Plant Physiol. (1983) 71, 425–431
0032-0889/83/71/0425/07/$00.50/0

Photosynthesis of Grass Species Differing in Carbon Dioxide Fixation Pathways[1]

VIII. ULTRASTRUCTURAL CHARACTERISTICS OF *PANICUM* SPECIES IN THE *LAXA* GROUP

Received for publication August 16, 1982 and in revised form November 10, 1982

R. HAROLD BROWN, JOSEPH H. BOUTON, LUANNE RIGSBY, AND MARK RIGLER
Department of Agronomy, University of Georgia, Athens, Georgia 30602

ABSTRACT

Ultrastructural studies of leaves of seven *Panicum* species in or closely related to the *Laxa* group and classified as C_3, C_4 or C_3-C_4 intermediate were undertaken to examine features associated with C_3 and C_4 photosynthesis. The C_3 species *Panicum rivulare* Trin. had few organelles in bundle sheath cell profiles (2 chloroplasts, 1.1 mitochondria, and 0.3 peroxisomes per cell section) compared to an average of 10.6 chloroplasts, 17.7 mitochondria, and 3.2 peroxisomes per bundle sheath cell profile for three C_3-C_4 species, *Panicum milioides* Nees ex Trin., *Panicum decipiens* Nees ex Trin. and *Panicum schenckii* Hack. However, two other C_3 species, *Panicum laxum* Sw. and *Panicum hylaeicum* Mez, contained about 0.7, 0.5, and 0.3 as many chloroplasts, mitochondria, and peroxisomes, respectively, as in bundle sheath cell profiles of the C_3-C_4 species. Chloroplasts and mitochondria in bundle sheath cells were larger than those in mesophyll cells for the C_4 species *Panicum prionitis* Griseb. and the C_3-C_4 species, but in C_3 species the organelles were similar in size or were smaller in the bundle sheath cells. The C_3-C_4 species and *P. laxum* and *P. hylaeicum* exhibited an unusually close association of organelles in bundle sheath cells with mitochondria frequently surrounded in profile by chloroplasts. The high concentrations in bundle sheath cells of somewhat larger organelles than in mesophyll cells correlates with the reduced photorespiration of the C_3-C_4 species.

Soon after discovery of the C_4 cycle, anatomical features different from those in C_3 leaves were described (1). Species with C_4 photosynthesis possess well-developed chlorophyllous, vascular bundle sheaths in contrast to poorly developed, or apparently nonfunctional sheaths in C_3 species. Greater concentration of organelles have been observed in BSC[2] of C_4 species, especially those of the NAD-malic enzyme type, than in MC of C_3 or C_4 species (3, 8, 13).

Chloroplasts and mitochondria in BSC are larger than in MC of C_4 plants (6, 13). Species of C_4 plants which possess NADP-malic enzyme as the decarboxylase in BSC have chloroplasts centrifugally arranged in the bundle sheath and little or no granal development (9). Another major C_4 group has granal chloroplasts, centripetally arranged in BSC, and NAD-malic enzyme.

The discovery and description of three closely related *Panicum* species as intermediate to C_3 and C_4 (14, 15) raise questions about their ultrastructural similarity to C_3 and C_4 plants. The intermediate nature of *Panicum milioides, Panicum schenckii,* and *Panicum decipiens* (hereafter referred to as C_3-C_4) was based on reduced O_2 inhibition of apparent photosynthesis, low CO_2 compensation concentrations and high concentrations of chloroplasts and mitochondria in BSC compared to C_3 species (4, 14, 15). In addition, *P. milioides* has carboxylase activities and other traits intermediate to C_3 and C_4 species (2, 12, 17, 18).

The C_3-C_4 species can be considered to belong to the *Laxa* group of *Panicum* (10) which also contains C_3 species (5, 14). *Panicum laxum* and *Panicum hylaeicum* of the *Laxa* group (5, 20) and *Panicum rivulare* (*Grandia* group; 5) were found to have photosynthetic and gross leaf anatomical characteristics typical of C_3 species (14, 15). Classification of species in the *Laxa* and *Grandia* groups is uncertain because of Brown's (5) proposed removal of the intermediate species from the *Panicum* genus, his inclusion of both C_3 (*P. rivulare*) and C_4 (*Panicum prionitis*) species in the *Grandia* group, and the close relationships indicated by Rosengurtt *et al.* (20) among the species in both the *Laxa* and *Grandia* groups. A study of leaf ultrastructure in species of these groups was carried out to assess (a) the presence of characteristics associated with C_3 and C_4 photosynthesis and (b) anatomical features which might clarify phylogenetic relationships among these species.

MATERIALS AND METHODS

Experiment 1.

Plant Material. The youngest fully expanded leaf on a stem was sampled on the following species: *Panicum laxum* Sw. (accession number 137), *Panicum hylaeicum* Mez (127), *Panicum rivulare* Trin. (107), *Panicum milioides* Nees ex Trin. (101), *Panicum schenckii* Hack. (109), *Panicum decipiens* Nees ex Trin. (136), and *Panicum prionitis* Griseb. (126). Plants were grown in pots in greenhouses under maximum daytime temperatures of about 35°C and minimum night temperatures of about 20°C. Day length and solar radiation levels were those prevailing during late spring in Athens, GA.

Fixation and Embedding. One- × one-mm pieces cut from the center of the lamina were fixed by immersion in 2% glutaraldehyde in 0.1 M sodium cacodylate or 50 mM K_2HPO_4 buffer (pH 7.2) at 4°C for 1.5 h. Tissue was washed twice in buffer for 1.5 h at 4°C prior to post-fixation in 2% OsO_4 in the same buffer at room temperature for 1 h. An overnight wash in buffer followed by a 20-min wash preceded dehydration in a graded series of ethanol. The series consisted of 50, 60, 70, 80, 90, and 95% ethanol for 1 h, each, followed by 100% for 1 d. Propylene oxide was used as a transitional solvent for 4 h followed by infiltration in a graded series of Polaron Ultra Low Viscocity embedding media. The

[1] Supported by state and Hatch funds allocated to the Georgia Agricultural Experiment Stations and the United States Department of Agriculture, Agricultural Research Service, under Grant 5901-0410-8-0181-0 from the Competitive Research Grants Office.

[2] Abbreviations: BSC, bundle sheath cells; MC, mesophyll cells.

series consisted of an infinite dilution scheme whereby one-half of the volume of fluid was removed from the sample vessel and replaced with resin. Two changes of this type were made, raising the resin concentration to approximately 75%. The tissue was infiltrated overnight and then four more changes were made over a period of 3 to 4 h (1 to 2 h/change). Resin (100%) was added and polymerized in a vacuum infiltrating oven overnight at 70°C. Thin (silver) transverse sections were cut and stained for viewing in a transmission electron microscope. Staining consisted of 3 min in methanolic uranyl acetate (saturated) and subsequently for 4 min in lead citrate (19). The sections were reviewed in a Philips EM200 electron microscope at 60 kv.

Cell Sampling. Samples were taken from three separate leaves of each grass and five micrographs each of BSC and MC were observed per leaf, a total of 15 observations per species. Determinations included cell diameters, number of plasmodesmata per BSC profile, BSC cell wall thickness, numbers of chloroplasts, mitochondria and peroxisomes per cell profile, and size of chloroplasts and mitochondria. Cell diameters were determined from two measurements perpendicular to each other. Size of chloroplasts was determined as lengths of a long axis or angular length of two straight lines best fitting the curvature of curved chloroplasts. Mitochondrial size was determined as the length of the longest axis. Wall thickness of BSC was determined from measurements at four random positions on each cell profile. The number of plasmodesmatal pit fields were counted per BSC profile. Counts were also made of the number of mitochondrial profiles per BSC which were surrounded (one-half or more) by chloroplasts. Measurements and counts were made in most cases from projections of negatives, but in a few cases from prints of the negatives.

Experiment 2. In this experiment, the same species used in experiment 1 were sampled. The plants were grown under greenhouse conditions similar to those in experiment 1. Leaves were sampled in a manner similar to that in experiment 1 except that five leaves were sampled instead of three.

Fixation and embedding were similar to that in experiment 1 with the following exceptions. Fixation was in 4% rather than 2% glutaraldehyde and specimens were stored in this fixative from 30 to 90 d. Samples were postfixed 4 h at 4°C in 1.5% rather than 2% OsO_4. Specimens were embedded in Spurr's (21) medium rather than the Polaron medium used in experiment 1. Thin (silver) sections were cut and stained for 20 min in saturated uranyl acetate and poststained 5 min in Reynolds' (19) lead citrate.

Thin sections were cut from five separate specimens of each of the grasses. Three to five micrographs of randomly selected BSC and MC were made of each of the five samples from each species. Prints (20 × 25 cm) were made of two to three each of BSC and MC from each sample and were used for organelle counts and measurements of cell and organelle size. The observations described for experiment 1 were also made in this experiment on 12 BSC and MC of each species. Sizes of chloroplasts and mitochondria were determined on three organelles per cell profile except when fewer organelles were present. In addition, MC profile areas and areas occupied by organelles were measured to determine vacuole size. Vacuole percentage was determined as total cell profile area minus area occupied by organelles.

RESULTS

Description of Cells. *Panicum* species examined in this study had well-developed vascular bundle sheaths (Figs. 1–4) as reported earlier (14). The bundle sheath in all of the species, except *P. prionitis*, was underlain in major veins by thick-walled mestome sheaths, indicating the origin of the bundle sheath to be parenchymatous tissue (5). Major veins of *P. prionitis*, an NADP-malic enzyme-type C_4 species (5, 11), were surrounded by two concentric sheaths, the inner containing quantities of chloroplasts, mitochondria, and peroxisomes and the outer sheath being composed of cells about twice as large and nearly devoid of organelles (Fig. 4). The inner chlorophyllous sheath apparently arose from the mestome sheath, since the mestome sheath is absent in this species (5). The outer sheath then apparently arose from parenchymatous tissue, although cell wall thickness was similar for the two sheaths (Table I). In addition to major veins, *P. prionitis* leaves possess many small veins with only a single sheath (Fig. 5). In the further assessment of cell types for this species, the small veins have been omitted, although they are probably important in photosynthesis by this plant.

Bundle sheath cells were similar in size in all of the species studied (18–24 μm diameter), except in *P. prionitis* whose inner sheath was composed of cells averaging 11.1 μm diameter (Table I). The outer sheath of *P. prionitis* had cells slightly smaller than BSC of the other species. Vacuolar size varied from very small in the small vein BSC of *P. prionitis* (Fig. 5) to nearly complete occupation of the outer BSC of major veins. In the other species, vacuoles made up from 50 to 75% of BSC profiles in *P. milioides, P. schenckii,* and *P. decipiens,* to near 100% in *P. rivulare.*

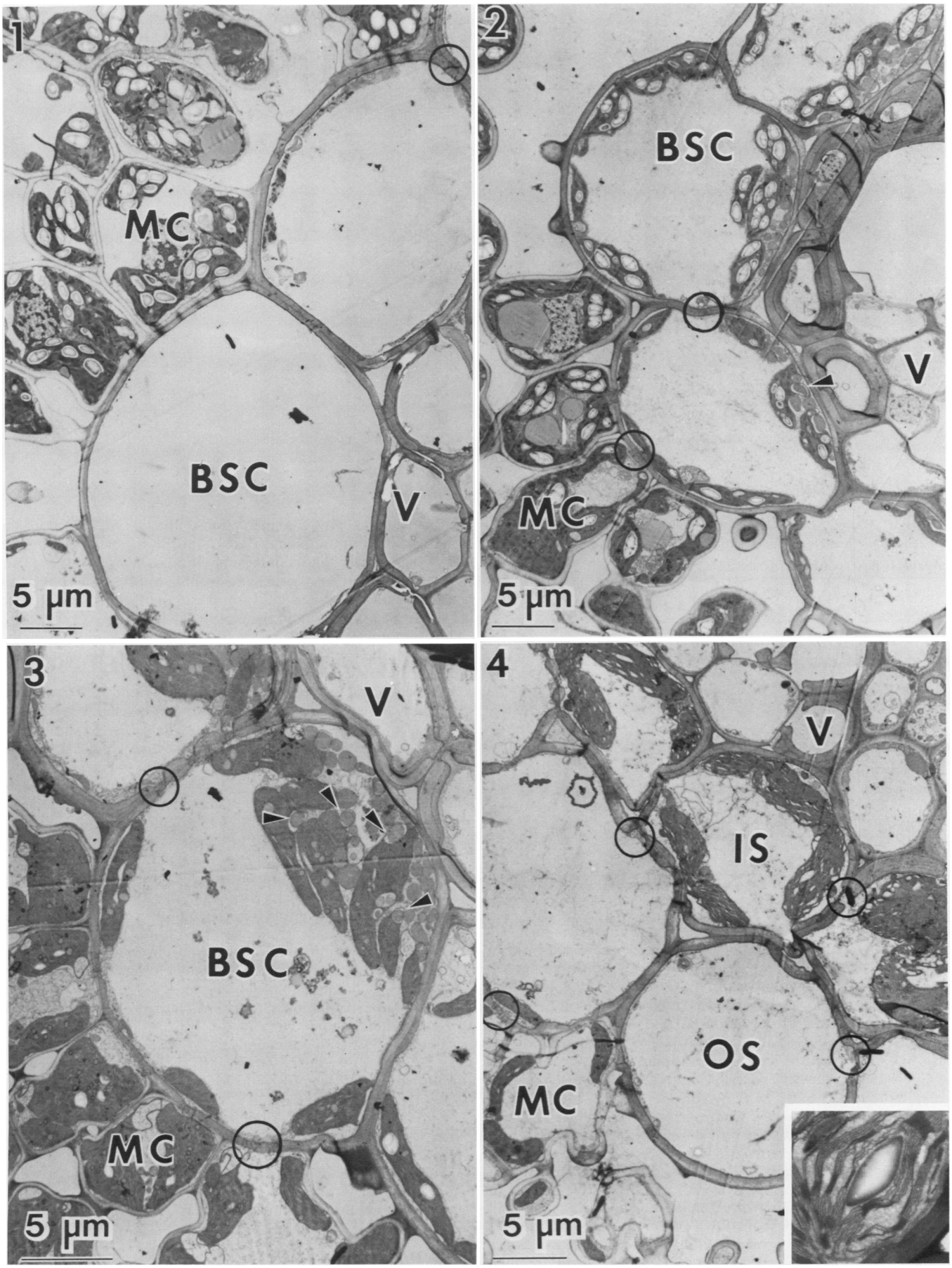
The number of plasmodesmatal pit fields per BSC profile varied from 2.2 in *P. rivulare* to 4.1 in *P. schenckii* and *P. decipiens* and to 5.7 in the outer sheath of *P. prionitis* (Table I). The C_3-C_4 species averaged 4.0 plasmodesmata per BSC profile compared to 2.5 for the C_3 species. The C_4 species *P. prionitis* had 3.1 plasmodesmata per BSC profile in inner sheaths, but these BSC are only one-half as large in diameter as BSC in the other species.

Mesophyll cells were irregular in size and shape (Figs. 1–4). Although MC diameters were significantly different among species, the range was only from 9.7 to 13.0 μm and no differences existed among photosynthetic types (Table II). Chloroplasts were arranged around the periphery of MC, but they occupied a high portion of MC profiles, except in *P. prionitis.* Estimates of vacuole area were from 20 to 43% of the MC profiles in the C_3 and C_3-C_4 species, but much higher (68%) in *P. prionitis* (Table II). In contrast to the BSC of C_3 and C_3-C_4 species in which mitochondria and peroxisomes were concentrated next to the centripetal walls, these organelles in MC were located between chloroplasts and away from the walls.

Organelle Numbers. Chloroplasts in MC varied from 4.5 per cell profile in *P. prionitis* to 7.1 in *P. hylaeicum* (Table III), but this difference is minimized if the difference in MC size in these two species is taken into account (Table II). The numbers of mitochondria and peroxisomes per MC profile tended to be higher in the C_3 than in the intermediate species, although significant differences were observed in only about one-half of the comparisons of species in the two groups. Mesophyll cells of *P. prionitis* contained 2.5 mitochondria and 1.0 peroxisome per profile, numbers similar to those of C_3-C_4 species.

Chloroplasts per BSC profile ranged from 2.0 in *P. rivulare* to 11.1 in *P. milioides* (Table III). The C_3-C_4 species contained, on the average, twice as many chloroplasts per BSC profile as C_3 species (10.6 *versus* 5.7), although within the C_3 group, numbers ranged from 2.0 in *P. rivulare* to 7.8 in *P. laxum.* The C_4 species, *P. prionitis,* had 3.7 chloroplasts per BSC profile (inner sheath), but when differences in cell profile area among species are accounted for (Table I), numbers of chloroplasts in BSC of *P. prionitis* are similar to the C_3-C_4 species.

Mitochondria were more numerous in BSC of the C_3-C_4 species than in C_3 species, but within the C_3 group, there was a 10-fold difference in numbers of mitochondria ranging from 1.1 in *P. rivulare* to 10.8 in *P. laxum* (Table III). The C_3-C_4 species had higher numbers of mitochondria in BSC profiles than *P. prionitis,* even when differences in cell size are accounted for. The number of peroxisomes in BSC profiles was also greatest in the C_3-C_4 species, ranging only from 3.1 to 3.4 per cell profile in this group. Peroxisomal profiles ranged from 0.3 to 1.1 in the C_3 species and



FIGS. 1–4. Electron micrographs of leaf cross-sections showing vascular, bundle sheath, and mesophyll tissues of *P. rivulare*, C_3 (1); *P. laxum*, C_3 (2); *P. schenckii*, C_3-C_4 (3); *P. prionitis*, C_4 (4). Note plasmodesmatal pitfields in BSC (circles) and mitochondrial profiles surrounded by chloroplasts in BSC of *P. schenckii* and *P. laxum* (arrows). (V), Vascular tissue; (OS), outer bundle sheath cells; (IS), inner bundle sheath cells. Inset at lower right of Figure 4 shows grana in BSC chloroplast (× 8840) of *P. prionitis*.

Table I. *Vascular Bundle Sheath Cell Characteristics of Panicum Species Differing in Photosynthetic Pathways*
Averages for experiments 1 and 2.

Pathway and Species	Cell Diameter	Wall Thickness	Plasmodesmata[a]	Mitochondria in Chloroplasts[b]
	μm		*no./cell profile*	
C_3				
P. rivulare	23.5	0.70	2.2	0.0
P. hylaeicum	21.2	0.45	2.6	0.3
P. laxum	20.9	0.40	2.7	0.7
C_3-C_4				
P. milioides	20.5	0.32	3.9	0.6
P. schenckii	20.7	0.42	4.1	2.4
P. decipiens	22.4	0.62	4.1	0.8
C_4				
P. prionitis (inner)	11.1	0.66	3.1	0.0
P. prionitis (outer)[c]	17.9	0.73	5.7	0.0
LSD (P = 0.05)	2.1	0.09	0.9	0.6

[a] Number of plasmodesmatal pitfields per cell profile.
[b] Number of mitochondria per cell profile one-half or more surrounded by chloroplasts.
[c] Outer bundle sheath cells evaluated only in experiment 2; not included in statistical analysis.

Table II. *Mesophyll Cell Diameters and Percentage of Cell Profile in Vacuoles for Panicum Species Differing in Photosynthetic Pathways*
Averages for experiments 1 and 2.

Pathway and Species	Cell Diameter	Vacuole Area
	μm	%
C_3		
P. rivulare	10.9	39
P. hylaeicum	13.0	35
P. laxum	11.4	20
C_3-C_4		
P. milioides	9.7	32
P. schenckii	10.9	35
P. decipiens	10.7	43
C_4		
P. prionitis	10.5	68
LSD (P = 0.05)	1.4	11

averaged 0.7 in *P. prionitis*.

Because there was a slight tendency for MC profiles in C_3-C_4 species to have fewer organelles than in C_3 species, the ratio of organelles in BSC to MC separated these two groups to a greater extent than organelle numbers in BSC alone (Table III). The greatest disparity between groups was in peroxisomal numbers, in which C_3 species varied from ratios of 0.3 to 0.7 and C_3-C_4 species ranged from 3.5 to 7.7. Although BSC to MC ratios for chloroplasts and mitochondria were similar among the C_3-C_4 species, in the C_3 group the values were 1.0 or higher for *P. hylaeicum* and *P. laxum*, but only 0.3 to 0.4 in *P. rivulare*.

Description of Organelles. Chloroplasts of both BSC and MC in all seven species possessed well-developed grana (Figs. 4 and 6). Very little peripheral reticulum was observed in chloroplasts of any of the species, the most being in MC of *P. prionitis*. In BSC of the C_3-C_4 species, chloroplasts were located centripetally as in those C_4 species for which NAD-malic enzyme is the C_4 acid decarboxylase. Chloroplasts in BSC of *P. laxum* (Fig. 2) and *P. hylaeicum* also tended to be centripetally located, but less conspicuously than in the C_3-C_4 species (Fig. 3) and in *P. rivulare*, they were more randomly arranged around the cell perimeter. Chloroplasts in the inner BSC of *P. prionitis* tended to be centripetally located (Fig. 4).

Differences among the three photosynthetic types in chloroplast lengths were not apparent, except that the C_4 species *P. prionitis* had smaller chloroplasts in mesophyll cells than C_3 species (Table IV). Ratios of chloroplast lengths in BSC to MC showed a clearer pattern, with values in the C_3 group ranging from 0.7 to 0.9, in the C_3-C_4 from 1.0 to 1.2, and the C_4 species exhibiting a value of 1.3. Thus, there was a tendency for BSC chloroplasts in intermediate and C_4 species to be larger relative to those in MC, while in C_3 species the tendency was reversed.

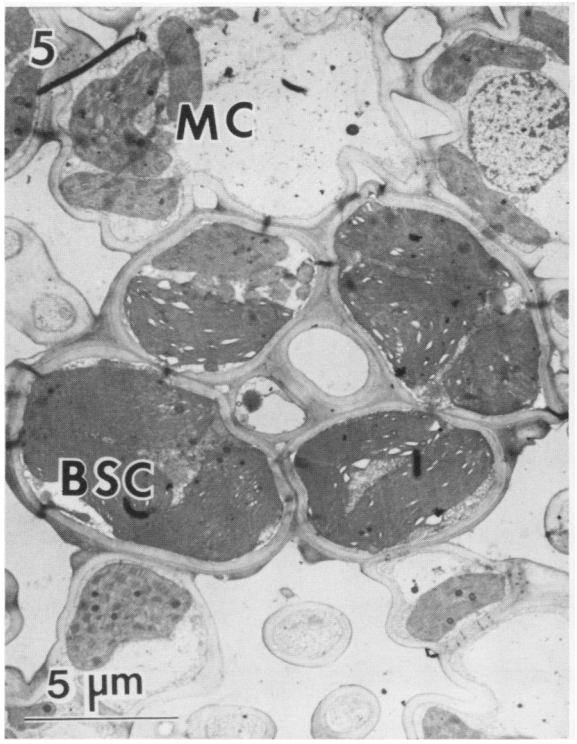
Mitochondria in BSC of the C_3-C_4 species and in *P. laxum* and *P. hylaeicum* were generally large and densely stained, with very translucent cristae (Fig. 6, a and b). They thus resemble mitochondria of BSC in NAD-malic enzyme-type C_4 species (6) and those described earlier for *P. milioides* and *Panicum hians* (7). Mitochondria in BSC in these plants were very closely associated with chloroplasts, in many cases being completely surrounded by them (Figs. 2; 3; 6, a and b). The frequency of mitochondria surrounded in profile by chloroplasts is shown in Table I. It occurred in all species except *P. rivulare* and *P. prionitis*. The high average value for this characteristic in *P. schenckii* is due mainly to the high frequency (4.1/cell profile) observed in experiment 2. Occasionally, peroxisomes (Fig. 6d) and oleosomes (Fig. 6c) were also observed to be surrounded by chloroplasts. Although the surrounding of mitochondria by chloroplasts was observed primarily in BSC, it also occurred at a much lower frequency in MC.

Differences in mitochondrial lengths among species were more clear cut than differences in chloroplast size (Table IV). The smallest mitochondria were in the BSC of *P. rivulare* with the second smallest BSC mitochondria in the C_4 species *P. prionitis*. The largest mitochondria were in BSC of the C_3-C_4 species. Ratios of mitochondrial size in BSC to MC were larger in the C_3-C_4 and C_4 species than in the C_3.

Peroxisomes appeared similar in structure for all the species. They were bounded by single membranes with edges of scalloped appearance in profile (Fig. 6d). The thread-like structures observed in microbodies of *Festucoid* species (8) were not clearly apparent. In a few cases, peroxisomes contained short rows of globular structure (not shown) similar to the structure described for C_4 species by Frederick and Newcomb (8).

DISCUSSION

The ultrastructural characteristics of *P. milioides, P. schenckii,* and *P. decipiens* support the CO_2 exchange and biochemical bases



for their classification as intermediate photosynthetic types. The most conspicuous trait is the concentration of organelles centripetally in the bundle sheath. The numbers of chloroplasts, peroxisomes, and mitochondria in BSC profiles were about 2, 5, and 7 times as great, respectively, as in MC. This compares to C_3 species which in this study and from inferences in published reports (8, 20) have organelles in BSC profiles nearly equal to or fewer than in MC. This greater concentration of organelles in BSC profiles in these C_3-C_4 species compared to C_3 suggests a greater metabolic role and perhaps a different one (18) than in C_3 species.

The large numbers of organelles in BSC profiles of C_3-C_4 species indicate that large percentages of total leaf organelles were in bundle sheaths. Ratios of the number of MC to BSC profiles determined from light micrographs of all of the species, except *P. prionitis,* averaged 10.2 and differed little among species. The percentages of organelles in the bundle sheath were estimated from the ratios of cell types and the organelle numbers in Table III. It was estimated that an average of 40, 32, and 17% of total leaf mitochondria, peroxisomes and chloroplasts, respectively, were in BSC of C_3-C_4 species compared to 14, 6, and 9% of corresponding organelles in BSC of C_3 species.

The differences between the C_3 and C_3-C_4 groups were not

FIG. 5. Minor vein in *P. prionitis*, with high concentration of chloroplasts in BSC.

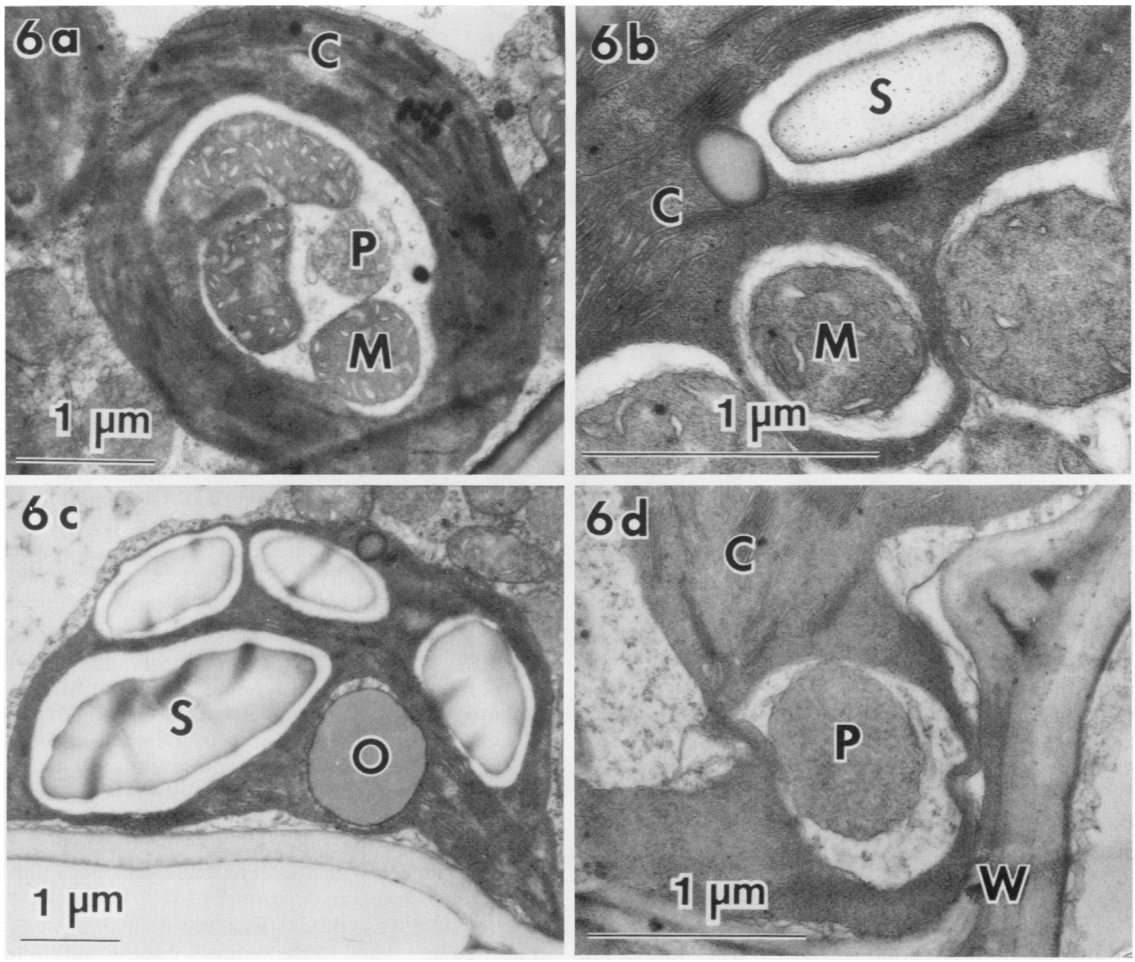


FIG. 6. Organelle associations in *Panicum* leaf cross-sections. Mitochondria surrounded by chloroplast in BSC of *P. milioides*, C_3-C_4 (a) and *P. laxum*, C_3 (b); oleosome surrounded by chloroplast in BSC of *P. laxum* (c); and peroxisome surrounded by chloroplast in BSC of *P. schenckii*, C_3-C_4 (d). (C), Chloroplast; (M), mitochondrion; (P), peroxisome; (S), starch grain; (W), cell wall; (O), oleosome.

Table III. *Numbers of Organelles per Cell Profile in BSC and MC of Panicum Species Differing in Photosynthetic Pathways*
Averages for experiments 1 and 2.

Pathway and Species	Chloroplasts			Mitochondria			Peroxisomes		
	BSC	MC	BSC:MC	BSC	MC	BSC:MC	BSC	MC	BSC:MC
				no./cell profile					
C_3									
P. rivulare	2.0	5.0	0.4	1.1	3.9	0.3	0.3	1.0	0.3
P. hylaeicum	7.4	7.1	1.0	8.7	4.8	1.8	0.9	1.4	0.6
P. laxum	7.8	5.7	1.4	10.8	4.3	2.5	1.1	1.5	0.7
C_3-C_4									
P. milioides	11.1	5.0	2.2	20.0	2.9	6.9	3.4	0.9	3.8
P. schenckii	10.6	5.6	1.9	19.5	2.4	8.1	3.2	0.9	3.5
P. decipiens	10.0	5.2	1.9	13.7	2.4	5.7	3.1	0.4	7.7
C_4									
P. prionitis	3.7	4.5	0.8	2.7	2.5	1.1	0.7	1.0	0.7
P. prionitis (outer)[a]	0.8			1.5			0.0		
LSD (P = 0.05)	1.5	1.0	0.4	2.9	1.5	2.8	0.8	0.6	0.7

[a] Outer bundle sheath cells evaluated only in experiment 2; not included in statistical analysis nor BSC to MC ratios.

Table IV. *Length of Organelles in BSC and MC of Panicum Speices Differing in Photosynthetic Pathways*
Averages for experiments 1 and 2.

Pathway and Species	Chloroplasts			Mitochondria		
	BSC	MC	BSC:MC	BSC	MC	BSC:MC
	μm		*ratio*	*μm*		*ratio*
C_3						
P. rivulare	4.0	5.8	0.7	0.5	0.8	0.6
P. hylaeicum	5.9	6.2	0.9	1.3	1.1	1.2
P. laxum	5.5	6.2	0.9	1.2	1.0	1.2
C_3-C_4						
P. milioides	6.8	5.7	1.2	1.5	0.9	1.7
P. schenckii	5.8	5.0	1.2	1.4	0.7	2.0
P. decipiens	5.9	5.6	1.0	1.5	0.9	1.7
C_4						
P. prionitis	6.0	4.6	1.3	1.0	0.6	1.7
P. prionitis (outer)[a]	3.3			0.4		
LSD (P = 0.5)	0.6	1.2	0.1	0.2	0.2	0.4

[a] Outer bundle sheath cells evaluated only in experiment 2; not included in statistical analysis nor BSC to MC ratios.

abrupt but characteristics appear to grade from C_3 into intermediate. For example, *P. rivulare* contained only about 20% as many chloroplasts per BSC profile as in C_3-C_4 species, but *P. hylaeicum* and *P. laxum* had about 75% as many (Table III). Similar trends were observed for increases in numbers of mitochondria and peroxisomes from *P. rivulare* to *P. hylaeicum, P. laxum*, and the C_3-C_4 species.

Species with C_4 photosynthesis have been observed to have larger chloroplasts, and in the case of NAD-malic enzyme types larger mitochondria, in BSC than in MC (3, 6, 16). A trend toward this inequality is noted in comparisons of the C_3-C_4 and C_3 species in this study with ratios of chloroplast lengths in BSC to MC varying from 0.7 in *P. rivulare* to 1.2 in *P. milioides* and *P. schenckii*. A similar trend in ratios of mitochondrial size was observed.

In the case of mitochondrial size, our data for C_3-C_4 species resemble closely those for two NAD-malic enzyme type C_4 species reported by Chapman *et al.* (6). The average mitochondrial lengths for *Amaranthus edulis* and *Atriplex spongiosa* were 1.46 μm for BSC and 0.69 μm for MC. Two NADP-malic enzyme-type species *Sorghum bicolor* L. Moench and *Zea mays* L. had smaller mitochondria in BSC (0.84 μm) which did not differ from those in MC. Thus, in several characteristics of BSC, including organelle numbers, size, and arrangement, grana in chloroplasts, and mitochondria with large translucent cristae, the C_3-C_4 species resemble NAD-malic enzyme type C_4 species.

The closeness of association of chloroplasts and mitochondria in the C_3-C_4 species and *P. laxum* and *P. hylaeicum*, especially the high frequency of mitochondrial profiles surrounded by chloroplasts, is unusual for C_3 or C_4 species. Although it may be observed in micrographs of *P. milioides* published by Kanai and Kashiwagi (11), and has been emphasized by Doohan (7), its physiological significance is not known. The surrounding of mitochondria by chloroplasts was found predominantly in BSC but also occurred in MC. The frequency of this configuration of chloroplast-mitochondrial association was variable, as indicated by the large LSD value in Table I, with less than one occurrence per BSC profile, except for *P. laxum* in experiment 1 (1.1/BSC) and *P. schenckii* in experiment 2 (4.1/BSC). This ultrastructural feature does not appear to be related to the reduced photorespiration in the C_3-C_4 *Panicum* species because it varied among the C_3-C_4 species and occurred in *P. laxum* and *P. hylaeicum* in a frequency not signifi-

cantly different from *P. milioides* and *P. decipiens.*

This comparative study of leaf ultrastructure does not explain the reason for reduced photorespiration in the C_3-C_4 species. If a large proportion of the photorespired CO_2 is released in BSC and if this CO_2 is not subject to loss to the atmosphere, considerable recycling of CO_2 should occur. The large proportion of organelles, particularly mitochondria and peroxisomes, in BSC of the C_3-C_4 species and the close association between chloroplasts and mitochondria may be related to the low photorespiratory CO_2 loss in these species. On the other hand, the high frequency of peroxisomes in these species is contrary to the trend expected in species with reduced photorespiration (8). The frequency of peroxisomes observed in the intermediate species in this study and that of Doohan (7) is much higher than normally seen in C_3 or C_4 species. So, if CO_2 loss in these species is due to low photorespiration *per se*, rather than increased recycling of photorespired CO_2, then it is not accompanied by a reduction in number of organelles involved.

The species in this study were selected on the basis of their assignment or close relationship to the *Laxa* group. The taxonomic grouping, with the exception of *P. prionitis*, tends to be confirmed by ultrastructure of leaves. The relationships among organelle size and numbers and carboxylase activities (15) are indicative of phylogenetic closeness. A more convincing characteristic of relationship is the surrounding of mitochondria by chloroplasts in BSC. This ultrastructural characteristic, as far as we know, is unique to the *Laxa* group and occurred in all of the species, except *P. rivulare* and *P. prionitis.*

P. prionitis does not appear closely related to the other species described. It is also not a very typical C_4 grass. The outer bundle sheath of large veins is composed of nonchlorophyllous cells twice as large as those of the inner sheath. There are numerous minor veins (14) which have only one sheath, composed of cells densely packed with chloroplasts (Fig. 5). Although it apparently is an NADP-malic enzyme type C_4 species (5, 15), it differs from other NADP-malic enzyme species in retention of an outer 'parenchyma' sheath with few organelles and in having granal chloroplasts, not centrifugally arranged in the inner BSC (Fig. 4). Brown (5) "tentatively proposed" that the *Grandia* group of *Panicum*, to which *P. prionitis* belongs, is more closely related to the intermediate species than to other non-kranz *Panicum* species. However, his proposed scheme for evolution in *Panicum* places the NADP-malic enzyme group, whose functional BSC (of large veins) developed from the mestome sheath, in a distant line from the NAD-malic enzyme, parenchyma sheath group which most closely resembles the C_3 and intermediate species in this study.

The data in this paper along with other characteristics of these species of *Panicum* (14, 15) lead to the conclusion that they are closely related, with the exception of *P. prionitis*, and possibly *P. rivulare.* Although leaf anatomical data place *P. rivulare* at the C_3 extreme of this group of species, evidence is not strong for its inclusion or exclusion from the *Laxa* group of *Panicum.* The gradation in organelle quantities and size in the BSC relative to MC from those typical of C_3 species toward C_4, may also indicate that species in this group include evolutionary intermediates.

LITERATURE CITED

1. Black CC 1973 Photosynthetic carbon fixation in relation to net CO_2 uptake. Annu Rev Plant Physiol 24: 253–256
2. Black CC, LD Goldstein, TB Ray, DP Kestler, BC Mayne 1976 The relationship of plant metabolism to internal leaf and cell morphology and to the efficiency of CO_2 assimilation. *In* RH Burris, CC Black, eds, CO_2 Metabolism and Plant Productivity. University Park Press, Baltimore, pp 113–139
3. Black CC Jr, HH Mollenhauer 1971 Structure and distribution of chloroplasts and other organelles in leaves with various rates of photosynthesis. Plant Physiol 47: 15–23
4. Brown RH, WV Brown 1975 Photosynthetic characteristics of *Panicum milioides*, a species with reduced photorespiration. Crop Sci 15: 681–685
5. Brown WV 1977 The kranz syndrome and its subtypes in grass systematics. Mem Torrey Bot Club 23: 1–97
6. Chapman EA, JM Bain, DW Gove 1975 Mitochondria and chloroplast peripheral reticulum in the C_4 plants *Amaranthus edulis* and *Atriplex spongiosa.* Aust J Plant Physiol 2: 207–223
7. Doohan ME 1978 Investigations of ultrastructural aspects of the $C_{(4)}$ syndrome. PhD Thesis. University of Wisconsin, Madison
8. Frederick SE, EH Newcomb 1971 Ultrastructure and distribution of microbodies in leaves of grasses with and without CO_2-photorespiration. Planta 96: 152–174
9. Guttierrez M, VE Gracen, GE Edwards 1974 Biochemical and cytological relationships in C_4 plants. Planta 119: 279–300
10. Hitchcock AA 1915 *Panicum.* North Am Flora 17: 198–288
11. Kanai R, M Kashiwagi 1975 *Panicum milioides,* a gramineae plant having kranz leaf anatomy without C_4-photosynthesis. Plant Cell Physiol 16: 669–679
12. Ku SB, GE Edwards 1978 Photosynthetic efficiency of *Panicum hians* and *Panicum milioides* in relation to C_3 and C_4 plants. Plant Cell Physiol 19: 665–675
13. Laetsch WM 1971 Chloroplast structural relationships in leaves of C_4 plants. *In* MD Hatch, CB Osmond, RO Slatyer, eds, Photosynthesis and Photorespiration. John Wiley and Sons, New York, pp 323–349
14. Morgan JA, RH Brown 1979 Photosynthesis of grass species differing in carbon dioxide fixation pathways. II. A search for species with intermediate gas exchange and anatomical characteristics. Plant Physiol 64: 257–262
15. Morgan JA, RH Brown, BJ Reger 1980 Photosynthesis of grass species differing in carbon dioxide fixation pathways. III. Oxygen response and enzyme activities of species in the *Laxa* group pf *Panicum.* Plant Physiol 65: 156–159
16. Pyliotis NA, KC Woo, WJS Downton 1971 Thylakoid aggregation correlated with chlorophyll a/chlorophyll b ratios in some C_4 species. *In* MD Hatch, CB Osmond, RO Slatyer, eds, Photosynthesis and Photorespiration. John Wiley and Sons, New York, pp 406–412
17. Rathnam CKM, R Chollet 1978 CO_2 donation by malate and aspartate reduces photorespiration in *Panicum milioides,* a C_3-C_4 intermediate species. Biochem Biophys Res Commun 85: 801–808
18. Rathnam CKM, R Chollet 1979 Photosynthetic carbon metabolism in *Panicum milioides,* a C_3-C_4 intermediate species: evidence for a limited C_4 dicarboxylic acid pathway of photosynthesis. Biochim Biophys Acta 548: 500–519
19. Reynolds ES 1963 The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J Cell Biol 17: 208–213
20. Rosengurtt B, B Arrillaga de Maffei, P Izaguirre de Artucio 1970 Gramineas Uruguayas. Universidad de la Republica, Montevideo, Uruguay
21. Spurr AR 1969 A low-viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res 26: 31–43