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Photosynthetic/Photorespiratory Carbon Metabolism in the C_3-C_4 Intermediate Species, *Moricandia arvensis* and *Panicum milioides*[1]

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ABSTRACT

The distribution of ^{14}C in photosynthetic metabolites of two naturally occurring higher plants with reduced photorespiration, *Moricandia arvensis* and *Panicum milioides*, in pulse and pulse-chase $^{14}CO_2$ incorporation experiments was similar to that for the C_3 species, *M. foetida* and *Glycine max*. After 6 seconds of $^{14}CO_2$ incorporation, only about 6% of the total ^{14}C fixed was in malate and aspartate in both *M. arvensis* and *P. milioides*. The apparent turnover of the C_4 acids was very slow, and malate accumulated during the day in *M. arvensis*. Thus, C_4 acid metabolism by *M. arvensis* and *P. milioides* had no significant role in photosynthetic carbon assimilation under the conditions of our experiments (310 microliters CO_2 per liter, 21% O_2, 1100 or 1900 micromoles photon per square meter per second, 27°C).

After a 36-second chase period in air containing 270 microliters CO_2 per liter, about 20% of the total ^{14}C fixed was in glycine with *M. arvensis*, as compared to 15% with *M. foetida*, 14% with *P. milioides*, and 9% with *G. max*. After a 36-second chase period in 100 microliters CO_2 per liter, the percentage in glycine was about twice that at 270 microliters CO_2 per liter in the C_3 species and *P. milioides*, but only 20% more ^{14}C was in glycine in *M. arvensis*. These data suggest that either the photorespiratory glycine pool in *M. arvensis* is larger than in the other species examined or the apparent turnover rate of glycine and the flow of carbon into glycine during photorespiration are less in *M. arvensis*. An unusual glycine metabolism in *M. arvensis* may be linked to the mechanism of photorespiratory reduction in this crucifer.

Leaf anatomical and photorespiratory CO_2 exchange characteristics of the crucifer, *Moricandia arvensis*, are intermediate between those of C_3 and C_4 plants (1–3, 8, 9, 17) and are strikingly similar to related features of the C_3-C_4 intermediate species, *Panicum milioides* (14). Rathnam and Chollet (13) proposed that a limited C_4 cycle, responsible for assimilating about 25% of the total CO_2 incorporated, reduces photorespiration in *P. milioides*. However, unlike the reported situation for *P. milioides* leaves (13), those of *M. arvensis* do not contain detectable activity of the key C_4 enzyme, pyruvate,Pi dikinase (9). Yet, the similarities of certain features of both species, such as the presence of bundle sheath cells with numerous centripetally arranged chloroplasts and mitochondria (9, 17), suggest that a C_4-type mechanism may be a factor in reducing photorespiration in *M. arvensis*. Leaves of this crucifer do accumulate substantial amounts of the C_4 acid, malate, in the light (17).

The recent findings of Winter *et al.* (17) lead one to conclude that any significant participation of C_4 acid metabolism in *M. arvensis* photosynthesis is unlikely. However, the data from their $^{14}CO_2$ incorporation studies are incomplete in that the length (5 min) of all of the chase periods used in their experiments is too long to critically evaluate C_4 acid or photorespiratory metabolism, the key photorespiratory metabolite, glycine, was not separated from other amino acids (*e.g.* glutamate, serine) in their analyses, and neither related nor representative C_3 species were used for direct comparative purposes. In addition, an atypically large portion (5–12%) of the total ^{14}C fixed after 10-s pulse photosynthesis was found in the water-insoluble fraction in their experiments with NO_3^--grown *M. arvensis*. This high percentage in the insoluble fraction following short-term photosynthesis is indicative of a poor recovery of water-soluble compounds during the extraction procedure.

Based on evidence from a recent $^{14}CO_2$ incorporation and enzyme study of *P. milioides*, Edwards *et al.* (6) disagree with the previous proposal that a limited C_4 cycle exists in this species (13) and suggest that possibly photorespiration is reduced by an efficient CO_2 recycling mechanism. However, no data are presented to support this suggestion. Thus, it is no longer certain that a C_4 cycle operates in *P. milioides*, but no information exists which indicates the presence of some other mechanism to reduce photorespiration in either this species or *M. arvensis*.

Our study examines the extent to which C_4 acid metabolism is involved in the photosynthetic/photorespiratory carbon metabolism of *M. arvensis* and *P. milioides*. Comparisons of $^{14}CO_2$ incorporation patterns during pulse-chase experiments with these C_3-C_4 intermediate species and representative C_3 and C_4 plants at atmospheric and subatmospheric levels of CO_2 suggest a possible mechanism for reducing photorespiration in *M. arvensis*.

MATERIALS AND METHODS

Plant Material. Plants of *Moricandia arvensis* (L.) DC. (C_3-C_4), *M. foetida* Bourg (C_3), *Glycine max* (L.) Merr. cv Williams (C_3), *Kalanchoë diagremontiana* Hammet et Perrier (CAM), and *Flaveria brownii* A. M. Powell (C_4) were grown in a controlled environment room at 21°C/16°C (day/night) (8, 9). *Panicum milioides* Nees ex. Trin. (C_3-C_4), P.I. No. 285220, and *Zea mays* L. (C_4) were similarly grown but at a 31°C/27°C (day/night)

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temperature regime. Some *P. milioides* plants were also grown in a greenhouse during the summer months. All plants were fertilized twice weekly with a modified Hoagland solution, which contained 13 mM NO_3^- and 2 mM NH_4^+ (*cf.* Ref. 17). Seeds of the two *Moricandia* species were generously supplied by Dr. P. Apel (Gatersleben, DDR). Dr. A. M. Powell (Sul Ross State University, Alpine, TX) graciously supplied seeds of *F. brownii*.

Determination of the Levels of Malate and PEP[4]. For each species examined, three leaves were removed from separate plants and pooled at various times during the diurnal cycle. The sampling and extraction procedures have been described in detail in Kenyon *et al.* (10). Chl concentrations in the 5% (v/v) $HClO_4$ extracts were determined indirectly by measuring the concentration of pheophytin (7, 16) before removal of the insoluble material by centrifugation. The extracts were neutralized with KOH and decolorized with activated charcoal prior to subsequent analysis (7, 10).

The enzymic assays for malate were performed at 30°C using the spectrophotometric method of Hatch (7). The PEP analyses were based on the incorporation of $H^{14}CO_3^-$ into malate via exogenous PEP carboxylase and NADH-malate dehydrogenase. The assay solution at pH 8.3 contained in 1 ml: 50 mM Tris-HCl, 10 mM $MgCl_2$, 5 mM DTT, 10 mM $NaH^{14}CO_3$ (0.5 Ci/mol), 1 mM NADH, 0.5 IU of wheat PEP carboxylase (Boehringer Mannheim), 3 IU of bovine heart NADH-malate dehydrogenase (Sigma), and 0.55 ml neutralized extract. All reactions had proceeded to completion within the 1-h assay period at 30°C, after which 0.1 ml 2 N HCl was added. The acidified solutions were dried completely to remove unfixed $^{14}CO_2$, and the amount of acid-stable ^{14}C was determined by liquid scintillation spectroscopy.

$^{14}CO_2$ Incorporation Studies. One leaf at a time was exposed to a 6-s pulse of $^{14}CO_2$ at an incident photon flux (400–700 nm) of either 1900, 1100, or 60 μmol m^{-2} s^{-1} and 27 ± 1.5°C in a 1-L plexiglass chamber. Leaves from all of the species, except *P. milioides*, remained attached to excised portions of stems, which were immersed in a reservoir of distilled H_2O in the chamber. The leaf blade was supported by a grid of nylon filaments. During the 40-min equilibration period prior to the introduction of $^{14}CO_2$, humidified air (21% O_2) containing either 270 or 100 μl CO_2/l (commercially prepared and periodically monitored with a Beckman model 215-B IR gas analyzer) was passed through the chamber at approximately 6 l/min. Incident illumination at 1100 or 1900 μmol photon m^{-2} s^{-1} was provided by 300-w Westinghouse, cool-temperature, spot lamps. Neutral density filters were used to reduce the incident photon flux to 60 μmol m^{-2} s^{-1} in some experiments.

After the 40-min equilibration period, the inlet and outlet ports of the chamber were sealed. An aliquot (100 μCi) of carrier-free $^{14}CO_2$, generated by mixing 50% (v/v) lactic acid with a $NaH^{14}CO_3$ solution (58 Ci/mol, Amersham) in a serum-stoppered vial, was injected immediately into the chamber using a gas-tight syringe, increasing the CO_2 concentration by about 40 μl/l. A small electric fan rapidly dispersed the $^{14}CO_2$. After 6 s of photosynthesis in $^{14}CO_2$, the upper portion of the chamber was lifted rapidly from its base so that the leaf could be removed and either killed in liquid N_2 or placed into another chamber containing $^{12}CO_2$-air for various chase periods before being killed. The 1-L chase chamber was flushed continuously at approximately 6 l/min with humidified air containing 270 or 100 μl CO_2/l and 21% O_2.

The frozen leaf material was ground at liquid N_2 temperature with a mortar and pestle. The leaf powder was sprinkled into a boiling 75% (v/v) ethanol/5% (v/v) HCOOH solution. HCOOH prevented the adsorption of a large percentage of the water-soluble, labeled metabolites (determined to be primarily phosphorylated sugars) onto the insoluble material. After homogenization of the ethanol-HCOOH suspension in a Ten Broeck glass homogenizer, the insoluble matter was pelleted and washed once with 50% ethanol/5% HCOOH and twice with water. During each wash, the resuspended material was heated at 70°C for 5 min before centrifugation. Most of the pigments in the 75% ethanol solution were removed by washing with petroleum ether. Less than 0.1% of the total ^{14}C fixed remained in the petroleum ether fraction after the two solutions separated.

The combined ethanol/water-soluble fractions were evaporated to dryness *in vacuo*, residual HCOOH was driven off under N_2, and the water-soluble compounds were redissolved in a known volume of water. Less than 0.05% of the total ^{14}C fixed remained in compounds that did not redissolve in water. Before determining the ^{14}C content of the ethanol/water-insoluble fraction, it was solubilized by heating in a tightly stoppered tube containing 1 ml of concentrated $HClO_4$ and 1 ml of 30% (v/v) H_2O_2 at 55°C.

An aliquot of the water-soluble fraction containing a known amount of ^{14}C radioactivity was passed successively through cation- and anion-exchange columns (Bio-Rad AG 50W-X8, 200–400 mesh [H^+ form] and AG 1-X8, 200–400 mesh [acetate form], respectively). The columns were washed with water to elute the neutral fraction. Then the basic fraction (amino acids) on the cation column was eluted with 2 N HCl. The total acidic fraction (organic acids and phosphorylated sugars) on the anion column was eluted with 3 N HCl. All of the fractions were evaporated to dryness *in vacuo* and redissolved in 0.2 ml of water after removal of residual HCl. The ^{14}C radioactivity of the redissolved basic, acidic, and neutral fractions was determined. More than 95% of the ^{14}C applied to the columns was recovered. No further analysis of the neutral fraction was performed.

Two-dimensional TLC was used to separate the labeled amino acids (5). Aliquots of the basic fractions containing known amounts of ^{14}C radioactivity were spotted on precoated plates of MN-300 cellulose (Analtech, Inc.). The plates were developed twice in the first dimension with 2-propanol/methyl ethyl ketone/1 N HCl (60:15:25, v/v) and then twice in the second dimension with *tert*-butyl alcohol/methyl ethyl ketone/acetone/methanol/water/NH_4OH (40:20:20:01:14:05, v/v). No equilibration period was required prior to the use of each solvent system.

The labeled compounds in the acidic fraction were separated by two one-dimensional chromatographic procedures. Sheets of Whatman No. 1 filter paper were developed with the solvent that formed as the upper phase after mixing equal volumes of *n*-pentanol and 5 N HCOOH (4). This solvent gave excellent separation of organic acids, but phosphorylated sugars and 3-PGA remained at the origin. Prior to development, the chromatogram was subjected to a 4-h equilibration period in an atmosphere saturated with the solvent. TLC plates coated with MN-300 cellulose were developed twice with Wood's GW3 solvent (18), which contained *n*-butanol/*n*-propanol/acetone/80% HCOOH/30% TCA (40:20:25:25:15, v/v) saturated with EDTA (free acid). No equilibration period was used. This solvent separated all of the acidic compounds well, especially 3-PGA and the phosphorylated sugars (18).

The labeled compounds on the chromatograms were located by fluorography using Kodak X-Omat AR film. By spraying the air-dried chromatograms with EN^{3}HANCE (New England Nuclear) and exposing the film at −80°C, spots containing as little as 70 ^{14}C-dpm could be detected within 1 week. The unknown, labeled metabolites were identified by comparing their positions on the chromatograms with the relative positions of co-chromatographed, authentic, labeled standards, with published R_F values

[4] Abbreviations: PEP, phosphoenolpyruvate; 3-PGA, 3-phosphoglycerate; RuBP, ribulose 1,5-bisphosphate; Γ, CO_2 compensation concentration.

(4, 18), and with positions of known compounds on published maps (5). The identified, labeled spots were removed by cutting or scraping and then placed into 20-ml scintillation vials with 1 ml of water for 48 h before their ^{14}C radioactivity was measured.

Approximately 4% or less of the unknown labeled compounds was lost during the extraction, separation, and identification procedures. Greater than 95% of the authentic, ^{14}C-labeled 3-PGA, malate, aspartate, and fructose 1,6-bisphosphate subjected to the same procedures as the unknown compounds was recovered.

RESULTS AND DISCUSSION

Diurnal Fluctuations in Malate and PEP Pools. According to Winter *et al.* (17), the finding that leaves of *M. arvensis* accumulate malate during the day is noteworthy. They suggest that the accumulation of organic acids is linked to the assimilation of NO_3^- in this species. In our plants, which are supplied with NO_3^- as the major nitrogen source and are grown at a day/night temperature regime favorable for producing diurnal fluctuations in malate concentration in the CAM plant, *K. daigremontiana* (Table I), the malate content of young, fully expanded leaves increases about 65% during the day (Table I). This situation is similar to that in the study by Winter *et al.* (17) and is clearly indicative of the absence of CAM in *M. arvensis*. However, the accumulation of malate in *M. arvensis* is not unusual. This organic acid also accumulates in leaves of the C_3 species, *M. foetida* and *G. max*, when they are grown under identical conditions (Table I). Malate accumulation occurs in *P. milioides* as well (6). The only difference between these species is that *M. arvensis* leaves contain higher concentrations of malate, on a Chl basis, than do those of the other plants. Although the malate level in *M. arvensis* is not nearly as high as in *K. daigremontiana*, it is 3-fold greater than the levels in the C_3 species at the end of the light period (Table I).

Malate synthesis in *M. arvensis* and the C_3 species presumably occurs via PEP carboxylase, thus utilizing PEP. However, no marked diurnal fluctuations in PEP concentration occur in the leaves of these species (Table I). In the light, the PEP concentration in *M. arvensis* leaves is 2- to 5-fold greater than the concentration in *G. max* leaves, but similar to those in *M. foetida* and *Z. mays* leaves. Obviously, the total pool size of PEP alone cannot be used as an indicator of the dominant pathway of CO_2 assimilation present.

$^{14}CO_2$ Incorporation into Photosynthetic Metabolites. The PEP carboxylase activity in leaf extracts of *M. arvensis* is twice that in extracts of *M. foetida* leaves (3, 9). This observation and the fact that the malate concentration in *M. arvensis* leaves at the end of the light period is 3-fold greater than in *M. foetida* (Table I) suggest that PEP carboxylase in *M. arvensis* may play a significant role in the initial assimilation of CO_2. However, since malate accumulates during the day in *M. arvensis*, it is more likely an end product of photosynthesis rather than an intermediate as in C_4 metabolism where the malate concentration remains constant in the light (Table I).

From our $^{14}CO_2$ incorporation studies, it is evident that PEP carboxylase in *M. arvensis* does not play a significant role in the initial assimilation of CO_2 at atmospheric levels of CO_2. After 6 s of photosynthesis in $^{14}CO_2$-air (310 μl CO_2/l and 1100 μmol photon m^{-2} s^{-1}), the percentage of label in the C_4 acids, malate and aspartate, for *M. arvensis* (6.5%) is as low as the percentages for *M. foetida* (5.1%) and *G. max* (2.6%) (Table II). Contrary to the results of an earlier study (13), this percentage for *P. milioides* is similarly low (5.2%) (Table II) and supports the recent findings of Edwards *et al.* (6). Subjecting the *P. milioides* plants to a high photon flux (~1900 μmol m^{-2} s^{-1}) during propagation and the $^{14}CO_2$ experiments, as well as increasing their nitrogen (as NO_3^-) fertilization, does not appreciably increase the percentage of $^{14}CO_2$ initially fixed into C_4 acids (data not shown).

For *M. arvensis*, during a 36-s chase period in $^{12}CO_2$-air (270 μl CO_2/l) following 6-s pulse photosynthesis, the percentage of ^{14}C in the C_4 acids increases to an average of about 10% (Tables II and III). Little change occurs in this percentage for *P. milioides*. For both C_3-C_4 intermediate species, the patterns of labeling occurring during the chase period with respect to the C_4 acids and Calvin cycle intermediates are similar to those for *M. foetida* and *G. max* (Table III). Thus, as Winter *et al.* (17) and Edwards *et al.* (6) recently concluded, CO_2 assimilation in *M. arvensis* and *P. milioides* occurs primarily via RuBP carboxylase and the C_3 photosynthetic carbon reduction cycle. No C_4 photosynthesis, as exemplified by *F. brownii* (Tables II and III), occurs in *M. arvensis* or *P. milioides* under the conditions of our experiments.

$^{14}CO_2$ Incorporation into Photorespiratory Metabolites. The labeling pattern for glycine in *M. arvensis* during chase periods in $^{12}CO_2$-air is markedly different from the patterns in the other species examined. As chase periods increase from 18 to 36 s, the percentage of label in glycine increases 60% in *M. arvensis*, while in *M. foetida* and *P. milioides* there is only a 25% increase (Table III). No increase occurs in *G. max*. After a 36-s chase period, the average percentage of ^{14}C in glycine is 20.2% for *M. arvensis*, 14.8% for *M. foetida*, 13.5% for *P. milioides*, and 8.9% for *G. max* (Table III). These data may reflect a larger glycine pool size in *M. arvensis* compared to the other species examined. On the other hand, they may suggest that the apparent turnover rate of photorespiratory glycine in *M. arvensis* is slower than in the other species, especially in *G. max*. Since photorespiratory CO_2 is released during the oxidative decarboxylation of glycine, a slower apparent turnover of glycine in *M. arvensis* relative to that in the C_3 species might be at least a partial reason for less

Table I. *Diurnal Levels of Malate and PEP in Leaves of Moricandia arvensis, M. foetida, Glycine max, Zea mays, and Kalanchoë daigremontiana*

The plants were grown in a controlled environment room under a 15-h photoperiod. The lights came on at 7:00 h and went off at 22:00 h. Mal., malate; D, dark; L, light.

Time of Day	*M. arvensis*		*M. foetida*		*G. max*		*Z. mays*		*K. daigremontiana*
	Mal.	PEP	Mal.	PEP	Mal.	PEP	Mal.	PEP	(Mal.)
h					*μmol/mg Chl*				
6:45 (D)	18.0[a]	0.09[b]	3.5	0.05	4.3	0.03	1.2	0.03	405
8:00 (L)	23.3	0.09	4.3	0.08	6.7	0.04	2.3	0.10	417
21:45 (L)	29.4	0.11	9.3	0.08	10.0	0.02	2.3	0.10	169
1:00 (D)	25.8	0.17	7.3	0.07	7.6	0.04			239
6:45 (D)	17.4		3.8						374

[a] The malate values for all species are from one representative diurnal sampling period. Five such sampling periods were performed for *M. arvensis* and *M. foetida*, one for *G. max*, *Z. mays*, and *K. daigremotiana*.

[b] The PEP values for *M. arvensis* and *M. foetida* are means from four separate diurnal sampling periods.

Table II. *Distribution of ^{14}C in Leaves of Moricandia arvensis, Panicum milioides, M. foetida, Glycine max, and Flaveria brownii following 6 Seconds of Photosynthesis in $^{14}CO_2$-Air*

The initial pCO_2 was 310 μl CO_2/l and the incident photon flux was 1100 μmol m^{-2} s^{-1}.

Metabolite	*M. arvensis*[a]	*P. milioides*[b]	*M. foetida*[a]	*G. max*[a]	*F. brownii*[c]
			% of total ^{14}C fixed		
3-PGA	28.3	26.0	21.0	↑	↑
Sugar phosphates	59.4	52.9	67.0	86.8	7.1
				↓	↓
Malate	4.9	3.0	3.4	1.7	45.5
Aspartate	1.6	2.2	1.7	0.9	38.1
Glycine	1.2	1.5	0.9	2.2	0.0
Serine	0.2	0.5	0.5	0.5	0.0
Alanine	0.1	0.5	0.6	0.2	0.0
Neutrals	1.2	2.0	0.7	2.8	1.4
Unidentified	3.3	6.7	3.1	3.6	9.0
Water-insoluble	0.3	1.5	0.5	2.6	0.2
Per cent recovered	100.5	96.8	99.4	101.3	101.3

[a] Percentages are the means of data from two separate experiments.

[b] Percentages are the means of data from three separate experiments.

[c] For *F. brownii*, only one experiment was performed.

Table III. *Distribution of ^{14}C in Leaves of Moricandia arvensis, Panicum milioides, M. foetida, Glycine max, and Flaveria brownii after 18 and 36 Seconds of Photosynthesis in $^{12}CO_2$-Air following Photosynthesis for 6 Seconds in $^{14}CO_2$-Air*

The initial pCO_2 during the 6-s pulse in $^{14}CO_2$-air was 310 μl/l and the pCO_2 during the 18- and 36-s chases in $^{12}CO_2$-air was 270 μl CO_2/l. The incident photon flux was 1100 μmol m^{-2} s^{-1}.

Metabolite	*M. arvensis*		*P. milioides*		*M. foetida*		*G. max*		*F. brownii*
	18 s	36 s	18 s	36 s	18 s	36 s	18 s	36 s	(18 s)
					% of total ^{14}C fixed				
3-PGA	13.1[a]	8.4	16.1	14.8	15.9	12.7	13.0	13.0	16.8
Sugar phosphates	48.7	38.9	38.3	37.0	52.1	42.6	50.8	44.4	30.6
Malate	5.4	5.8	2.6	1.8	3.5	2.6	2.2	3.0	23.6
Aspartate[b]	3.0	4.1	2.7	2.6	2.8	3.3	0.7	0.9	6.8
Glycine[b]	12.6	20.2	10.8	13.5	11.8	14.8	8.8	8.9	4.5
Serine[b]	2.1	4.6	4.5	6.2	2.3	5.4	4.5	4.4	2.3
Alanine[b]	0.8	1.7	0.7	2.3	1.5	1.6	0.8	1.0	4.9
Neutrals	4.3	6.2	4.6	5.8	2.2	6.6	4.4	5.6	1.0
Unidentified	5.4	3.6	11.2	4.8	4.8	2.9	6.3	6.3	3.9
Water-insoluble	3.8	5.0	6.1	12.2	2.2	5.2	8.8	12.2	5.5
Per cent recovered	99.2	98.5	97.6	101.0	99.1	97.7	100.3	99.7	99.9

[a] Percentages, unless noted otherwise, are the means of data from two separate experiments with the exception of those for *F. brownii* which are the results of one experiment.

[b] Percentages for these metabolites are the means of data from two to four separate experiments, except in the case of *F. brownii*.

photorespiratory CO_2 release in this crucifer. The difference in the percentages in glycine for the two C_3 species examined detracts from this idea, however. *Moricandia foetida* and *G. max* exhibit similar Γ-values at 21% O_2 and 25°C (46 and 45 μl CO_2/l, respectively) (A. S. Holaday and R. Chollet, unpublished; 8), but the average percentage of ^{14}C in glycine for *M. foetida* after 36-s chase periods is 1.7-fold greater than that for *G. max* (Table III). Still, the observed difference in glycine labeling between *M. arvensis* and *M. foetida* (Table III) may be sufficient to account for the difference in photorespiratory activity exhibited by these related species.

The percentage of ^{14}C in serine does not increase more slowly in *M. arvensis* than in *M. foetida* or *G. max* with increasing duration of the chase as one might predict from the glycine data (Table III). The changes in serine labeling may be complicated, however, by serine synthesis from labeled 3-PGA rather than phosphoglycolate (12, 15).

After a 36-s chase period in air containing 100 μl CO_2/l (Table IV), the percentages of label in glycine for *P. milioides* and the C_3 species are approximately twice those occurring after a 36-s chase at 270 μl CO_2/l (Table III). Although these data at low pCO_2 are from a single experiment and should be considered preliminary, the increased percentage of label in glycine at 100 μl CO_2/l is indicative of increased O_2 fixation by RuBP carboxylase/oxygenase due to the decreased concentration of the alternate substrate, CO_2. Interestingly, the increase in glycine labeling for *M. arvensis* is much smaller, being only about 20% (Tables III and IV). Again, this low percentage increase could be the result of a larger glycine pool size in *M. arvensis* relative to that in the other species examined. However, this observation may also suggest that the increase in carbon flow into the C_2 photorespiratory carbon oxidation pathway caused by lowering the pCO_2 is considerably less in *M. arvensis* than in the other species. Because of the difficulty in determining carbon flux through photorespiratory glycine from changes in the percentage distribution of ^{14}C during chase periods, our present investigations concern the determination of the relative rates of glycine synthesis and metabolism in addition to the relative glycine pool sizes in the C_3-C_4 intermediate species and representative C_3 and C_4 plants.

Since our study does not include any C_3 *Panicum* species for direct comparison, it is difficult to speculate as to whether the

Table IV. *Distribution of ^{14}C in Leaves of Moricandia arvensis, Panicum milioides, M. foetida, and Glycine max*

Values are taken after 36 s of photosynthesis at 100 μl CO_2/l and 1100 μmol photon m^{-2} s^{-1} following photosynthesis for 6 s in $^{14}CO_2$-air containing 140 μl CO_2/l.

Metabolite	*M. arvensis*	*P. milioides*	*M. foetida*	*G. max*
		% of total ^{14}C fixed		
3-PGA	↑	↑	↑	↑
Sugar phosphates	|	|	|	|
Malate	57.3[a]	57.3	56.1	61.6
Neutrals	|	|	|	|
Unidentified	↓	↓	↓	↓
Aspartate	7.3	3.8	2.5	2.1
Glycine	24.6	24.6	32.1	22.7
Serine	5.9	9.8	4.2	9.6
Alanine	1.5	1.7	1.8	1.2
Water-insoluble	3.0	1.8	2.9	2.5
Per cent recovered	99.6	99.0	99.6	99.7

[a] All percentages are from one experiment.

reduction of photorespiration in *P. milioides* may also be linked to the apparent rate of glycine turnover. The C_3-like percentage of ^{14}C in glycine after 36-s chase periods at 270 μl CO_2/l (Table III) and the marked increase in glycine labeling after 36 s at 100 μl CO_2/l (Table IV) suggest, however, that some other mechanism may be involved in this species.

Effect of Light Intensity on the Initial Distribution of ^{14}C. As the incident photon flux decreases from 700 to 50 μmol m^{-2} s^{-1}, Γ at 21% O_2 and 25°C for *M. arvensis* and *P. milioides* increases from 14 to 44 μl CO_2/l and 12 to 36 μl/l, respectively (8). Under the same conditions, the Γ-value for *G. max* remains constant. The biochemical basis for this atypical response of Γ to light intensity is unknown, but it might be related to the mechanism(s) for reducing photorespiration in these C_3-C_4 intermediate species. When *M. arvensis* and *P. milioides* assimilate $^{14}CO_2$ for 6 s at 1100 μmol photon m^{-2} s^{-1} and 140 μl CO_2/l (conditions which approximate those present during the determination of Γ above 700 μmol photon m^{-2} s^{-1}), the initial distribution of ^{14}C in photosynthetic metabolites is similar to that found at 1100 μmol photon m^{-2} s^{-1} and 310 μl CO_2/l (Tables II and V). Under conditions approximating those present during Γ-determinations at low light intensity (60 μmol photon m^{-2} s^{-1} and 140 μl CO_2/l), a somewhat greater proportion of the initial ^{14}C fixed occurs in the C_4 acid fraction, especially in aspartate, indicating an increase in the proportion of the total assimilated CO_2 that is fixed via PEP carboxylase (Table V). One might predict that if 60 μmol photon m^{-2} s^{-1} enhances PEP carboxylation relative to RuBP carboxylation, Γ-values would be lower than at 1100 μmol m^{-2} s^{-1}. Yet Γ is actually higher at low light intensity in both *M. arvensis* and *P. milioides* (8). Thus, the effect of light intensity on Γ for these C_3-C_4 intermediate species does not appear to involve changes in the activity ratio of the primary carboxylation reactions. Nor does the effect involve other changes in photosynthetic carbon metabolism in these species. The patterns of ^{14}C incorporation into photosynthetic metabolites in comparative pulse-chase experiments at 100 μl CO_2/l and 1100 or 60 μmol photon m^{-2} s^{-1} are similar to those of the C_3 species examined (data not shown).

Reportedly, at low pCO_2 PEP carboxylase assimilates a much greater proportion of the initial carbon fixed in *P. milioides* than at atmospheric pCO_2 (11). Our comparative results at 1100 μmol photon m^{-2} s^{-1} (Tables II and V) show that no marked increase in CO_2 assimilation via PEP carboxylase occurs in *P. milioides* and *M. arvensis* as the pCO_2 is lowered from 310 to 140 μl CO_2/l. These data are an additional indication that although the PEP carboxylase activity *in vitro* in leaf extracts of these C_3-C_4 intermediate species is about twice that in C_3 leaf extracts (3, 6, 9, 17), the activity *in vivo* remains as low as in C_3 species.

Table V. *Distribution of ^{14}C in Leaves of Moricandia arvensis and Panicum milioides*

Values are taken after 6 s of photosynthesis in air at an initial pCO_2 of 140 μl/l and high and low light intensities.

Metabolite	*M. arvensis*		*P. milioides*	
	1100[a]	60[a]	1100[a]	60[a]
	% of total ^{14}C fixed			
3-PGA + sugar phosphates	83.9[b]	75.7	79.0	72.2
Malate	7.5	9.0	5.1	1.6
Aspartate	2.3	9.1	3.1	7.5
Glycine	0.8	0.6	2.3	1.8
Serine	0.2	0.0	0.4	1.5
Alanine	0.2	0.4	0.4	1.7
Neutrals	1.0	0.7	1.0	1.1
Unidentified	3.5	3.8	5.9	9.8
Water-insoluble	0.4	0.2	1.5	0.5
Per cent recovered	99.8	99.5	98.7	97.7

[a] Incident photon flux (400–700 nm) in μmol m^{-2} s^{-1}.

[b] Percentages are the means of data from two separate experiments.

CONCLUSIONS

Consistent with the findings from our earlier enzymic studies of *M. arvensis* leaf extracts (9), the results from this *in vivo* study confirm and extend the recent findings of Winter *et al.* (17) showing that the pathway for the initial assimilation of CO_2 by this crucifer is similar to that in representative C_3 species (Table II). There is no evidence for the operation of a C_4 cycle in *M. arvensis* (Tables II and III) which could account for the reduced photorespiratory activity in this species relative to that in C_3 plants (1, 8). As with representative C_3 species, the slow or negligible consumption of malate in the light results in its accumulation during the day (Table I; Ref. 17). Malate accumulation is greater in *M. arvensis* than in the C_3 species examined, however.

Our study supports the recent findings of Edwards *et al.* (6) that the pathway of CO_2 assimilation in *P. milioides* is also similar to that in C_3 species. No C_4 cycle operates in this species under the conditions of our experiments (Tables II and III). Consistent with this finding, we have repeatedly observed very low levels of ATP-dependent pyruvate,Pi dikinase activity (an average of 7.9 ±0.3 μmol mg^{-1} Chl h^{-1} for four separate experi-

ments [assayed at 30°C as in Ref. 9]) in crude leaf extracts of *P. milioides* plants which displayed reduced photorespiration (R. Chollet and A. S. Holaday, unpublished). These results are contrary to those reported earlier (13) which indicate that a limited C_4 cycle is responsible for assimilating ~25% of the initial CO_2 fixed by *P. milioides* at atmospheric levels of CO_2. No differences in the environmental conditions used in these studies during the growth of the plants and during the $^{14}CO_2$ incorporation experiments are known to have produced these seemingly contradictory results. While this apparent variability in the results from $^{14}CO_2$ incorporation studies exists, no similar variability occurs in leaf anatomical features or photorespiratory CO_2 exchange characteristics of *P. milioides* (8, 14). Thus, although the potential for limited C_4 photosynthesis may exist under certain conditions, it is clearly *not* required for reducing photorespiration in *P. milioides*.

Similarly, under the conditions of our study, the functioning of PEP carboxylase *in vivo* in *P. milioides* and *M. arvensis* is responsible for assimilating only a small fraction of the initial CO_2 incorporated at 1100 μmol photon m^{-2} s^{-1} and either atmospheric or subatmospheric levels of CO_2 (Tables II and V). Therefore, it is highly unlikely that PEP carboxylase could be responsible for recycling large amounts of photorespiratory CO_2 in these species. An obvious, but noteworthy, corollary to these conclusions is that a *novel* mechanism(s) must exist in *M. arvensis* and *P. milioides* to account for the reduced photorespiratory activity in these two unrelated terrestrial plants.

The pattern of ^{14}C incorporation into the key photorespiratory metabolite, glycine, during chase periods at 270 or 100 μl CO_2/l following pulse-photosynthesis by *M. arvensis* is different from the patterns with *M. foetida*, *G. max*, and *P. milioides*. These differences may be due to the presence of a larger glycine pool size in *M. arvensis* leaves than exists in the leaves of the C_3 species and *P. milioides*. However, they may also reflect a slower turnover of the photorespiratory glycine pool in *M. arvensis*. Studies are continuing in our laboratory to determine whether the apparent difference in glycine metabolism for *M. arvensis* relates directly to the reduction of photorespiration in this crucifer.

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