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Photosynthetic Plasticity in *Flaveria brownii*

Growth Irradiance and the Expression of C_4 Photosynthesis[1]

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ABSTRACT

Photosynthesis was examined in leaves of *Flaveria brownii* A. M. Powell, grown under either 14% or 100% full sunlight. In leaves of high light grown plants, the CO_2 compensation point and the inhibition of photosynthesis by 21% O_2 were significantly lower, while activities of ribulose 1,5-bisphosphate carboxylase/oxygenase and various C_4 cycle enzymes were considerably higher than those in leaves grown in low light. Both the CO_2 compensation point and the degree of O_2 inhibition of apparent photosynthesis were relatively insensitive to the light intensity used during measurements with plants from either growth conditions. Partitioning of atmospheric CO_2 between Rubisco of the C_3 pathway and phosphoenolpyruvate carboxylase of the C_4 cycle was determined by exposing leaves to $^{14}CO_2$ for 3 to 16 seconds, and extrapolating the labeling curves of initial products to zero time. Results indicated that ~94% of the CO_2 was fixed by the C_4 cycle in high light grown plants, versus ~78% in low light grown plants. Thus, growth of *F. brownii* in high light increased the expressed level of C_4 photosynthesis. Consistent with the carbon partitioning patterns, photosynthetic enzyme activities (on a chlorophyll basis) in protoplasts from leaves of high light grown plants showed a more C_4-like pattern of compartmentation. Pyruvate,Pi dikinase and phosphoenolpyruvate carboxylase were more enriched in the mesophyll cells, while NADP-malic enzyme and ribulose 1,5-bisphosphate carboxylase/oxygenase were relatively more abundant in the bundle sheath cells of high light than of low light grown plants. Thus, these results indicate that *F. brownii* has plasticity in its utilization of different pathways of carbon assimilation, depending on the light conditions during growth.

Plants in the genus *Flaveria* assimilate atmospheric CO_2 through different pathways including C_3, C_3-C_4 and C_4 photosynthesis (6). Though classified as a typical C_4 species in the past (1, 25), *Flaveria brownii* is now considered to be C_4-like (5, 13) in that it incorporates a significant portion of the atmospheric CO_2 by an active C_3 pathway present in mesophyll cells (5). While possessing well differentiated Kranz cells (5, 15), it lacks complete compartmentation of key enzymes in both the C_3 and C_4 pathways (2, 5, 13, 26), and exhibits substantial O_2 inhibition of AP[3] and quantum yield (5, 20). Recently some evidence has been presented to suggest that the expression of certain C_4 characteristics in *F. brownii* is under environmental control (13, 17). The activities of PEPC and especially PPDK in *F. brownii* were higher in plants grown under high light with a long photoperiod than those grown under low light with a short photoperiod (13). Furthermore, when grown in a greenhouse during summer (under high light, high temperature, and a long photoperiod) leaves of *F. brownii* exhibited a $\delta^{13}C$ value of −14.5‰, typical of C_4 plants; but, when grown during winter (under low light, low temperature, and a short photoperiod) the leaf $\delta^{13}C$ value shifted to −21‰ (17), which is intermediate to values of C_3 and C_4 species (−25 to −35‰ *versus* −10 to −17‰) (9). However, in another study Monson *et al.* (21) found no difference in carbon isotope composition in greenhouse grown *F. brownii* with leaf samples collected in March and July.

Leaf photosynthetic characteristics are dependent upon the incident light received during growth (3). Studies with leaves of maize and *Amaranthus* indicate that high growth irradiance increases the activities of several key C_4 enzymes, and changes both the maximum photosynthetic rates and the light-saturation characteristics of leaves (10). However, $^{14}CO_2$-labeling experiments provided no evidence for significant changes in the pathway of photosynthesis in either species grown under high or low light regimes (10). Environmental variables such as light and temperature generally exert little influence on leaf $\delta^{13}C$ during growth of C_3 and C_4 plants, there being only a few species with values shifted by as much as 2‰ (12, 23). The shift of carbon isotope composition (by 6.5‰) between winter and summer growth conditions suggests that the mode of photosynthesis in *F. brownii* may be altered by environmental conditions. However, the specific environmental factor(s) responsible for this alteration is not known. In this study, light intensity during growth was examined as a possible factor contributing to such a change in photosynthetic pathway.

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[3] Abbreviations: AP, apparent photosynthesis; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase; PEPC, phosphoenolpyruvate carboxylase; $\delta^{13}C$, carbon isotope composition; PPFD, photosynthetic photon flux density; Γ, photosynthetic CO_2 compensation point; LMP, protoplasts from larger mesophyll cells; SMP, protoplasts from smaller mesophyll cells; BSP, protoplasts from bundle sheath cells; PPDK, pyruvate, Pi dikinase; NADP-ME, NADP-malic enzyme; NADP-MDH, NADP-malate dehydrogenase.

MATERIALS AND METHODS

Plant Material

Plants of *Flaveria brownii* A. M. Powell (clone B6), *Flaveria cronquistii* A. M. Powell, *Flaveria sonorensis* A. M. Powell, *Flaveria floridana* J. R. Johnson and *Flaveria trinervia* (Spreng.) Mohr were propagated vegetatively from cuttings and were grown in a greenhouse during summer (from June to August), under full sunlight with a maximum midday PPFD of 1750 $\mu E\ m^{-2}\ s^{-1}$ (high light grown plants). Following initiation of growth under full sunlight some plants were transferred to an adjacent cheesecloth covered cage (1 × 1 × 1 m), with a maximum midday PPFD of 250 $\mu E\ m^{-2}\ s^{-1}$ (14% of full sunlight; low light grown plants). During the summer months, the natural photoperiod was ~16 h and the thermoperiod was controlled at ~25°C day/18°C night. Leaf (or air) temperature did not appear to vary between light regimes. Plants were watered with nutrient solution once a week. Leaves from the third node from the apex were used for all experiments. Plants were grown in each light regime for at least 6 weeks before being used for experiments. Generally, it takes about 10 d for first node leaves to become third node leaves. Thus, the leaves used for experiments were developed entirely under their respective light regimes.

CO_2 Gas Exchange Measurement

Γ was determined following procedures described by Rumpho *et al.* (27). Intact leaves were sealed in a plexiglass chamber and illuminated with a PPFD of either 240 or 1150 $\mu E\ m^{-2}\ s^{-1}$. Gas samples were withdrawn from the chamber and injected into an IR gas analyzer to determine the $[CO_2]$ at equilibrium.

AP of whole leaves was measured with an open IRGA systems as described previously (18). PPFD inside the leaf chamber was either 300 or 1650 $\mu E\ m^{-2}\ s^{-1}$. The CO_2 concentration in the leaf chamber was maintained at 330 ± 5 $\mu L\ L^{-1}$ and the O_2 level was either 2 or 21%. The gas mixtures were generated using Wosthoff pumps by mixing gases from cylinders containing 1% CO_2 in N_2 and 2 or 21% O_2 in N_2. All CO_2 exchange measurements were conducted at 30 ± 0.2°C.

Protoplast Isolation and Purification

Previously, leaves of *F. brownii* were shown to contain bundle sheath cells and two types of mesophyll cells (5). Smaller mesophyll cells surround bundle sheath cells, and larger mesophyll cells are located between epidermis and smaller mesophyll cells. Protoplasts of these three cell types were isolated similarly to Cheng *et al.* (5) with some modifications. All the media used in isolating and purifying protoplasts are the same as described previously unless otherwise indicated (5). In the digestion medium 0.5% (w/v) Rohament P pectinase was used in place of 0.1% Pectolyase, and leaf segments were digested for 4 h instead of 2.5 h. After filtering and washing the leaf segments in a 0.5 M sucrose medium (5), 2-mL aliquots of the filtrate were poured into 15 × 125 mm glass tubes which were overlaid with 1 mL of sorbitol medium. After centrifugation at 200*g* for 1 min, three fractions of protoplasts were obtained, one at the sorbitol/sucrose interface (source of LMP), one suspended in the original filtrate (source of protoplasts from SMP), and the other pelleted at the bottom of the tube (source of BSP).

The fraction which partitioned at the sorbitol/sucrose interface consisted of purified LMP. These protoplasts were collected, resuspended in 3 volumes of sucrose medium, and finally spun at 150*g* for 1 min to concentrate the LMP at the top of the solution.

Protoplasts remaining in the filtrate after the initial centrifugation (200*g*, 1 min) were mostly SMP, with a very small portion from the other two cell types. Dextran powder was added to this fraction, giving a final concentration of 15% (w/v). This dextran-supplemented fraction (3-mL aliquots) was overlaid sequentially with 2 mL each of 10.7% dextran, sucrose, sorbitol-sucrose (a mixture of an equal volume of sorbitol and sucrose media), and sorbitol media (5). Purified SMP were collected between the interface of the sorbitol-sucrose/sucrose media after centrifugation at 300*g* for 10 min.

The pellet after the initial centrifugation contained ~70% BSP and ~30% SMP. This was first dispersed in 15% dextran medium and then overlaid sequentially with 10.7% dextran (5), 5.4% dextran (sucrose medium plus 5.4% dextran) and sucrose media. Purified BSP were obtained from the interface between the 5.4 and 10.7% dextran layers after centrifugation at 300*g*, 10 min. Since dextran interferes with Chl determination, it was removed by two washes of the BSP with 0.25 M Hepes medium (150*g*, 1 min) (5). The two mesophyll cell types and bundle sheath cells can be distinguished visually as they are very different in size, and in organelle content and distribution (for details, see Ref. 5). The purity of these protoplasts was thus determined, from replicate preparations, based on protoplast counts under a microscope. The cross contamination was estimated to be less than 5%.

Enzyme Extraction and Assays

Photosynthetic enzymes from both whole leaves and protoplasts were extracted as in Cheng *et al.* (5), except 80 μM pyridoxal 5-phosphate was added to the extraction media. Protoplasts were illuminated to activate certain enzymes prior to extraction (5). All enzyme assays were conducted at 30°C. PPDK (EC 2.6.1.2) was assayed as described by Edwards *et al.* (8); PEPC (EC 4.1.1.32) was determined after Uedan and Sugiyama (29); and NADP- ME (EC 1.1.1.40) and NADP-MDH (EC 1.1.1.82) were assayed according to Kanai and Edwards (16). The NADP-MDH in the whole leaf extract was fully activated by DTE under an atmosphere of N_2 (22). Rubisco (EC 4.1.1.39) was measured radiometrically using $NaH^{14}CO_3$ (19). Aspartate (:2-oxoglutarate) aminotransferase (EC 2.6.1.1) and alanine (:2-oxoglutarate) aminotransferase (EC 2.6.1.2) were assayed following procedures described by Edwards and Gutierrez (7). All reaction rates were linear for 2 to 6 min, and the enzymes used in all coupling assays were verified to be nonlimiting.

$^{14}CO_2$ Labeling and ^{14}C-Product Identification

$^{14}CO_2$-labeling experiments were conducted with excised, whole leaves. After the indicated times of continuous exposure

to $^{14}CO_2$ (425 μL L^{-1}, 14.2 Ci mol^{-1}), the leaf was quickly killed in boiling 80% (v/v) ethanol. The conditions and procedures for these experiments followed those of Moore *et al.* (22). Soluble ^{14}C-products were extracted from leaves as described by Rumpho *et al.* (27), with the radioactivity in the final insoluble fraction being less than 3%. Labeled metabolites were separated and identified by two-dimensional thin-layer electrophoresis and chromatography on cellulose plates, according to Schürmann (28). Recovery of radioactivity from the plates was essentially 100%. ^{14}C-label in glycerate was counted with that in 3-phosphoglycerate.

Chl Measurement.

Chl contents were determined according to Wintermans and De Mots (32).

RESULTS

CO_2 Gas Exchange Characteristics

When measured at a light intensity of either 240 or 1150 μE m^{-2} s^{-1}, Γ of *F. brownii* was lower (3–5 μL L^{-1}) in leaves grown under high light than in leaves developed under low light (10–12 μL L^{-1}) (Table I). The light intensity used during measurement (240 *versus* 1150 μE m^{-2} s^{-1}) had no apparent effects on Γ in leaves of *F. brownii* from either light regime. These data indicate that apparent photorespiration in this species is lower when plants are grown at the higher light intensity. In contrast, there was no effect of different light intensities during growth on Γ of several other *Flaveria* species including C_3, C_3-C_4, and C_4 species (Table I). However, Γ in the two C_3-C_4 species from both light regimes decreased as the measuring light intensity increased, similar to those reported previously for C_3-C_4 species from other genera (4, 14).

The degree of inhibition of AP by 21% O_2 in *F. brownii* was also influenced by the light intensity during growth. In leaves from high light grown plants, AP was inhibited only 11 to 13% by 21% O_2 compared to 17 to 19% in leaves from low light grown plants (Table II), whether using a high (1650 μE m^{-2} s^{-1}) or low (300 μE m^{-2} s^{-1}) light intensity during measurements. The light levels used in the experiments, however, did influence the rates of AP. When measured under 300 μE m^{-2} s^{-1}, rates of AP were equivalent in plants from either light regime. However, when measured at 1650 μE m^{-2} s^{-1}, the rate of AP was about twice as high in plants grown in high light compared with those grown in low light (Table II).

Activities of Photosynthetic Enzymes in Whole Leaves

The activities of various enzymes of the C_3 and C_4 pathways of photosynthesis were found to be lower in low light grown plants than in high light grown plants (Table III). Depending on the enzyme, the decrease ranged from about 20 to 60% on a Chl basis or about 10 to 50% on a leaf area basis. Notably, activities of PPDK, PEPC, and NADP-MDH were suppressed the most, while the activity of aspartate aminotransferase was affected the least. PEPC activities were suppressed more under the low light regime than was Rubisco.

Table I. *Influence of Light Intensity during Growth on the Photosynthetic CO_2 Compensation Point in Leaves of Various Flaveria species*

The maximum midday PPFD were 1750 and 250 μE m^{-2} s^{-1} in the high and low light regimes, respectively. For details on growth conditions see "Materials and Methods." The CO_2 compensation points were measured at 30 ± 0.2°C. Values are the means of measurements on three to four leaves ± SE.

Species	Measuring Light Intensity (μE m^{-2} s^{-1})			
	High light grown		Low light grown	
	240	1150	240	1150
	μL L^{-1}			
C_3:				
F. cronquistii	58.2 ± 1.8	56.2 ± 1.8	56.9 ± 1.0	57.5 ± 0.8
C_3-C_4:				
F. sonorensis	51.1 ± 1.1	30.0 ± 1.7	49.9 ± 0.6	29.6 ± 1.3
F. floridana	18.9 ± 0.9	8.5 ± 1.0	20.4 ± 0.9	8.4 ± 1.4
C_4-like:				
F. brownii	4.9 ± 0.8	3.2 ± 1.3	11.5 ± 1.3	10.0 ± 1.6
C_4:				
F. trinervia	6.4 ± 1.1	4.5 ± 0.3	3.9 ± 0.3	3.9 ± 0.3

Table II. *Influence of Light Intensity during Growth on Rates and O_2 Inhibition of Apparent Photosynthesis in Leaves of F. brownii*

The net photosynthetic rates were measured at 30 ± 0.2°C, 21% O_2 and 330 ± 5 μL L^{-1} CO_2. The O_2 inhibition is expressed as the % reduction in photosynthetic rate at 21% O_2 relative to the rate measured at 2% O_2, with other conditions held constant. Values are the means of measurements on three to four leaves ± SE. The light condition for growth is as described in Table I.

Measuring Light Intensity	High Light Grown		Low Light Grown	
	Net photosynthesis	O_2 inhibition	Net photosynthesis	O_2 inhibition
$\mu E\ m^{-2}\ s^{-1}$	$\mu mol\ dm^{-2}\ h^{-1}$	%	$\mu mol\ dm^{-2}\ h^{-1}$	%
300	309 ± 23	12.9 ± 0.7	275 ± 32	19.3 ± 2.1
1650	907 ± 20	11.4 ± 0.5	493 ± 25	17.5 ± 1.5

Table III. *Activities of Various Photosynthetic Enzymes in Leaves of F. brownii Grown under Different Light Regimes*

Leaves were harvested *in situ* after 4 to 6 h into the photoperiod and were stored in liquid N_2 until used. Values are the means from three separate extractions. Numbers in parentheses are the percent of expressed activities, relative to the high light grown leaves.

Enzyme	High Light Grown		Low Light Grown	
	$\mu mol\ mg\ Chl^{-1}\ h^{-1}$	$\mu mol\ dm^{-2}\ h^{-1}$	$\mu mol\ mg\ Chl^{-1}\ h^{-1}$	$\mu mol\ dm^{-2}\ h^{-1}$
PPDK	210	859	121 (58)	577 (67)
PEPC	521	2131	210 (40)	1002 (47)
NADP-MDH	1357	5550	581 (43)	2771 (50)
Aspartate aminotransferase	908	3714	709 (78)	3382 (91)
Alanine aminotransferase	964	3943	601 (62)	2867 (73)
NADP-ME	804	3288	535 (67)	2552 (78)
Rubisco	375	1534	228 (61)	1088 (71)

CO_2 Labeling

To examine if light influences the partitioning of atmospheric CO_2 between PEPC and Rubisco, whole leaves from plants grown under the two light regimes were continuously exposed to $^{14}CO_2$ for up to 16 s and the initial photosynthetic products were analyzed (Fig. 1). Extrapolation of the labeling curves to zero time showed that in the high light grown plants about 94% of the initial ^{14}C-products were C_4 acids (malate plus aspartate), and about 6% were C_3 products (3-PGA plus sugar-phosphates). However, in low-light grown plants only about 78% of the initial products were C_4 acids, while about 20% were C_3 products. Replicate experiments were performed with plants from each light regime, and the carbon partitioning into the C_4 acids was estimated to be 94 and 81% in high light and low light grown plants, respectively.

Intercellular Localization of Photosynthetic Enzymes

Our previous study has shown that leaves of *F. brownii* contain bundle sheath cells, smaller mesophyll cells surrounding the bundle sheath cells, and larger mesophyll cells external to the smaller mesophyll cells (5). In this study, protoplasts of these cell types were isolated in order to evaluate whether the light intensity during growth may affect the intercellular compartmentation of certain photosynthetic enzymes. The purity

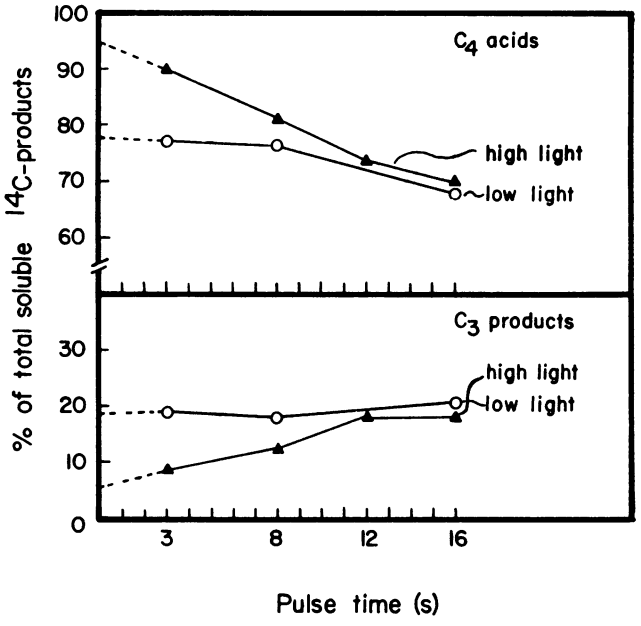

Figure 1. Change in percent distribution of radioactivity among soluble ^{14}C-products in a $^{14}CO_2$ (425 μL L^{-1}) pulse series in leaves of *F. brownii* grown under high (▲) or low (○) light conditions. See "Materials and Methods" for the light intensity during growth. The pulse experiments were performed with excised leaves at ~25°C and with a PPFD of ~1000 to 1200 μE m^{-2} s^{-1} on both upper and lower leaf surfaces. Dashed lines indicate linear extrapolation of the labeling curves to zero time (based on the shorter exposures to $^{14}CO_2$). Metabolites grouped as C_4 acids include malate and aspartate. Those grouped as C_3 products include 3-phosphoglycerate and sugar monophosphates plus sugar bisphosphates. Data presented are from one set of experiments; similar results were obtained from another replicate.

of the preparations of each protoplast type from leaves grown under high or low light intensity was similar to that reported previously (5) (also see "Materials and Methods"). In terms of size and Chl content, the protoplasts of each cell type showed no noticeable differences with respect to growth conditions, and were similar to those isolated from leaves grown in a growth chamber (5).

On a Chl basis, photosynthetic enzyme activities in protoplasts isolated from leaves grown in both light regimes showed three general patterns of incomplete compartmentation (Tables IV and V). First, PPDK, PEPC, and NADP-MDH, enzymes for the carboxylation phase of the C_4 cycle, were highest in SMP, intermediate in LMP, and lowest in BSP (with the exception of PPDK from the high light grown leaves). Second, aspartate and alanine aminotransferases were present in higher activities in SMP and LMP, and in lower activities in BSP. Third, in contrast to the aminotransferases, activities of Rubisco and NADP-ME exhibited a reverse gradation with the highest activities being in BSP. In leaves from plants of both light regimes PEPC was mainly localized in the SMP and Rubisco and NADP-ME were predominantly found in the BSP, but the other enzymes showed substantial activities in all three cell types.

In general, compared to low light grown plants, enzyme activities were significantly higher in all leaf cell types from high light grown plants. Furthermore, leaves from the high light regime did have a more C_4-like compartmentation of PPDK, PEPC, Rubisco, and NADP-ME between cell types than did leaves from the low light regime. Relative to low light grown leaves, leaves grown under high light have higher activity ratios of MP to BSP for PPDK and PEPC, and lower activity ratios for Rubisco and NADP-ME. Although the absolute activities of PEPC in BSP and Rubisco in mesophyll protoplasts were similar for both growth conditions, one should note that the activity ratios of PEPC to Rubisco were higher in mesophyll protoplasts and lower in BSP in high light grown leaves than in low light grown leaves. In contrast, the distributions of activities of NADP-MDH and the aminotransferases among cell types were similar in leaves from both light regimes.

DISCUSSION

At ambient levels of O_2, plants grown under the two light regimes had similar rates of apparent photosynthesis when measured at a low light intensity. However, when measured under a high light intensity the high-light grown plants had much higher rates of photosynthesis (Table II). Thus, leaves of *F. brownii* do show some capacity for photosynthetic light acclimation.

The activity of Rubisco has been shown to correlate with both the photosynthetic capacity of many sun-adapted species with C_3 photosynthesis and with the variation in photosynthesis due to light acclimation (see Ref. 3 for review). Usuda *et al.* (31) have suggested that PPDK and Rubisco are rate-limiting enzymes in C_4 photosynthesis during light acclimation in maize. In the present study, the activities of all the photosynthetic enzymes examined were well in excess of the photosynthetic rates, except that of PPDK from high light grown plants, which was very similar to the photosynthetic

Table IV. *Intercellular Localization of Photosynthetic Enzymes in Leaf Protoplasts of F. brownii Grown under Full Sunlight*

Protoplasts were illuminated with a PPFD of 800 μE m^{-2} s^{-1} for 1 min and subsequently were osmotically shocked (see Ref. 5). Chloroplasts were ruptured by two cycles of freeze/thaw, and spun at 15,000g for 1 min. The clarified supernatants were immediately used for enzyme assays. Values are the means of four separate experiments. In all cases, the standard errors were less than 5% of the means (not shown).

Enzyme or Enzyme Ratio	Cell Type			Ratio	
	LMP[a]	SMP	BSP	LMP/BSP	SMP/BSP
	μmol mg Chl^{-1} h^{-1}				
PPDK	86	75	20	4.3	3.8
PEPC	410	931	83	4.9	11.2
NADP-MDH	431	486	322	1.3	1.5
Aspartate aminotransferase	897	902	487	1.8	1.9
Alanine aminotransferase	728	1024	471	1.6	2.2
NADP-ME	30	188	1328	0.02	0.14
Rubisco	56	224	453	0.1	0.5
PEPC/Rubisco	7.32	4.16	0.18		

[a] LMP, larger mesophyll protoplast extract; SMP, smaller mesophyll protoplasts extract; BSP, bundle sheath protoplast extract.

Table V. *Intercellular Localization of Photosynthetic Enzymes in Leaf Protoplasts of F. brownii Grown under 14% Full Sunlight*

Values are the means of three separate experiments; standard errors were less than 7% of the means. Other conditions are as in Table IV.

Enzyme or Enzyme Ratio	Cell Type			Ratio	
	LMP	SMP	BSP	LMP/BSP	SMP/BSP
	μmol mg Chl^{-1} h^{-1}				
PPDK	36	51	18	2.0	2.8
PEPC	240	311	87	2.8	3.6
NADP-MDH	145	155	78	1.9	2.0
Aspartate aminotransferase	735	637	439	1.7	1.5
Alanine aminotransferase	636	544	346	1.8	1.6
NADP-ME	166	265	745	0.2	0.4
Rubisco	66	184	268	0.3	0.7
PEPC/Rubisco	3.64	1.69	0.32		

rate measured at near full sunlight (1650 μE m^{-2} s^{-1}) (Tables II and III). PPDK may restrict the extent of rate adjustments during light acclimation in leaves of *F. brownii* (also see Ref. 13).

When measured over a broad range of light intensities, Γ in leaves of high light grown *F. brownii* was always similar to that of C_4 species, while Γ in low light grown leaves was markedly higher and more similar to values of some C_3-C_4 *Flaveria* species (Table I) (see Ref. 6 for review). Also, the inhibition of AP by 21% O_2 was higher in leaves of low light grown *F. brownii*, independent of whether the measurements were done with a light intensity of 300 or 1650 μE m^{-2} s^{-1} (Table II). However, in the two C_3-C_4 *Flaveria* species included in this study, Γ increased as the light intensity was decreased during the assays, but was not influenced by the light intensity imposed during leaf growth (Table I). Similar observations have been made in other studies on C_3-C_4 plants, *Panicum milioides* and *Moricandia arvensis* (4, 14). It is not clear why Γ in these C_3-C_4 plants does vary with light intensity. Brown and Morgan (4) have suggested that a light-dependent CO_2-recycling mechanism or a reduced rate of photorespiration at higher light intensity may occur in *P. milioides*. In *F. brownii*, apparent photorespiration (as determined from Γ) in high light grown leaves may in part be reduced by the mechanisms suggested by Brown and Morgan (4) and/or by a decrease in dark respiratory CO_2 loss, but these factors cannot also explain the concomitant decrease in the O_2 sensitivity of AP. Rather, in *F. brownii* these may be largely reduced by an increased expression of C_4 photosynthesis when grown under high light (Table IV and V and Fig. 1) (see below for discussion).

It is intriguing why apparent photorespiration in the two C_3-C_4 *Flaveria* species examined is not influenced by growth irradiance as occurs in *F. brownii* (Table I). Perhaps, unlike the case of *F. brownii* which has a relatively high capacity for C_4 photosynthesis, growth irradiance may exert little effect on the expression of C_4 photosynthesis in these two C_3-C_4 species. Whether growth irradiance influences photorespiration in other *Flaveria* species remains to be investigated.

F. brownii was initially classified as a typical C_4 species (1, 25). However, recent reports showed that leaves of *F. brownii* lack strict intercellular compartmentation of several photosynthetic enzymes, including Rubisco and PEPC (2, 5, 13, 26), and show a CO_2 gas exchange response atypical of C_4 plants (5, 13, 20). Additionally, ~20% of the atmospheric CO_2 was estimated to be directly fixed into the C_3 cycle by Rubisco in leaf mesophyll cells of chamber-grown (400 μE m^{-2} s^{-1}) plants of *F. brownii* (5). Thus, more recently *F. brownii* has been suggested to be a C_4-like species (5, 13) or a C_4-like C_3-C_4 intermediate (20). In this study, the carbon

partitioning pattern was shown to be altered by the light intensity during growth: a more typical C_4 pattern in high light grown leaves *versus* a partial fixation of CO_2 directly through the C_3 cycle in leaves grown in low light (Fig. 1). Thus, light appears to increase the expression of the C_4 cycle in *F. brownii*. These results are consistent with the shift of $\delta^{13}C$ values resulting from changing growth conditions (see the introduction), and indicate that the photosynthetic mode in leaves of *F. brownii* is altered by the light intensity imposed during growth. This alteration in photosynthetic pathway has not been observed in other nonsucculent higher plants during light acclimation (10).

The biochemical basis for this environmental regulation in carbon metabolism in *F. brownii* is uncertain. The direct entry of CO_2 into the C_3 pathway could be due to a more leaky bundle sheath compartment which developed under low light during growth, thus allowing external CO_2 to enter both mesophyll and bundle sheath cells simultaneously. The variation in $\delta^{14}C$ values among different C_4 subgroups is thought to be related to the degree of leakage in the bundle sheath (11). Thus, the shift in $\delta^{13}C$ values in *F. brownii* could reflect variations in leaf anatomy which affect bundle sheath conductance to CO_2.

It is more likely that the direct fixation of some CO_2 by Rubisco is due to the limited activity of a functional C_3 cycle in mesophyll cells, as suggested to occur in chamber-grown leaves of *F. brownii* (5). Leaves grown in either high or low light contain similar activities of Rubisco in the mesophyll cells, which presumably are capable of fixing atmospheric CO_2 into C_3 products (Tables IV and V). However, the decreased labeling of C_3 metabolites in high light grown leaves may be attributed to the much higher activity ratio of PEPC to Rubisco in these mesophyll cells, which allows atmospheric CO_2 to be predominantly fixed into C_4 acids. High light grown leaves also exhibited a more C_4-like compartmentation of photosynthetic enzymes than did leaves grown in low light (Tables IV and V). These results are in accord with the more C_4-like carbon partitioning pattern observed in leaves grown under high light and the less C_4-like pattern in leaves developed under low light (Fig. 1). Thus, the more negative $\delta^{13}C$ value in leaves of *F. brownii* when grown in winter may be explained by the co-functioning of C_3 and C_4 pathways in the mesophyll cells. This, presumably, would allow Rubisco to incorporate part of the CO_2 in an "open system," resulting in a greater discrimination against $^{13}CO_2$.

Photosynthesis in C_3 plants is especially limited under high light where the capacity for generating assimilatory power is excessive relative to the CO_2 available (9). The increased expression of C_4 photosynthesis in high light grown *F. brownii* may be most advantageous for providing a high concentration of CO_2 at the site of Rubisco under such high light conditions. Since *F. brownii* is a perennial species with a relatively long growing season (25), the ability to light-acclimate could be beneficial during seasonal changes in the environment.

The results obtained from this study indicate that *F. brownii* has some plasticity in its ability to fix atmospheric CO_2 through different pathways, depending on the light environment. This plasticity may be due to a difference in the intercellular expression of principal photosynthetic enzymes. Among terrestrial plants, shifts in photosynthetic mode are well documented in facultative CAM plants (24), but are rarely found in nonsucculent higher plants. To date, *Eleocharis vivipara* is the only nonsucculent species discovered which has a habitat-dependent shift in photosynthetic pathways (30). It performs C_4 photosynthesis when inhabiting a terrestrial environment and utilizes the C_3 pathway when in a submerged aquatic habitat. To our knowledge, the present study is the first report of plasticity in the pathway of CO_2 assimilation for terrestrial plants that principally use the C_4 pathway. Whether other environmental variables, such as temperature and photoperiod, can cause a further shift between C_3 *versus* C_4 photosynthesis in this species awaits further investigation.

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