ADAPTATIONS AT THE CELL AND ORGANELLE LEVEL FOR UTILIZING SUNLIGHT[1, 2]

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ABSTRACT

The discovery of multiple pathways of carbon dioxide assimilation and dissimilation in higher plants has drastically changed the research and thinking in plant biology. The theme is developed that adaptations within photosynthesis are of fundamental importance in plants and that changes in this dominant metabolic process are apt to result in strong and immediate selection advantages in the course of plant evolution. Data are presented on the variations in photosynthetic CO_2 assimilation in the reductive pentose phosphate cycle, the C_4-dicarboxylic acid cycle, and in Crassulacean acid metabolism. Photosynthetic studies with primitive plants such as *Psilotum nudum* indicate a metabolism similar to C_4-dicarboxylic acid may have arisen in this primitive plant.

In order to establish a perspective on the metabolic processes in plants which might be subject to adaptation, we have considered the activities of major metabolic processes such as photosynthesis, photorespiration, dark respiration, transpiration, nitrogen fixation, ion uptake, and the synthesis of proteins, lipids, cell walls. Many of these metabolic activities are common to all of life forms and probably have been subject to common adaptations; for example, respiration or protein, lipid, and polysaccharide synthesis. Certain metabolic processes however, are unique to plants and to certain bacteria; among these are photosynthesis, photorespiration, transpiration, nitrogen fixation, and ion uptake from soil. Unfortunately, quantitative data on these activities are not available on a comparable basis with a single plant or on a single plant organ, such as a leaf or a fruit. Photosynthesis probably is the most important process in plant metabolism and the best comparative quantitative data are available on this process. In this paper we will contend that changes in photosynthesis resulted in a strong and immediate selection advantage during the course of plant evolution. This is in contrast to the traditional idea that plant adaptations within secondary metabolic substances such as alkaloids or terpenes are apt to be more important in plant evolution.

Tables 1 and 2 assemble some representative data on the rates and amounts of photosynthetic CO_2 uptake and on the loss of CO_2 through photorespiration and respiration in intact plants or portions of plants. Clearly large variations exist in the maximum rates of photosynthesis observed between specific plants and between portions of the same plant. If the CAM plants are exempted (see table 2) then one can conclude that the rate of photosynthesis will be 10 to 30 times faster than respiration; particularly in leaves. One could ask such questions as: "If a plant is adapting to a change, what process is a likely site of adaptation?"; or "If you tried to grow a plant in a given environment, what process would you expect to be most critical?" Our contention is that data on photosynthesis is a likely answer to such questions, since it is a crucial process which must be maintained if the plant is to survive.

In historical perspective, prior to the discovery of C_4 photosynthesis, research in photosynthesis was surprisingly monolithic in its thought pattern, outlook, and concepts on basic reactions. Indeed the ideas of van Niel (1941) in the late 1930's

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[2]Abbreviations used in the manuscript: C_3=reductive pentose phosphate; C_4=C_4-dicarboxylic acid; CAM=Crassulacean acid metabolism; 3-PGA=3-phosphoglyceric acid; RuDP=ribulose-1,5-diphosphate; R-5-P=ribose-5-phosphate; MDH=malic dehydrogenase; MAL=malate; ASP=aspartate; HMP=hexose monophosphate; HDP=hexose diphosphate; Chl=chlorophyll.

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TABLE 1

Typical carbon dioxide assimilation and release rates in higher plant tissues

Plant	CO_2 uptake	CO_2 release		
	Photosynthesis	Post-illumination CO_2 burst (maximum)	Photorespiration (estimated)	Dark respiration
	mg CO_2/dm² of surface/hr			
C_3 Plants:				
Tobacco leaves[a]	16.6	5.4	3.4	.97
Wheat ear (grain)[b]	3.0[c]	——	3.0[c]	1.5[c]
Panicum bisulcatum leaves[d]	34	7.0	——	3.5
Stylosanthes humilis shoot[e]	25	——	3.1	1.6
C_4 Plants:				
Panicum maximum leaves[d]	68	10	——	5
Digitaria sanguinalis leaves[d]	45	3.5	——	2.5
Cynodon dactylon leaves[d]	85	6.5	——	3.5

[a]Decker, 1959; [b]Evans and Rawson, 1970; [c]mg of CO_2/ear/hr; [d]Brown and Gracen, 1972; [e]Begg and Jarvis, 1968.

comparing bacterial photosynthesis with green plant photosynthesis were so clear, simplistic, and satisfying that research in photosynthesis has been guided by these concepts for over 3 decades:

Bacterial Photosynthesis:

$$CO_2+2H_2A \xrightarrow[\text{Bchl}]{\text{Light}} [CH_2O]+H_2O+2A$$

Plant Photosynthesis:

$$CO_2+2H_2O \xrightarrow[\text{Chl}]{\text{Light}} [CH_2O]+H_2O+O_2$$

Today most people working on the light reactions of photosynthesis only consider two basic possibilities—bacterial photosynthesis with its one light reaction or plant and algal photosynthesis with their two light reactions. Some, however, do consider three light reactions in plants and several in bacteria. The course of photosynthesis evolving from one light cyclic electron flow system in bacteria to a two-light-noncyclic electron flow system in algae and higher plants was considered in some detail by Olson (1970). Unfortunately many workers believe that studies on the light reactions in spinach or *Chlorella* are entirely applicable to photosynthesis in all plants or algae.

An analogous situation existed in regard to photosynthetic CO_2 fixation, so that the formulation of the C_3 cycle in the 1950's, primarily utilizing algae (Bassham and Calvin, 1957), was questioned by few workers. Some disturbing irregularities arose in CO_2 fixation with photosynthetic bacteria, but despite such attacks the C_3 cycle has remained essentially unchanged for two decades. Thus, most workers accepted the C_3 cycle as being the pathway of carbon assimilation in photosynthesis and, in addition, accepted the C_3 cycle as present in all photosynthetic organisms. With the discovery of the C_4 cycle in 1965 (Kortshak *et al.*, 1965) and subsequent demonstration that it occurred in a wide variety of higher plants (Hatch *et al.*, 1967), the thinking of many plant scientists was radically modified, particularly in regard to considering the C_3 cycle as the only pathway of CO_2 assimilation. As the C_4 pathway of carbon assimilation was unravelled, it became clear that

TABLE 2
Rates of photosynthesis and dark CO_2 exchange in higher plants

Plant	Net photosynthesis (CO_2 uptake)	Dark respiration (CO_2 release)	Reference
	mg of CO_2/dm^2 of surface/hr		
C_3 Plants:			
Rice plants	15	1.1	Murata, 1961
Cotton leaf	38	3.0	El-Sharkaway and Hesketh, 1965
Douglas-fir	10.5	1.2	Brix, 1971
Stylosanthes humilis-stem	8	1.5	Begg and Jarvis, 1968
Total leaf canopy[a]	89.9 (day)	12.1 (night)	Begg and Jarvis, 1968
Total stem[a]	.91 (day)	5.9 (night)	Begg and Jarvis, 1968
Alfala canopy[a]	45.1	5.9	Brown *et al.*, 1971
Sunflower intact plant tops	15	1.9	Neales *et al.*, 1968
Tobacco intact plant tops	14.5	1.8	Neales *et al.*, 1968
C_4 Plants:			
Corn leaves	63	3.0	El-Sharkaway and Hesketh, 1965
Corn leaves attached	45	1.2	Heichel, 1971
Corn ears attached	−122[b]	−152[b]	Hesketh and Musgrave, 1966
CAM Plants:			
Aeonium haworthii[c]	4	+ 3.2[d]	Neales *et al.*, 1968
Ananas comosus[c]	.94	+ .48[d]	Neales *et al.*, 1968
Agave americana[c]	6.7	+ 11.8[d]	Neales *et al.*, 1968

[a]gm of CO_2/m^2 of ground; [b]mg of CO_2 evolved/ear/hr; [c]Intact plant tops; [d]CAM plants takeup CO_2 at night also.

earlier work with CAM plants (Ranson and Thomas, 1960) had many features, particularly organic acid synthesis, which resembled C_4 carbon assimilation. From the continued work on C_4 and CAM plants we can conclude with reasonable confidence that at least three major pathways exist for carbon assimilation in higher plants and even these pathways vary in specific plants (Black, 1973).

PLANT LEAF ANATOMY

In early research on photosynthesis, the relationship of plant structure to photosynthesis was not generally considered in the formulation of ideas and theories concerning higher plant metabolism. A notable exception to this was provided by the work of Prof. G. Haberlandt (1884), who formulated principles related to physiological anatomy based on light microscopy studies with a wide variety of green cells. One of these principles states that green leaves tend to expose a maximum of their chlorophyll-containing surface to the sun. Examples of this principle are that chloroplasts frequently are near the cell walls of plants, and cell walls may be folded to expose a larger internal surface area.

A resurgence of interest in the relationship of leaf anatomy to photosynthesis in higher plants came with the notable discovery of the "Kranz type" leaf anatomy (Haberlandt, 1884) being associated with the "new" C_4 cycle of photosynthesis (Downes and Hesketh, 1968). It has been found that Kranz anatomy is a necessity for C_4 photosynthesis (Hatch *et al.*, 1971); however, a type of Kranz cell anatomy can be present in plant leaves having the C_3 cycle of photosynthesis. It is common for C_3 monocots to have a layer of bundle sheath cells (which usually do not contain chloroplasts) surrounding the vascular tissue. Thus, the C_4 pathway of photosynthesis has been related to a unique internal leaf anatomy where at

least two major types of photosynthetic cells, mesophyll and bundle sheath cells, are found.

Other higher plant leaves were reconsidered in looking for differences in internal leaf anatomy. C_3 cycle plants are characterized by a layering of cells which have a wide variety of sizes and shapes. Haberlandt (1884), in pursuing his principle of maximum exposure of cell surfaces to sunlight, described a number of photosynthetic cell shapes: the simpliest are isodiametric, with a tendency to be rounded off at the edges; many are commonly elongated, cylindrical, or tubular. Special cells are funnel-shaped as in *Selaginella* species where the cell act as condensing lenses with the light reflected toward the base of the cells where the chloroplasts reside; other plants have arm-palaside cells. Some plants, particularly conifers, have polyhedral shaped cells with flanges projecting in from the cell walls often to give H-shaped cells. Spongy parenchyma with numerous radiating branches is common in higher plant leaves. Thus, a wide variety of shapes exist in photosynthetic cells, particularly in C_3 plants, but at this point no clear pattern of function or adaptation in this variety of cell shapes in evident.

A third type of leaf anatomy has been described which is often found in desert or arid land plants; commonly referred to as CAM plants. Similar size and shape of photosynthetic cells in many layers surrounding the vascular tissue is characteristic of CAM plants (Black, 1973; Black *et al.*, 1973). Other features associated with CAM plants are a thick epidermis and cuticle. Presumably, this functions to retard loss of water during the hot, dry days often experienced in arid climates. The leaves of CAM plants are frequently described as fleshy and with only one major type of photosynthetic cell, spongy mesophyll. These characteristics probably are related to the high content of water and consequently the large amount of cell sap found in these leaves. It is worth noting that a number of higher plants with known fleshy or succulent leaves do not appear to exhibit CAM; their leaf and organelle anatomy and their metabolic activities such as photosynthesis have not been investigated extensively.

CO_2 ASSIMILATION PATHWAYS IN LEAVES, CELLS, AND ORGANELLES

Today we know that multiple pathways of carbon assimilation do exist in individual plant leaf cells or chloroplasts (Hatch *et al.*, 1971; Black, 1971, 1972, 1973) and we now will consider some special features of C_4, C_3, and CAM plants as well as speculate on other types of plants found in specific environments.

Features of C_4 Plants. The photosynthetic cells of C_4 plants are of two types: mesophyll and bundle sheath cells. The evidence for the distinct nature of these cells is of two kinds. First, light and electron microscopy was used to describe

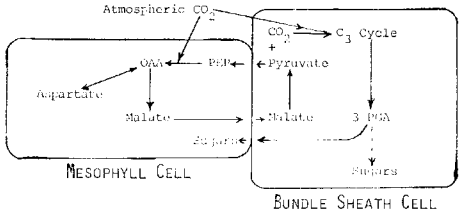

FIGURE 1. Outline of a proposed carbon-flow scheme in crabgrass.

the differences in the chloroplasts of these two types of cells, and further investigations of other cellular organelles (mitochondria and peroxisomes) by electron microscopy indicate other organelle differences in these two cells types (Hatch *et al.*, 1971; Black, 1972, 1973; Black *et al.*, 1973). Secondly, the current proposal of carbon flow in the C_4 pathway of photosynthesis involves distinctly different roles for the two cell types. The proposed scheme of carbon flow in crabgrass (a

C_4 plant) is outlined in figure 1. This proposed scheme is based on the location of enzymes in the isolated and separated cells of crabgrass as shown in table 3 (Edwards and Black, 1971; Chen *et al.*, 1973). Since chlorophyll is about equally distributed between the mesophyll and bundle sheath cells of crabgrass (Black, 1973), the specific activities presented represent the distribution of the total

TABLE 3

Distribution of enzymes in cell types and organelles of mature Digitaria sanguinalis *(crabgrass) leaves*

	Enzyme	Mesophyll cells	Bundle sheath cells	Probable subcellular location[1]
		μmoles/mg Chl/hr		
A.	C_4 Cycle Enzymes:			
	PEP Carboxylase	1,220	22	?
	Pyruvate, P_i, Dikinase	290	25	Ch,?
	Malic Enzyme	40	850	M,Ch,?
	MDH (NADP)	390	10	Ch
	Adenylate Kinase	1,900	1,500	Ch
	Pyrophosphatase	1,800	2,700	Ch
B.	C_3 Cycle Enzymes:			
	RuDP Carboxylase	24	450	Ch
	Ru-5-P Kinase	80	1,430	Ch
	R-5-P Isomerase	50	970	Ch
	Fructose DP Aldolase	50	1,000	Ch,Cy
	Glyceraldehyde-3-P-Dehydrogenase (NADP)	206	284	Ch
C.	Respiratory Enzymes:			
	Cytochrome *c* Oxidase	4	45	M
	Enolase	180	180	Cy
	Phosphoglycerate Mutase	120	100	Cy
	MDH (NAD)	620	1,130	M,Cy,Ch,P
	Catalase	13,000	55,000	P
	Phosphoglycolate Phosphatase	16	20	Ch
	Glycolate Oxidase	1.4	11	P
	Glyoxylate Reductase (NAD)	19	26	P
	Hydoxypyruvate Reductase (NAD)	30	170	P

[1]Subcellular location is designated as: Ch=chloroplast; M=mitochondria; P=peroxisome; and Cy=cytosol.

enzyme activities between the cell types in an intact leaf. Recently, we presented similar enzyme distribution data for *Cyperus rotundus* (purple nutsedge) which is also a C_4 plant (Chen *et al.*, 1974). The scheme is further supported by the fixation of CO_2 via PEP carboxylase in isolated mesophyll cell preparation in the presence of PEP and the fixation of CO_2 by bundle sheath cells in the presence of R–5–P or RuDP. In addition, we have demonstrated that isolated mesophyll cells of crabgrass fix $^{14}CO_2$ in the light in a stoichiometry of 1 mole of CO_2 fixed: 1 mole of malate reduced: 1 atom of O_2 evolved (Salin *et al.*, 1973). Finally, it has been shown that isolated bundle sheath strands of crabgrass can transfer carbon atom 4 of malic acid to compounds of the C_3 cycle, such as 3–PGA, and that the transfer process is enhanced by light (Dittrich *et al.*, 1973).

These data indicate that within a C_4 plant leaf, photosynthetic cells exist which have different characteristics and distinct roles in photosynthesis. However, the proposed pathway of carbon assimilation in C_4 plant leaves requires a strict dependence of the two cell types. The concept of the mesophyll cells acting as the "CO_2 trapping antennas" in the C_4 leaf and the bundle sheath cells serving the role of the "CO_2 reducing sites" of the C_4 leaf has been proposed (Black, 1973).

In addition to the distinct roles of these cell types in leaf photosynthesis we have studied the role of peroxisomes and mitochondria in each cell type. The data in table 3 and other data by Liu and Black (1972) on peroxisomal metabolism indicate that photorespiration is primarily in bundle sheath cells. Unpublished data on mitochondrial activity (Whitworth and Black) indicate that mitochondria in bundle sheath cells may be active enough to participate directly in leaf photosynthesis.

As work on C_4 photosynthesis expanded to a variety of plants it has become clear that several variations in the pathway of carbon assimilation exist (Black, 1973). In table 4 an up-to-date status of the variations known to exist in C_4

TABLE 4

Variations in carbon flow pathways in C_4 plant photosynthesis[1]

	Sugarcane crabgrass	*Panicum maximum* *Sporobolus poiretii*	*Atriplex* sp. *Portulaca* sp.
Primary product of $^{14}CO_2$ fixation accumulating (<10 sec photosynthesis)	Malate	Aspartate, 3-PGA	Aspartate
Likely intermediate moving from MC or BSC	Malate	OAA or Aspartate	Aspartate or Malate
Decarboxylating enzyme in BSC	Malic Enzyme ($NADP^+$)	PEP Carboxykinase	Malic Enzyme (NAD^+)
Likely intermediate moving from BSC to MC	Pyruvate or PEP 3-PGA	PEP 3-PGA	Pyruvate or PEP 3-PGA
Carboxylase in MC	PEP	PEP	PEP
Level of pyruvate Pi diKinase	=PS	<30% PS	=PS

[1]MC=mesophyll cell; BSC=bundle sheath cell; PS=photosynthesis.

photosynthesis is presented. No doubt this table will be modified and expanded as work continues and we feel that such variation is good evidence for presuming that C_4 photosynthesis is a relatively recent event in evolution.

Features of C_3 Plants and the C_3 Cycle. When one microscopically examines a C_3 leaf in cross section, usually two major cell types with chloroplasts are observed, spongy mesophyll and palisade. Haberlandt (1884) counted the chloroplast levels in these cells and the leaf distribution is shown in table 5. Prior to

TABLE 5

Number of chloroplasts in leaf palisade and spongy cells

Plant	Tissue	
	Palisade	spongy
	% of total	
Fragaria elatior	86	14
Pulmonaria officinalis	85	15
Ricinus communis	82	18
Brassica Rapa	80	20
Galeopsis Tetrahit	79	21
Tropaeolum majus	77	23
Helianthus annuus	73	27
Phaseolus multiflorus	69	31
Bellis perennis	67	33

discovery of C_4 photosynthesis, one expected both of these cell types to be C_3. Today we would question that assumption. W. Outlaw and D. Fisher (personal communication) have recently shown that palisade and spongy mesophyll cells of *Vicia faba*, a C_3 plant, do in fact conduct C_3 photosynthesis. However, in the epidermis of C_3 plants the guard cells are photosynthetic and our recent work shows that 4-carbon metabolism is active in the epidermal tissues of dayflower and tulip, both C_3 plants (Willmer *et al.*, 1973). In addition, the mesophyll cells of C_4 plants have a portion of the C_3 cycle; from 3–PGA to starch (table 6, and Salin and Black, 1974).

TABLE 6

Variations in carbon flow pathways in pentose plants or specific cell types

Pathway	Cell Types
Classical reductive pentose phosphate cycle	In a wide variety of higher plants including CAM and C_4 bundle sheath cells
3–PGA→Starch	In mesophyll cells of C_4 plants
4-carbon	Epidermal tissue including guard cells

Even in the C_3 cycle and in C_3 plants one finds variations in carbon flow pathways as outlined in table 6. Hence the function of the epidermal photosynthetic cells in C_3 plants may involve 4-carbon metabolism to open and close stomata. Mesophyll cells of C_4 plants may use their chloroplasts to reduce the 3-carbon acid, 3–PGA, to an aldehyde and then to directly form sugars completely omitting the generation of RuDP and the CO_2-fixing portion of the C_3 cycle (Salin and Black, 1974).

Features of CAM. CAM plants have not been investigated on a biochemical level in as much detail as the plants just discussed, but they are clearly unique. They will fix net quantities of atmospheric CO_2 over 24 hours if grown in the proper environment (Neales *et al.*, 1968). In addition, they adjust their pathway of carbon assimilation with environmental growth conditions (Bender *et al.*, 1973). In CAM plants some variables in the pathways of carbon assimilation are outlined in table 7; these are discussed in detail by Bender *et al.* (1973) and Dittrich *et al.* (1973).

TABLE 7

Tentative variations in carbon flow pathways in CAM plants

	Bromeliaceae Euphorbiaceae	*Cactaceae Crassulaceae*
Decarboxylase activity:		
PEP carboxykinase	High	Not detectable
Malic enzyme, $NADP^+$	Low	High
$\delta^{13}C$ indicative of:		
PEP carboxylase	Yes	Both and variable
RuDP carboxylase	No	with environment

On the subcellular level, CAM plants have the three major leaf organelles: chloroplasts, mitochrondria, and peroxisomes; however, another cell feature appears to be of great importance to CAM plants. This is the large cell vacuole which appears to occupy 90 to 95% of the cell in CAM plants. A major function

of this vacuole may be to store the C_4-dicarboxylic acids which are synthesized during dark CO_2 fixation (Ranson and Thomas, 1960). The current proposal of CAM operation is a diurnal cycle of acid production at night followed by a decarboxylation and CO_2 fixation and reduction to form starch during the day (Black, 1973). This scheme is based on $^{14}CO_2$ fixation studies and chemical studies of acid accumulation in CAM plants. Recently, an investigation of enzyme activities in CAM plants has suggested that the daylight catabolism of the dark accumulated acid may be by one of two pathways (Dittrich *et al.*, 1973). These two pathways are clearly separated among CAM genera and probably reflect an adaptive process of ancient origin. It has been suggested that CAM plants are performing carbon assimilation via a process quite similar to C_4 photosynthesis. However, the spatial separation found in the photosynthetic cells of a C_4 leaf is not found in a CAM leaf, where the processes of CO_2 trapping and reduction have a temporal separation while occurring in the same cell.

Other Pathways of Photosynthesis in Higher Plants. Currently, we are aware of three major types of photosynthetic mechanisms: the C_3 cycle, C_4 cycle, and CAM. These three plant types have distinct leaf anatomical features at both the cellular and subcellular levels. Other features of these three types may yet be discovered, such as distinct functional differences among the cells of C_3 or CAM leaves, and other major types of photosynthetic mechanisms. Green algae have a photosynthetic metabolism very similar to higher C_3 plants but may have distinct structures and functions which occur in response to an aqueous environment. In addition, other aquatic plants, particularly angiosperms such as *Zostera capricorni* or *Cymodocea serrulata* with their unique leaf structure, may represent a distinct group of photosynthetic plants. Leaves of alpine plants develop massive palisade tissues and high rates of photosynthesis (Black, 1971; 1973) which may indicate other distinct functions yet to be elucidated.

Subcellular Structures of Higher Plant Leaves. Turning briefly to the subcellular level of plant cells, compartmentation of the major metabolic pathways into organelles is as important in plant leaves as it has been found to be in animal cells. Certainly one site of adaptation appears evident in the wide variety of chloroplast ultrastructures observed in C_4 plants. Indeed within C_4 chloroplasts there is evidence that adaptive changes have occurred in the ratio of photosystem I to II (Black, 1973).

The role of the leaf peroxisome appears to be related to photorespiration (Tolbert, 1971). However, photorespiration appears to require a complex interplay of three cellular organelles, the chloroplast, the peroxisome, and the mitochondrion. The proposal is based mainly on subcellular location of the enzymes involved in the photorespiratory pathways (Table 3, Tolbert, 1971). Photorespiration has been thought at times to be absent from C_4 plants, but a number of investigations have revealed that the bundle sheath cells or strands of C_4 plants are capable of a photorespiratory type of activity (Black, 1973; Liu and Black, 1972). Furthermore, it has recently been shown that nutsedge (a C_4 plant) mesophyll cells lack activity for certain key enzymes of photorespiration and actually may not photorespire (Chen *et al.*, 1974). The function of photorespiration in plants remains uncertain, but the separation of this activity between the cell types of a C_4 plant implies a unique adaptation of these plants. Neither photorespiration nor respiration has been examined on a biochemical level in CAM plants.

CO_2 ASSIMILATION PATHWAYS IN PRIMITIVE PLANTS

In an effort to learn more about the evolution of photosynthetic carbon assimilation pathways, we have initiated a study with a wide variety of nonangiosperm tracheophytes. Our early studies have been short-time $^{14}CO_2$-pulse experiments followed by a $^{12}CO_2$-chase in which we identified initial products labeled with

^{14}C and observed the movement of ^{14}C into other products in a $^{12}CO_2$ atmosphere during constant illumination.

The prevailing idea today concerning the evolution of photosynthesis is that C_3 photosynthesis arose prior to 4-carbon photosynthesis. Indeed a careful check of the known C_4 plant species will reveal that it is present only in specific angiosperms (Hatch *et al.*, 1971; Black, 1973). Angiosperms are generally considered to have arisen in the Cretaceous period of the geologic time table. We have worked with the primitive plants shown in figure 1 and in table 8 which contains some

TABLE 8

Products of $^{14}CO_2$ fixation after 10 seconds of Photosynthesis

Plant name	MAL	ASP	PGA	HMP, HDP	Glycerate	Other amino acids
			% of Total $^{14}CO_2$ Fixed			
C_3 Plants:						
Gnetum montanum	—	—	47	53	—	—
Campyloneurum sp	—	—	33	67	—	—
Polypodium sp (20 sec)	—	—	23	52	12	8
Pueraria thunbergiana (Kudzu)	—	—	44	20	9	26
C_4 plants:						
Anthephora cristata	64	22	12	2	—	—
Uniola paniculata	43	37	3	—	—	17
Pectis leptocephala	62	34	4	1	—	—
Alternanthera repens	46	41.5	2	—	10	—

control experiments with Kudzu (C_3) and four C_4 plants. All of the more primitive plants in table 8 and figure 2 exhibit early ^{14}C labeling of 3-PGA, hexose monophosphates, and hexose diphosphates which turn over in $^{12}CO_2$ (fig. 2). We interpret all of these experiments as showing that the C_3 cycle is operating in these primitive plants and that 4-carbon photosynthesis is not a major CO_2 fixation pathway.

We have detected however, an exception in *Psilotum nudum* (fig. 3). The preliminary results indicate that *Psilotum* possesses some of the characteristics typical of a C_4 plant (Black, 1973). The primary labeled products of $^{14}CO_2$-photosynthesis are aspartate and 3-PGA. Aspartate rapidly loses ^{14}C in *Psilotum* as it does in certain C_4 plants (Chen *et al.*, 1971) while 3-PGA does not lose label as rapidly (Black, 1973). These studies are part of a preliminary survey and we are now gathering more data on photosynthesis and related characteristics such as those considered by Black (1973). We would tentatively conclude that plants long in the fossil record evolved C_3 photosynthesis which is still retained in many present day plants. C_4 photosynthesis appears to be relatively new having evolved only in angiosperms. If *Psilotum nudum* is conducting 4-carbon photosynthesis, it may be a rather recent development. Further studies in progress with other *Psilotum* species should yield more insight into their evolution. Other species of non-angiosperm tracheophytes also are being studied to assess the pathway(s) of CO_2 assimilation in a wide variety of primitive plants.

GENERAL CONCLUSIONS

Interaction of the three major types of higher plants is an important feature of our environment. It appears that each plant type has adapted to a specific environment and probably thrives best under specific climatic conditions. Only under extremes of climate or habitat (i.e., severe moisture stress, low temperatures, a salt marsh, or poor soil nutrition) does one type of plant tend to dominate. In

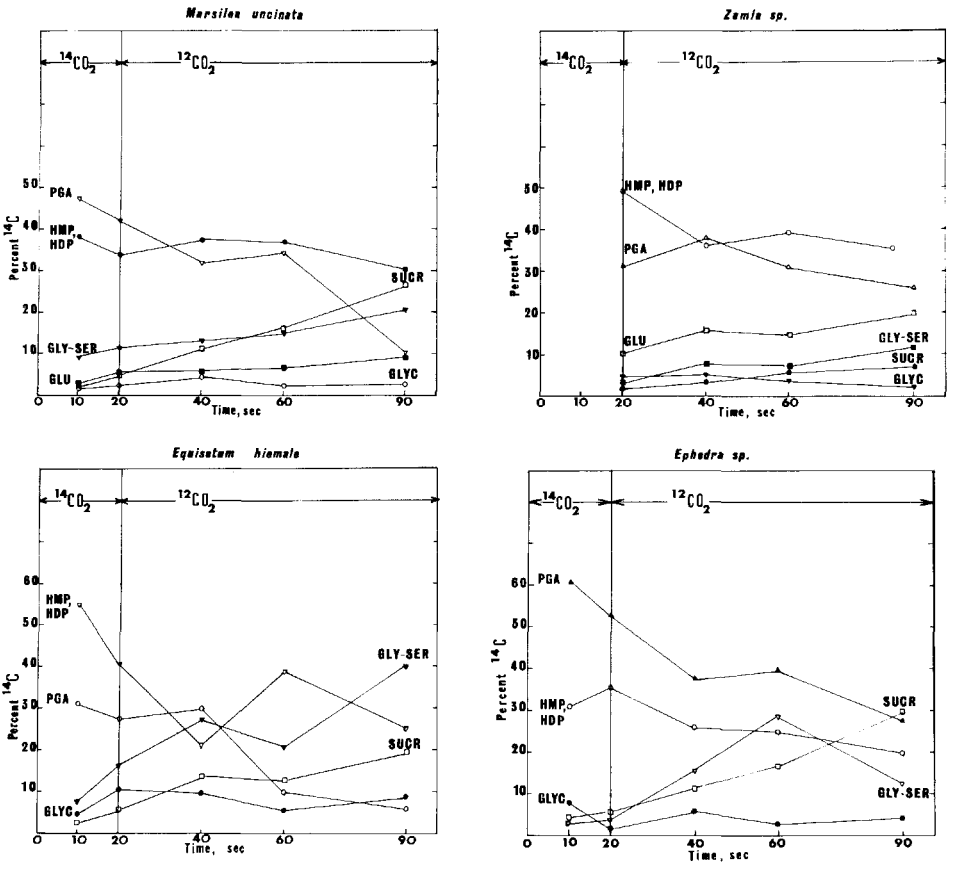

FIGURE 2. Pulse-chase experiments with green portions of: *Marsilea uncinata*, upper left; *Zamia* sp., upper right; *Equisetum hiemale*, lower left; and *Ephedra* sp., lower right. Illumination 5,000 foot candles at a temperature of 23–25° C.

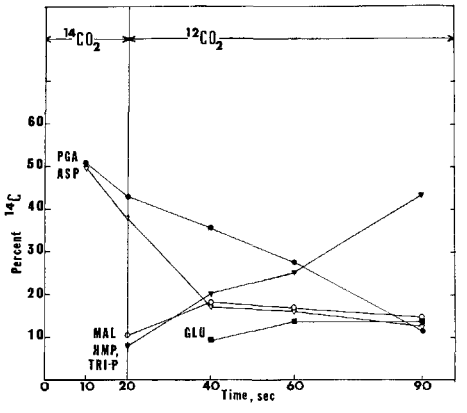

FIGURE 3. Pulse-chase experiment with *Psilotum nudum*. Illumination 5,000 foot candles at a temperature 25° C.

most locales of tropical and temperate zones, all three plant types coexist in apparent harmony. The appearance of a dominant photosynthetic producer in any community is a function of growth conditions to a great extent. One should note that the apparent rate of photosynthesis among the three types of plants indicates the following order: $C_4 > C_3 \gg CAM$.

Dampness, moderate temperatures, and light will bring terrestrial C_3 plants into dominance while terrestrial C_4 plants will dominate during periods of higher temperatures, high light, and moderate drought (Black *et al.*, 1969; Black, 1971). Primarily CAM plants will survive and thrive as perennials under extremely arid conditions, but CAM plants can grow well under a wide variety of conditions (Black, 1972). Thus, these three types of higher plants have distinct adaptive differences but have not become so specialized that they cannot survive under a variety of conditions. They are not so competitive on a year round basis that they eliminate other higher plants except in stress environments. For example, the salt marshes of the Southeastern United States can be monocultures of *Spartina alterniflora*, a C_4 plant.

Finally, the data presently available on photosynthetic cell metabolism in all higher plants clearly indicate that environmental pressure has resulted in considerable diversity of metabolic types. For example, we have described two different photosynthetic cell types of C_4 plants, mesophyll and bundle sheath. However, we have also indicated that there are at least three types of metabolism among the C_4 plants. We interpret this as an indication of the adaptive ability on the cellular level of the higher plant. The large number of higher plant species appear then to represent a complex heterogenous group of organisms when examined at the level of cellular metabolism.

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Glacial Geology of Highland County, Ohio. *Theodore E. Rosengreen.* Ohio Department of Natural Resources, Division of Geological Survey, Fountain Square, Columbus, OH 43224. 1974. iv+36 p., 19 figs., 1 folded colored map. $3.50 plus 14 cents tax in Ohio and 35 cents mailing charge. (Report of Investigations 92).

Glacial drift deposits cover nearly all of Highland County, located in southwestern Ohio. Late Wisconsinan drift mantles the northern third of the county, and, except for the southeasternmost corner, Illinoian drift covers the remainder. Soil analyses, radiocarbon dating, and geomorphology all are used to unravel the glacial history of this county. Sand and gravel deposits and their suitability for exploitation are discussed.

A colored map, scale one inch equals one mile, shows the several moraines, kames, shallow till areas, outwash, and alluvial deposits. This report will be of use to land-use planners, researchers, earth science teachers, consultants, and others needing information on the glacial geology of Highland County.

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