more effectively against O_2 for RuDP (4, 6, 15). The high PEP carboxylase activity in the C_4 mesophyll cells can refix photorespiratory CO_2 which might be produced before it can escape from the leaf. In plants with an intermediate Γ, it has been suggested that the reduced O_2 sensitivity is due to increased PEP carboxylase fixation of CO_2 (8, 11). The investigations reported here were undertaken to characterize further the reduced O_2 sensitivity of photosynthesis and Γ in *Panicum milioides*, compared to C_3 plants, and relate these characteristics to our understanding of the regulation of the O_2 response in C_3 and C_4 photosynthesis.

MATERIALS AND METHODS

Plant Material. Soybean (*Glycine max* [L.] Merr. cv. Wayne) and maize (*Zea mays* [L.] cv. WF9×M14) seeds were germinated and grown in vermiculite in growth chambers (30 C day/20 C night, 14 hr photoperiod, 550 μeinstein m^{-2} sec^{-1}) and subirrigated with modified Hoagland solution. Seeds of *P. milioides* (P.I. 310042) were germinated on a paper wick and, after 10 to 14 days, transplanted into vermiculite. Subsequent growth conditions were identical to those for soybean and maize. Experiments were conducted with the most recent fully expanded soybean trifoliate leaf, 10- to 14-day-old maize leaves, and 4- to 5-week-old leaves of *P. milioides*.

Gas Exchange Measurements. Net photosynthesis and Γ were measured with excised leaves at 25 C. In Figure 1, experiments were done at 15, 20, 25, 30, and 35 C. The closed system used in these measurements has been described elsewhere (13). In experiments using variable O_2 concentrations, desired gas mixtures were made from cylinders of N_2, O_2, and 1% CO_2 in N_2. Carboxylation efficiency was calculated as described previously (13).

Enzyme Measurements. Crude leaf extracts were prepared by grinding about 200 mg (fresh weight) of leaf tissue in a TenBroeck homogenizer for 2 min in ice, in a grinding medium of 50 mM tris-Cl, pH 8, 10 mM $MgCl_2$, 5 mM dithiothreitol, 5 mM isoascorbate, and 0.1 mM EDTA. The homogenizer was rinsed with additional grinding medium and added to the extract to bring the final volume to 10 ml. The crude extract was assayed for RuDP carboxylase (13) and PEP carboxylase (5) activities.

Partially purified RuDP carboxylase, for the determination of *Km* and *Ki* values, was prepared as follows. About 1 g (fresh weight) of leaf tissue was ground in a TenBroeck homogenizer in ice, in 5 mM K phosphate, pH 7.6, 5 mM mercaptoethanol, 0.1 mM EDTA, and 6% PVP. After grinding, the extract was centrifuged at 18,000*g* for 10 min, and the pellet discarded. Cold, saturated $(NH_4)_2SO_4$ solution was slowly added to the supernatant to give 35% saturation. After stirring for 20 min in ice bath, the suspension was centrifuged at 10,000*g* for 10 min, and the pellet discarded. Saturated $(NH_4)_2SO_4$ was added to bring the supernatant to 50% saturation. After stirring for 20 min, the suspension was centrifuged at 18,000*g* for 10 min, and the supernatant discarded. The pellet was dissolved in 1 ml of 25 mM tris-Cl, pH 8, 10 mM $MgCl_2$, and 0.25 mM EDTA, and added to a Sephadex G-25 column (1 × 10 cm) equilibrated with

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[2] Abbreviations: RuDP: ribulose 1,5-diphosphate; PEP: phosphoenolpyruvate; Γ: CO_2 compensation concentration. Abbreviations used in equations are defined in the text.

the same buffer. The column was eluted with the same buffer and RuDP carboxylase was collected in the first 2 ml after the void volume. After dilution to 10 ml with suspension buffer, the enzyme solution was stored on ice for 2 hr. $Km(CO_2)$ and $Ki(O_2)$ values were determined as described previously (13) under N_2, 50% O_2, and 100% O_2 at pH 8.

RESULTS

The Γ in *P. milioides* was substantially less than in soybean at all temperatures between 15 and 35 C, ranging from 8 to 36 μl/l CO_2 over this temperature range for *P. milioides*, and from 26 to 69 μl/l CO_2 for soybean (Fig. 1).

The Γ of *P. milioides* was also less than that in soybean over the range of 2 to 100% O_2 at 25 C (Fig. 2). Two significant differences in the response of Γ to increasing O_2 concentration were observed. In soybean, Γ was directly proportional to O_2 concentration. In *P. milioides*, however, the plot of Γ as a function of O_2 concentration was curvilinear, with a break at about 10% O_2 (Fig. 2). At less than 10% O_2, Γ was close to zero. At O_2 concentrations between 10% and 100% O_2, Γ was proportional to O_2, but the slope was considerably less than that observed with soybean.

The curvilinear response curve in *P. milioides* suggests to us that at less than 10% O_2, much of the CO_2 was fixed in a carboxylation reaction not linked to photorespiration. At about 10% O_2, this CO_2 fixation system became saturated. The low Γ observed in C_4 species has been attributed to an increased PEP carboxylase activity in the mesophyll, which concentrates CO_2 in the bundle sheath, thereby reducing synthesis of photorespiratory substrate, and refixes any photorespiratory CO_2 that is produced (4, 6, 15). The PEP carboxylase activity in extracts of *P. milioides* leaves was found to be intermediate to the activity in leaf extracts of soybean, a C_3 plant, and maize, a C_4 plant (Table I; refs. 8 and 11). The intermediate PEP carboxylase activity in *P. milioides* may explain in part the intermediate Γ observed for this species.

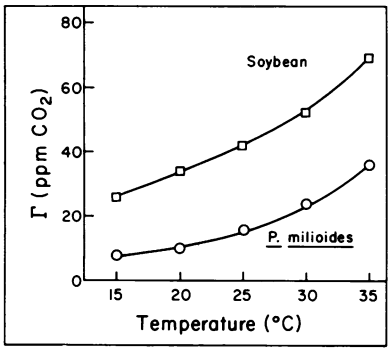

FIG. 1. Temperature dependence of Γ in *P. milioides* and soybean at 21% O_2.

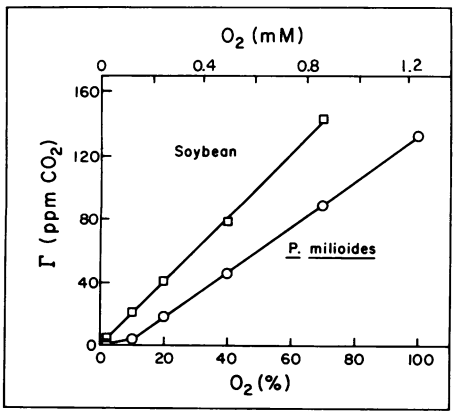

FIG. 2. Response of Γ to O_2 concentration in *P. milioides* and soybean at 25 C.

Above 10% O_2, Γ in *P. milioides* increased linearly with increasing O_2 concentration, but the slope of the line was considerably less than that observed with soybean (Fig. 2). The reduced slope of *P. milioides* indicates that photosynthesis in *P. milioides* is less sensitive to O_2 than is photosynthesis in soybean, and that photorespiration, relative to photosynthesis, is reduced in *P. milioides*. To examine further differences in O_2 sensitivity of photosynthesis in *P. milioides* and soybean, a more complete analysis of gas exchange characteristics in these two species was made. The O_2 inhibition of photosynthesis at 300 μl/l CO_2 in soybean was greater than in *P. milioides* (Fig. 3). The differential inhibition observed is similar to that reported for *P. milioides* and the C_3 plant tall fescue (3).

Oxygen inhibition of net photosynthesis comprises two components, direct O_2 inhibition of photosynthesis and O_2 stimulation of photorespiratory CO_2 evolution (7, 13, 16). The photorespiration component of inhibition can be removed by determining the effect of O_2 on carboxylation efficiency (Fig. 4). This analysis further indicates that photosynthesis in *P. milioides* is less sensitive to O_2 than is soybean photosynthesis.

We have previously suggested that Γ and O_2 sensitivity in C_3 plants are functions of the kinetic characteristics of RuDP carboxylase with respect to CO_2 and O_2 (13, 15). In this context, the reduced O_2 response of *P. milioides* (Figs. 2–4) suggests that the kinetic constants of soybean and *P. milioides* RuDP carboxylase may be different. To examine this possibility, Km (CO_2) and Ki (O_2) were determined for enzymes from the two species. *P. milioides* RuDP carboxylase exhibited a slightly greater affinity for CO_2 than soybean RuDP carboxylase (Table II) but the Ki (O_2) values for both enzymes were similar.

DISCUSSION

In a previous analysis of O_2 sensitivity of soybean photosyn-

Table I. *PEP and RuDP Carboxylase Activities in Crude Leaf Extracts of Soybean, P. milioides, and Maize*

Species	Carboxylase Activity		
	μmol CO_2/mg Chl·hr		Ratio
	PEP	RuDP	PEP/RuDP
Soybean	26	180	0.14
P. milioides	65	134	0.48
Maize	281	98	2.87

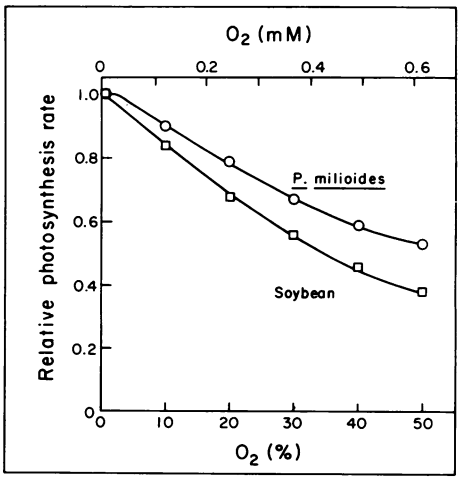

FIG. 3. Effect of O_2 concentration on relative net photosynthesis rate in *P. milioides* and soybean at 25 C. Photosynthesis rates at 21% O_2 were 31.2 mg CO_2 $dm^{-2}hr^{-1}$ for *P. milioides*, and 30.5 mg CO_2 $dm^{-2}hr^{-1}$ for soybean.

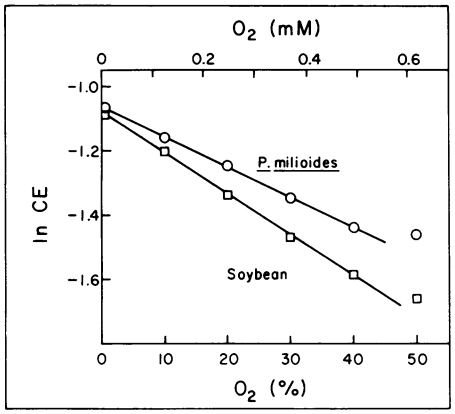

FIG. 4. Effect of O_2 concentration on carboxylation efficiency in *P. milioides* and soybean at 25 C.

Table II. *Km (CO_2) and Ki (O_2) Values for Partially Purified RuDP Carboxylase from Soybean and P. milioides*

Km and Ki values are the mean of three enzyme preparations for each species. Experiments were conducted under N_2, 50% O_2/50% N_2, and 100% O_2 at pH 8.

Species	Km (CO_2)	Ki (O_2)
	μM	mM
Soybean	25	0.78
P. milioides	17	0.81

thesis (13), an expression of Γ in C_3 plants was derived from RuDP carboxylase kinetics with respect to CO_2 and O_2. In this analysis, Γ was considered to be the CO_2 concentration, at a given O_2 concentration, where the rate of CO_2 evolution by photorespiration equals the rate of CO_2 uptake by photosynthesis. If the rate of photorespiration is regulated by RuDP oxygenase activity, and the rate of photosynthesis is regulated by RuDP carboxylase activity, then at Γ,

$$t\nu_o = \nu_r \quad (1)$$

where ν_o is the velocity of the oxygenase reaction, ν_r is the rate of CO_2 fixation by RuDP carboxylase, and t is the proportion of glycolate oxidized to CO_2. Previously (13, 15), t was defined as the proportion of glycolate carbons released as CO_2 in photorespiration, 0.25. A more correct kinetic definition of t is the ratio of mol of CO_2 released in photorespiration to the mol of O_2 consumed in RuDP oxygenase reaction. According to the stoichiometry between O_2 uptake and CO_2 evolution in photorespiration proposed by Tolbert (18), $t = 0.5$. Expressing ν_o and ν_r in terms of RuDP carboxylase kinetics (13), equation 1 becomes

$$tV_oK_rO/(K_rK_o + K_rO + K_oC) = V_rK_oC/(K_rK_o + K_rO + K_oC) \quad (2)$$

where V_o and V_r are the maximum velocities of RuDP oxygenase and RuDP carboxylase, K_o and K_r are the Michaelis constants for O_2 and CO_2, O is the O_2 concentration, and C is the CO_2 concentration. At Γ, $C = \Gamma$. Solving,

$$\Gamma = tV_oK_rO/V_rK_o \quad (3)$$

This equation predicts the linear response of Γ to increasing O_2 concentration observed in C_3 plants (Fig. 2, 9, 13).

It is evident that RuDP carboxylase kinetics alone does not account for the O_2 response of Γ in *P. milioides*, because Γ is not directly proportional to O_2 concentration (Fig. 2). Figure 2 suggests an additional fixation reaction, and the carboxylase analysis (Table I) suggests that an increased PEP carboxylase activity may account for this fixation. If PEP carboxylase is fixing a significant amount of CO_2 at Γ, then, at Γ, the rate of CO_2 evolution in photorespiration is equal to the combined rate of CO_2 fixation by both RuDP and PEP carboxylases, or,

$$t\nu_o = \nu_r + \nu_p \quad (4)$$

where t, ν_o, and ν_r are as defined above, and ν_p is the velocity of the PEP carboxylase reaction. Expansion of equation 4 in terms of RuDP and PEP carboxylase kinetics,

$$tV_oK_rO/(K_rK_o + K_rO + K_oC) = V_rK_oC/(K_rK_o + K_rO + K_oC) + V_pC/(K_p + C) \quad (5)$$

where t, V_o, V_r, K_r, K_o, O, and C are defined above, and V_p is the maximum velocity of PEP carboxylase, and K_p is the Km (HCO_3^-) of PEP carboxylase expressed as the equivalent CO_2 concentration. At Γ, $C = \Gamma$. Solving,

$$\Gamma = [-b + (b^2 - 4ac)^{1/2}]/2a \quad (6)$$

where

$$a = (V_r + V_p)K_o \quad (7)$$

$$b = (V_p - tV_o)K_rO + (V_rK_p + V_pK_r)K_o \quad (8)$$

and

$$c = -tV_oK_rK_pO. \quad (9)$$

The response of Γ to O_2 concentration is not obvious from equation 6, so some arbitrary solutions to the equation are given in Figure 5. Curve A simulates the observed response of soybean, and curve C simulates the response of *P. milioides*. In curve A, it was assumed that no PEP carboxylase recycling occurs. This assumption is based on the observation that only a negligible amount of CO_2 is fixed into C_4 acids at Γ in the C_3 plant barley (8, 11). Curve C assumes some PEP carboxylase recycling at Γ, as has been demonstrated for *P. milioides* (8, 11).

The difference in response of Γ to O_2 in soybean and *P. milioides* is not adequately explained solely on the basis of differences in extractable PEP carboxylase activity. If CO_2 fixation by PEP carboxylase *in vivo* were proportional to the extractable activity of this enzyme in both species, the Γ response curve ought to intercept the abscissa in soybean as it does for *P. milioides*, although the intercept would be much closer to the origin (Fig. 5, curve B). However, the soybean response curve intercepts the origin (Fig. 2), an observation typical for C_3 plants. The response of Γ to O_2 in *P. milioides* cv. P.I. 285220 (17), a cultivar in which the PEP carboxylase to RuDP carboxyl-

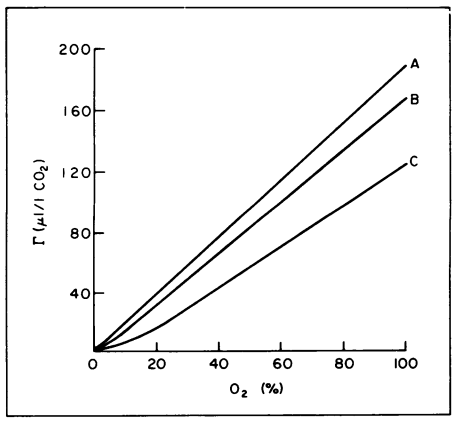

FIG. 5. Simulation of response of Γ to O_2 concentration at 25 C. These curves represent solutions to equation 6. Constants used in all curves are: V_r, 2000 μmol/dm²·hr; V_o, 1000 μmol/dm²·hr; K_r, 16 μM; K_o, 0.75 mM; t, 0.50. Values of V_p (μmol/dm²·hr) and K_p (μM CO_2) are, respectively: curve A: 0 and 0; curve B: 33 and 0.2; curve C: 100 and 0.2.

ase ratio is equal to that in soybean (R. Chollet and B. Quebedeaux, personal communication) is described by curve B in Figure 5.

The positive intercept obtained by extrapolating Γ at high O_2 to low O_2 in *P. milioides* species (Figs. 2 and 5; ref. 17) indicates that the $Km(HCO_3^-)$ of PEP carboxylase, when expressed as the CO_2 concentration in equilibrium with HCO_3^-, is substantially less then the $Km(CO_2)$ of RuDP carboxylase. The $Km(HCO_3^-)$ for spinach leaf PEP carboxylase is very low (14), and a similar enzyme may be present in *P. milioides*. The absence of a curvilinear response curve in soybean indicates that either the $Km(HCO_3^-)$ of soybean PEP carboxylase is higher than the $Km(CO_2)$ of RuDP carboxylase and $Km(HCO_3^-)$ of *P. milioides* PEP carboxylase, or soybean PEP carboxylase is largely inactive in the light. The latter possibility is supported by the observations that a smaller percentage of CO_2 is fixed into C_4 acids at air levels of CO_2 than at Γ (11), and that PEP carboxylase in spinach, also a C_3 plant, has a low $Km(HCO_3^-)$ (18).

The reduced O_2 sensitivity of photosynthesis and carboxylation efficiency in *P. milioides* (Figs. 3 and 4; ref. 3) may be ascirbed in part to PEP carboxylase fixation of CO_2. Because PEP carboxylase activity is not affected by O_2 (1), O_2 would inhibit only a part of CO_2 uptake. A second component of reduced O_2 sensitivity in *P. milioides* may be a greater affinity of *P. milioides* RuDP carboxylase for CO_2 (Table II). In C_3 plants, Γ is determined by the kinetic constants of RuDP carboxylase with respect to CO_2 and O_2 (13; equation 3). If PEP carboxylase fixation were reducing Γ from 40 μl/l CO_2, typical for C_3 plants, to 20 μl/l CO_2, as found in *P. milioides* (Fig. 2, ref. 3), then PEP carboxylase must fix at least one-half of the CO_2 incorporated at Γ. Furthermore, if PEP carboxylase fixation were the major factor in reduced O_2 sensitivity of photosynthesis in *P. milioides* at 300 μl/l CO_2, then a significant fraction of the products must be C_4 acids. However, product analysis of *P. milioides* photosynthesis indicates that only 15% of the products at Γ were C_4 acids, and at 300 μl/l CO_2, no C_4 acids were synthesized (11). Thus, PEP carboxylase fixation is a minor component of CO_2 uptake at Γ and is negligible at air levels of CO_2, so cannot account for all the difference in Γ and O_2 inhibition observed in *P. milioides* and soybean.

The conclusions drawn from the data presented here and reported elsewhere (3, 8, 11) are that at O_2 concentration up to 10% in *P. milioides*, much of the photosynthetic CO_2 fixation is catalyzed by PEP carboxylase. Above 10% O_2, the slope of the Γ response curve and O_2 inhibition of photosynthesis is less than in soybean because of, in small part, PEP carboxylase fixation. A second, more important factor is also present, and this may be an increased affinity of *P. milioides* RuDP carboxylase for CO_2. The measured $Km(CO_2)$ differences in RuDP carboxylase isolated from the two species (Table II) were less than could be reliably established by standard assay techniques, yet this small variation is sufficient to account for all of the observed differences in O_2 inhibition of photosynthesis and carboxylation efficiency. The O_2 response of Γ and O_2 inhibition of photosynthesis and carboxylation efficiency is about 50% greater in soybean than in *P. milioides*, and this is equal to the measured difference in $Km(CO_2)$. Because the initial products of photosynthesis in *P. milioides* at Γ and atmospheric CO_2 concentration are predominantly products of C_3 photosynthesis, and not C_4 acids (8, 10, 11), the reduced O_2 sensitivity in this species is more consistent with the concept of an altered RuDP carboxylase than with increased PEP carboxylase fixation. Quantitation of the two potential components of reduced O_2 sensitivity in *P. milioides* will require more precise data.

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