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Photosynthetic $^{14}CO_2$ Fixation Products and Activities of Enzymes Related to Photosynthesis in Bermudagrass and Other Plants[1]

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ABSTRACT

After a 5-second exposure of illuminated bermudagrass (*Cynodon dactylon* L. var. 'Coastal') leaves to $^{14}CO_2$, 84% of the incorporated ^{14}C was recovered as aspartate and malate. After transfer from $^{14}CO_2$-air to $^{12}CO_2$-air under continuous illumination, total radioactivity decreased in aspartate, increased in 3-phosphoglyceric acid and alanine, and remained relatively constant in malate. Carbon atom 1 of alanine was labeled predominantly, which was interpreted to indicate that alanine was derived from 3-phosphoglyceric acid. The activity of phosphoenolpyruvate carboxylase, alkaline pyrophosphatase, adenylate kinase, pyruvate-phosphate dikinase, and malic enzyme in bermudagrass leaf extracts was distinctly higher than those in fescue (*Festuca arundinacea* Schreb.), a reductive pentose phosphate cycle plant. Assays of malic enzyme activity indicated that the decarboxylation of malate was favored. Both malic enzyme and $NADP^+$-specific malic dehydrogenase activity were low in bermudagrass compared to sugarcane (*Saccharum officinarum* L.). The activities of NAD^+-specific malic dehydrogenase and acidic pyrophosphatase in leaf extracts were similar among the plant species examined, irrespective of the predominant cycle of photosynthesis. Ribulose-1,5-diphosphate carboxylase in C_4-dicarboxylic acid cycle plant leaf extracts was about 60%, on a chlorophyll basis, of that in reductive pentose phosphate cycle plants.

We conclude from the enzyme and ^{14}C-labeling studies that bermudagrass contains the C_4-dicarboxylic acid cycle and that pyruvate-phosphate dikinase does not exist exclusively in C_4-dicarboxylic acid cycle plants, and we propose that in C_4-dicarboxylic acid cycle plants the transfer of carbon from a dicarboxylic acid to 3-phosphoglyceric acid involves a decarboxylation reaction and then a refixation of carbon dioxide by ribulose-1,5-diphosphate carboxylase.

Previous experiments with isolated chloroplasts from *Cynodon dactylon* L. (bermudagrass, var. 'Coastal') leaves indicated an active cyclic and noncyclic photophosphorylation which might meet the ATP requirements for photosynthetic CO_2 metabolism in bermudagrass (8). However, the photosynthetic pathway(s) whereby bermudagrass metabolizes CO_2 have not been established experimentally. From preliminary results comparing the 5-sec $^{14}CO_2$ fixation products of bermudagrass with sugarcane, it was assumed that bermudagrass possessed the C_4 cycle[3] (8). Bermudagrass has several other characteristics which indicate that it may possess the C_4 cycle, including a CO_2 compensation concentration of 0.0004%, a high rate of photosynthesis at 0.03% CO_2 (60 to 80 mg of CO_2 dm^{-2} hr^{-1}), a rate of photosynthesis approaching saturation at intensities near 10,000 to 12,000 ft-c, a temperature optimum for photosynthesis of about 35 C, and a high concentration of organelles such as chloroplasts, mitochondria, and peroxisomes in the leaf bundle sheath cells (3, 4, 7, 9, 20). This report presents data to establish the pathway of photosynthetic CO_2 metabolism in bermudagrass, and comparative data are presented on several other plants which possess either the C_4 cycle or the pentose cycle of photosynthesis.

MATERIALS AND METHODS

$^{14}CO_2$ Fixation Chamber. A Plexiglass chamber which consisted of two main parts was used for $^{14}CO_2$ fixation experiments. The upper part was a box (17 × 17 × 10 cm) with the bottom side open. A 15-cm fan blade was suspended beneath the top side and was spun at 500 rpm throughout the experiment to maintain a homogenous atmosphere in the upper chamber. For $^{14}CO_2$ release, a 10-ml beaker was glued to one corner of the box, with a magnetic stirrer installed beneath for immediate mixing of $NaH^{14}CO_3$ and acid. A 16- × 13.5-cm platform for plant tissue made of Plexiglass and nylon wiring, with one corner notched for a 10-ml beaker, rested 1.5 cm above the lower edge of the box. The other main part of the CO_2 fixation chamber was a lower container (27 × 27 × 4.5 cm) designed to accomodate the upper part. The lower container served three purposes: (*a*) maintenance of constant temperature (30 C) in the chamber obtained by running water through the container, (*b*) partial sealing of $^{14}CO_2$ in the upper chamber from the atmosphere, and (*c*) a supply of water for the detached plant tissues during the equilibration and fixation periods. Illumination at about 4000 ft-c at the leaf level was provided by a 300-w incandescent flood lamp installed under the assembled chamber. The volume of the assembled CO_2 fixation chamber was 2300 cm^3.

$^{14}CO_2$ Fixation. Detached coastal bermudagrass, sugarcane, or tall fescue leaves were inserted into the chamber and were rested on the nylon wiring from the side of the platform with a

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[3] Abbreviations: C_4 cycle: C_4-dicarboxylic acid cycle; pentose cycle: reductive pentose phosphate cycle; 3-PGA: 3-phosphoglyceric acid; PEP: phosphoenolpyruvate; RuDP: ribulose-1,5-diphosphate; OAA: oxaloacetic acid; DCMU: 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

short section of tissue extending outside the chamber into water for quick retrieval. Leaves were equilibrated in the chamber 25 to 30 min before releasing $^{14}CO_2$ with a stream of air being pulled through the chamber, in addition to illumination, air mixing, and water cooling. The air stream and cooling water were stopped immediately before the release of $^{14}CO_2$.

One hundred microcuries of $NaH^{14}CO_3$ solution, specific radioactivity 20 mc/mmole, were injected into the beaker, followed by 1 ml of concentrated lactic acid. After the acid release of $^{14}CO_2$, all plant tissues were removed at timed intervals from the chamber and were immediately killed in boiling 80% ethanol.

In the experiment reported in Figure 1 all leaves were exposed to $^{14}CO_2$ for 5 sec and were then removed from the fixation chamber into air ($^{12}CO_2$) and under a similar illumination intensity for 0, 30, 60, 90, and 120 sec before killing in boiling 80% ethanol (v/v).

Extraction, Separation, and Identification of Labeled Compounds. Each tissue sample was extracted twice with boiling 80% ethanol, followed by two extractions with 25% ethanol. Extracts were combined and evaporated to dryness with a flash evaporator under 40 C and taken up in a small volume of 25% ethanol. Two-dimensional descending paper chromatography was employed to separate labeled products. No-Screen x-ray film was exposed to the chromatograms (35.6 × 43.2 cm) to locate radioactive compounds. Each radioactive spot then was cut out and counted in a liquid scintillation spectrometer. Corresponding radioactive spots on other chromatograms were eluted with 25% ethanol and then concentrated. Individual eluants were cochromatographed with L-aspartate-^{14}C, L-malate-^{14}C, D-3-PGA-^{14}C, sucrose, and alanine in 1-dimensional paper chromatography in at least two solvent systems (Table I). Positions of radioactive spots were determined by a Packard 7201 radiochromatogram scanner. The position of authentic nonlabeled sucrose and amino acids was detected by resorcinol and 0.25% ninhydrin in acetone, respectively (5).

Cultivation of Plants and Preparation of Crude Enzyme Extracts. Plants were grown in the greenhouse with supplementary incandescent light at about 3000 ft-c at the top of plants. Lights were turned on at midnight each night, and the leaves harvested about 9 AM for each day's experiment. The temperature varied from 20 C at night to 30 C at midday.

Leaf laminae were ground in a prechilled mortar and pestle with 0.1 M tris-HCl buffer, pH 8.0, containing 10 mM dithiothreitol. Considerable effort was expended during grinding to ensure a complete cell breakage and subsequent enzyme extraction. Nitrogen gas was flushed continuously over the mortar when preparing extracts for the pyruvate-Pi dikinase assays. The homogenate was squeezed through one layer of muslin cloth; a sample was removed for chlorophyll determination; the remainder was centrifuged at 20,000 *g* for 10 min. All preparations were done at 3 C, except for pyruvate-Pi dikinase preparations which were made at room temperature (15).

Table I. *Solvent Systems Employed for the Paper Chromatography Using Whatman No. 1 Filter Paper*

Solvent A (first dimension) and B (second dimension) were used for 2-dimensional separation, and all solvents were employed for 1-dimensional cochromatography of known compounds with unknown labeled products. Aspartate, solvent C, D, and E; malate, D, E, and F; PGA, C, D, and E; sucrose, A, B, and C; alanine B, C, and D.

	Solvent Systems
A	Phenol-water (72:28)
B	1-Butanol-propionic acid-water (623:310:418)
C	1-Butanol-acetic acid-water (250:60:250)
D	Methanol-water-pyridine (80:20:4)
E	Methanol-formic acid-water (80:15:5)
F	Pentanol-water-formic acid (20:12:1)

Assay of PEP Carboxylase. The reaction mixture contained 5 μmoles of tris-HCl buffer, pH 8.3, 1 μmole sodium 2-PGA, excess enolase, 0.5 μmole $NaH^{14}CO_3$, 0.5 μmole sodium glutamate and the leaf extract in a total volume of 0.1 ml. The reaction mixtures were incubated at 30 C for a time period within the linear range (5 to 10 min). An aliquot of the mixture, 0.04 ml, was then pipetted into a scintillation vial containing 0.04 ml of 10% (w/v) trichloroacetic acid by which the unincorporated $H^{14}CO_3$ was converted to CO_2 and evolved. The samples, after being flushed with a stream of CO_2 for at least 1 min, were counted in a liquid scintillation spectrometer.

Assay of RuDP Carboxylase. The reaction mixture contained 10 μmoles of tris-HCl buffer, pH 8.3, 1 μmole of $MgCl_2$, 1 μmole of of dithiothreitol, 0.2 μmole of RuDP, 7.5 μmoles of $NaH^{14}CO_3$, and the leaf extract in a total volume of 0.13 ml. Reaction mixtures were incubated, stopped, and counted as described above.

Assay of Pyrophosphatase. Hydrolysis of pyrophosphate was determined in a 1-ml reaction mixture containing 1 μmole of tetrasodium pyrophosphate, 100 μmoles of tris-HCl buffer, pH 8.9, 10 μmoles of $MgCl_2$, and appropriate amounts of leaf extract (17, 24). After incubation at 30 C, the reaction was stopped by the addition of 1 ml of 10% (w/v) trichloroacetic acid. Acidic pyrophosphatase (13) was assayed in the same way except acetate buffer at pH 5.0 was used. Pi released was determined colorimetrically.

Assay of Malic Enzyme, Malic Dehydrogenase, Pyruvate-Pi Dikinase, and Adenylate Kinase. Established procedures were employed for the assay of malic dehydrogenase (22), pyruvate-Pi dikinase (15), and adenylate kinase (17). Correction for a trace of adenylate kinase activity in the coupling enzymes, pyruvate kinase and lactic dehydrogenase, was made by measuring the activity in a reaction mixture without the leaf extract. Malic enzyme was assayed in the direction of malate decarboxylation (21) and malate formation (25).

RESULTS

^{14}C-Labeled Compounds and Distribution of Radioactivity. Preliminary photosynthetic experiments established that the rate of $^{14}CO_2$ incorporation by bermudagrass in the fixation chamber was linear with time up to 60 sec when the leaves were preilluminated for 25 to 30 min as described in Materials and Methods.

By paper chromatographic separation and identification, the major ^{14}C-labeled compounds in sugarcane and bermudagrass leaves immediately after 5-sec exposure to $^{14}CO_2$ were aspartate, malate, and 3-PGA. The percentage of distribution of radioactivity in aspartate, malate, 3-PGA, sucrose, and unidentified compounds was 22, 62, 7, 7, and 2%, respectively in sugarcane, which agreed with the results of Kortschak, Hartt, and Burr (18). In bermudagrass, the percentage of distribution of radioactivity was 54, 33, 4, 1, and 8%, respectively (Fig. 1B). This was in contrast with the early and major production of 3-PGA (59%) in tall fescue (Fig. 1A).

Under steady state photosynthetic conditions, bermudagrass and tall fescue leaves were exposed to $^{14}CO_2$ for 5 sec; then the changes in products containing ^{14}C were followed after the leaves were transferred to $^{12}CO_2$-air (Fig. 1A and B). The most striking product changes in bermudagrass leaves were: the de-

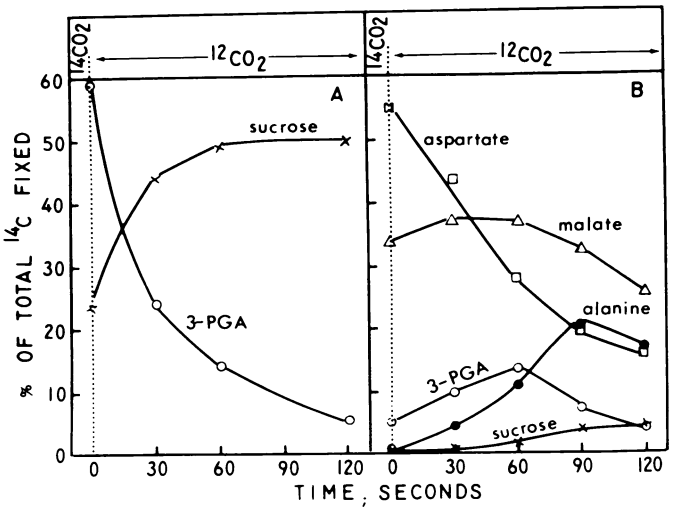

FIG. 1. Changes in the distribution of radioactivity among ^{14}C-labeled compounds in tall fescue (A) and coastal bermudagrass (B). Leaves were exposed to air containing $^{14}CO_2$ for 5 sec and were then transferred to $^{12}CO_2$-air. Leaves were illuminated throughout the experiment at an intensity of 4,000 ft-c at 30 C. With the tall fescue extracts on an equal chlorophyll basis the total radioactivity spotted on each chromatogram at each (time) was: 30,020 (0); 30,000 (30); 30,900 (60); and 30,880 (120). Similarily with bermudagrass extracts the total radioactivity was 107,400 (0); 105,400 (30); 113,400 (60); 113,600 (90); and 111,200 (120).

Table II. *Changes in the Distribution of Radioactivity following a 5-sec Pulse of ^{14}C between the C-1 and C-2 + 3 Atoms of Alanine in Coastal Bermudagrass from the Experiment Described in Figure 1*

Degradation of the carboxyl group of alanine was made as follows: One milliliter of 0.1 M citrate buffer with 1% ninhydrin and an aliquot of paper chromatographically purified, labeled alanine from different time periods were pipetted into scintillation vials. The mixtures were heated and evaporated to dryness on a steam bath. The residue was dissolved in 0.2 ml of water and then counted in a liquid scintillation spectrometer. The carboxyl group (C-1) of alanine was released by ninhydrin. The remaining radioactivity was therefore in C-2 plus C-3. An equal volume of the alanine solution from each time period was counted without degradation to determine total radioactivity.

Purified Alanine	Time in $^{12}CO_2$-Air (sec)				
	0	30	60	90	120
	cpm				
Minus ninhydrin	7670	7500	6540	6670	6330
Plus ninhydrin	78	150	208	300	724
% of Total Radioactivity in C-1 and C-2 + C-3					
Carbon position					
C-1	99.0	98.0	96.8	95.5	88.6
C-2 + 3	1.0	2.0	3.2	4.5	11.4

crease of radioactivity in aspartate, a concurrent increase of radioactivity in 3-PGA (up to 60 sec), and a similar increase of ^{14}C in alanine (up to 90 sec). The radioactivity in malate was relatively unchanged for 2 min aftter switching to $^{12}CO_2$. About 20% of the total radioactivity accumulated in alanine 90 sec after the transfer to $^{12}CO_2$ air. Sucrose accounts for only 4% of the total radioactivity even 120 sec after the transfer.

In tall fescue, a pentose cycle plant, radioactivity in 3-PGA apparently is quickly diverted to the synthesis of sucrose, which accounts for about 50% of ^{14}C incorporated within 1 min (Fig. 1A).

The distribution of labeled carbon atoms in alanine in C_4 cycle plants has not been reported previously. The present study (Table II) showed that initially alanine was labeled in the carboxyl group (C-1). The percentage of radioactivity in C-1 position relative to C-1+2+3 was 99% immediately after a 5-sec pulse of $^{14}CO_2$. Only at 120 sec after the transfer into $^{12}CO_2$ air did the C-2 and C-3 positions have appreciable labeling (11.4%).

Comparison of Enzyme Activities in Bermudagrass and Other Plants. PEP carboxylase activity in C_4 cycle plants was much higher than in pentose cycle plants, whereas RuDP carboxylase was not. The level of RuDP carboxylase in tall fescue, curled dock, and barley was about 40 to 50% higher than that in bermudagrass, sugarcane, and maize (Table III). Alkaline pyrophosphatase of bermudagrass and sugarcane was five to six times higher than that of tall fescue. Acidic pyrophosphatase activity was lower than alkaline pyrophosphatase in all plants and no major differences were observed between the two groups of plants (Table IV). The adenylate kinase of bermudagrass and sugarcane ranged from 50 to 80 times higher than that of tall fescue (Table IV). The activities of enzymes in bermudagrass extract which may be involved in the operation of C_4 cycle of photosynthesis. *i.e.*, PEP carboxylase, pyrophosphatase, adenylate kinase, and pyruvate-Pi dikinase were similar to those of sugarcane, but were different from

Table III. *Comparison of Phosphoenolpyruvate Carboxylase and Ribulose Diphosphate Carboxylase Activities in Leaf Extracts of Bermudagrass with those of Other Plants with the C_4-dicarboxylic Acid Cycle or the Reductive Pentose Phosphate Cycle of Photosynthesis*

The figures are the average of at least three separate tests.

Plant	PEP Carboxylase	RuDP Carboxylase
	μmoles/mg chl·hr	
C_4-dicarboxylic acid cycle		
Cynodon dactylon L.	680	170
Saccharum officinarum L.	830	130
Zea mays L.	680	180
Reductive pentose phosphate cycle		
Festuca arundinacea Schreb.	21	380
Rumex crispus L.	6	276
Hordeum vulgare L.	14	296

Table IV. *Activities of Pyrophosphatase, Adenylate Kinase, Pyruvate-Phosphate Dikinase, Malic Dehydrogenase, and Malic Enzyme in Bermudagrass, Sugarcane, and Tall Fescue Leaf Extracts*

Enzymes	Bermuda-grass	Sugarcane	Tall Fescue
	μmoles/mg chl·hr		
Pyrophosphatase (pH 8.9)	2400	2630	400
Pyrophosphatase (pH 5.0)	280	170	270
Adenylate kinase	2304	4090	47
Pyruvate-Pi dikinase	105	(130)[1]	4
Malic dehydrogenase (NADH)	5520	4170	4740
Malic dehydrogenase (NADPH)	26	293	20

[1] This value is from Hatch and Slack (16).

Table V. *Malic Enzyme Activities in Plant Extracts*

Figures on line a for each plant species were obtained from assays in the direction of malate decarboxylation (21); line b from the direction of malate formation (22).

Plant Species	Activity		
	μmoles/mg chl·hr	*μmoles/mg protein·hr*	*μmoles/g fr. wt·hr*
Bermudagrass			
a	11	0.78	0.44
b	3	...	...
Sugarcane			
a	394	28	13
b	30	...	...
Maize			
a	576	39	19
b	31	...	...
Tall Fescue			
a	3	0.13	0.06
b	0.1	...	...

those of tall fescue. The NAD^+-specific malic dehydrogenase was active at a similar level in these three plants. However, $NADP^+$-specific malic dehydrogenase and malic enzyme activity varied widely among plant species (Table IV and V).

When assayed in the direction of decarboxylation, the activity of malic enzyme in extracts of sugarcane and corn was almost as high as PEP carboxylase. When assayed in the direction of malate formation, malic enzyme activity was about 3 to 20 times lower than when assayed in the direction of malate decarboxylation. Ochoa (21) has noted that the equilibrium constant is 19.6 in the direction of decarboxylation with the wheat germ enzyme.

Although the malic enzyme activity in bermudagrass was quite low in comparison to those of sugarcane and corn, it was more active than in tall fescue, a pentose cycle plant. We considered that the low malic enzyme activity in bermudagrass or tall fescue might be due to an inhibitor present in the extracts. However, when malic enzyme activity was assayed in sugarcane extracts alone or in combination with other plant extracts, neither the extract from bermudagrass nor from tall fescue inhibited the sugarcane malic enzyme.

DISCUSSION

The early production of C_4-dicarboxylic acids (18) instead of 3-PGA (1, 6) has implied the existence of a photosynthetic CO_2 fixation cycle other than the pentose cycle in specific plants. However, the strongest evidence for the C_4 cycle has been derived from the demonstration of enzymes activities at levels sufficient for the partial operation of the C_4 cycle (2, 11, 15–17, 23–26). The activity of PEP carboxylase in C_4 cycle plants was high enough to accomodate the maximal rate of photosynthesis. The enzyme pyruvate-Pi dikinase (PEP synthetase), at levels from 84 to 132 μmoles/mg chl·hr, reportedly exists exclusively in C_4 cycle plants (15). Adenylate kinase, which catalyzes the regeneration of ADP from AMP plus ATP, was much higher in C_4 cycle plants in pentose cycle plants and alkaline pyrophosphatase was more than five times higher.

The rate of photosynthetic CO_2 fixation in bermudagrass leaves is about 400 to 500 μmoles/mg chl·hr (8). From the $^{14}CO_2$-labeling studies and the demonstration of active PEP carboxylase, adenylate kinase, pyruyate-Pi dikinase, and alkaline pyrophosphatase bermudagrass definitively can be classified as a C_4 cycle plant. These enzyme activities *in vitro* are sufficiently high to support leaf photosynthesis although the level of pyruvate-Pi dikinase is sufficient for only about one-fifth of the rate of leaf photosynthesis. This may simply reflect enzyme instability *in vitro* (15).

The enzyme and $^{14}CO_2$-labeling data indicated that tall fescue is a pentose cycle plant which is consistent with other characteristics of tall fescue. Tall fescue has a high CO_2 compensation concentration (0.0045%, v/v), a low rate of photosynthesis (31 mg of CO_2 dm^{-2} hr^{-1}), a low light saturation (3000 ft-c) and a low temperature optimum (20–25 C) for photosynthesis (9).

An important enzyme in the C_4 cycle plants, pyruvate-Pi dikinase, has been reported to be exclusively present in C_4 cycle plants (15). The activity of pyruvate-Pi dikinase in bermudagrass was 105 μmoles/mg chl·hr, close to the 132 reported for sugarcane (15). Tall fescue, a pentose cycle plant, contains a measurable amount of enzyme activity (4 μmoles/mg chl·hr, Table IV). Osmond *et al.* (23) also observed some pyruvate-Pi activity in *Atriplex hastata*, a pentose cycle plant. Apparently, this enzyme does not exist exclusively in C_4 cycle plants. Thus the enzyme difference between leaf extracts of C_4 cycle plants and pentose cycle plants appear to be differences in levels of enzymes. In addition, we have recently shown that specific enzymes are concentrated in specific cells within a fully differentiated leaf (11, 19); hence, enzyme assays on leaf extracts may show little correlation with enzyme levels in specific cells or with rates of leaf photosynthesis.

Slack and Hatch (25) reported that the activity of RuDP carboxylase of sugarcane, maize, and sorghum is 18, 37, and 21 μmoles/mg chl·hr, respectively. Our repeated tests always demonstrated that the activity in sugarcane and bermudagrass was 5 to 10 times higher than these values (Table III). Since high RuDP carboxylase activity is located in the bundle sheath cells (11), an insufficient grinding of leaf tissue would leave more bundle sheath cells unbroken and result in less RuDP carboxylase activity. Recently Bjorkman and Gauhl (2) and Osmond *et al.* (23) reported the activity of RuDP carboxylase in leaves of other C_4 cycle plants to be of the same magnitude as those in Table III. Therefore in C_4 cycle plants this enzyme should play an important role in CO_2 fixation. One role could be in photosynthesis as in pentose cycle plants (1). Another role could be an involvement in a transcarboxylation reaction as postulated by Hatch and Slack (14). We propose that the enzyme also could fix CO_2 from such sources as dicarboxylic acid decarboxylation (Table V), or mitochondrial or peroxisomal respiration. Some evidence has been presented for the refixation of CO_2 evolved during illumination in C_4 cycle plants with no apparent photorespiration by using DCMU to inhibit photosynthesis (12). In addition, an abundant number of peroxisomes are present in the bundle sheath cells (4), indicating the possible presence of photorespiration in the same cells which contain the RuDP carboxylase. A burst of CO_2 by illuminated bermudagrass leaves also has been observed after extinguishing the light, which was interpreted as indicating the presence of photorespiration (R. H. Brown, unpublished data).

The $NADP^+$-specific malic dehydrogenase and malic enzyme have been implicated in CO_2 assimilation by C_4 cycle plants, the former in the formation of malate in the mesophyll chloroplasts and the latter in the decarboxylation of malate in the bundle sheath cells (16, 26). In isolated cells of crabgrass (*Digitaria sanguinalis* (L.) Scop.), $NADP^+$-malic dehydrogenase was found only in the mesophyll cells, whereas NAD^+-malic dehydrogenase was uniformly distributed in both cell types (19). On the other hand, the malic enzyme has been found predominantly in the bundle sheath cells of crabgrass (unpublished data). $NADP^+$-malic dehydrogenase is quite active in C_4 cycle plants such as sugarcane; however, bermudagrass only contains about one-tenth the activity found in sugarcane (Table IV).

Malic enzyme activity in bermudagrass also is much lower than in sugarcane (Table V).

Malic enzyme levels appear to be sufficient to support the proposed role of decarboxylation in sugarcane and in maize (Table V), but levels in bermudagrass appear to be too low to play a major role. A comparison of the $^{14}CO_2$ pulse-chase experiment in Figure 1 with the data of Hatch and Slack (Fig. 4 in Ref. 14) indicates that in sugarcane malate rapidly (15 sec) loses label in $^{12}CO_2$, whereas in bermudagrass the label in malate is relatively constant for over 1 min in $^{12}CO_2$. In contrast, aspartate is not maximally labeled in sugarcane until 15 sec after transferring to $^{12}CO_2$ (14), while in bermudagrass the aspartate contains about 55% of the total ^{14}C immediately after a 5-sec exposure to $^{14}CO_2$ and the label rapidly is depleted in $^{12}CO_2$ (Fig. 1). We tentatively conclude that in bermudagrass malate decarboxylation is not a major process in photosynthesis and we propose that a decarboxylation of aspartate or oxaloacetate is possible. We are investigating these possibilities.

In Figure 1 we interpret the labeling data on 3-PGA and alanine as indicating that alanine is derived from 3-PGA possibly by the route of: 3-PGA→PEP→pyruvate→alanine as proposed by Smith *et al.* (27). The labeling data of Hatch and Slack (14) show early C-1 labeling in 3-PGA, which also is consistent with this pathway as is the early C-1 labeling of alanine (Table II).

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