Plant Physiol. (1979) 64, 257-262
0032-0889/79/64/0257/06/$00.50/0

Photosynthesis in Grass Species Differing in Carbon Dioxide Fixation Pathways

II. A SEARCH FOR SPECIES WITH INTERMEDIATE GAS EXCHANGE AND ANATOMICAL CHARACTERISTICS[1]

Received for publication January 24, 1979 and in revised form April 5, 1979

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ABSTRACT

Thirty-three grass species were examined in two experiments in an attempt to locate plants with photosynthetic responses to O_2, CO_2 compensation concentrations, and leaf anatomy intermediate to those of C_3 and C_4 species. Species examined included seven from the *Laxa* group in the *Panicum* genus, one of which, *P. milioides* Nees ex Trin., has been reported earlier to have intermediate characteristics. The species with O_2-sensitive photosynthesis typical of C_3 plants showed more than 37% increase in apparent photosynthesis at 2% O_2 compared to 21% O_2 at 25 C and 335 microliters per liter CO_2, whereas in *Panicum milioides*, *P. schenckii* Hack., and *P. decipiens* Nees ex Trin., members of the *Laxa* group of *Panicum*, increases ranged from 25 to 30%. The remainder of the species did not respond to O_2. Species with O_2 responses characteristic of C_3 plants exhibited CO_2 compensation concentrations of 44 microliters per liter or higher at 21% O_2 and 25 to 27.5 C and species characterized as O_2-insensitive had values of microliters per liter or less. The CO_2 compensation concentration (Γ) values of *P. milioides*, *P. schenckii*, and *P. decipiens* ranged from 10.3 to 23.3 microliters per liter. Other species of the *Laxa* group of *Panicum* exhibited O_2 response and Γ values of either C_3 (*P. laxum* Sw., *P. hylaeicum* Mez., and *P. rivulare* Trin.) or C_4 (*P. prionitis* Griseb.) plants. Leaves of species with O_2 response and CO_2 compensation values typical of C_3 plants had poorly developed or nearly empty bundle sheath cells, and much larger distances and mesophyll cell numbers between veins than did the O_2-insensitive ones. Vein spacings in *P. milioides*, *P. schenckii*, and *P. decipiens* ranged from 0.18 to 0.28 millimeter and mesophyll cell number between veins from 5.2 to 7.8. While these vein spacings are closer than those of most C_3 grasses, two O_2-sensitive species of *Dactylis* had vein spacings similar to these *Panicums* and veins in *Glyceria striata*, another O_2-sensitive plant, were separated by only four mesophyll cells and 0.12 millimeter. Bundle sheath cells of the three intermediate *Panicums* contained greater quantities of organelles than are typical for C_3 grasses.

The best documented example of a naturally occurring intermediate plant is the tropical grass, *Panicum milioides*. Response of AP[2] to O_2, CO_2 evolution in the light, and Γ of *P. milioides* were found to be intermediate to those observed in *P. maximum*, a C_4 species and *Festuca arundinacea*, a C_3 species (4). The intermediate gas exchange characteristics of *P. milioides* have been confirmed in other laboratories (17, 18, 20, 22). Leaf anatomy of *P. milioides* is Kranz-like in appearance, but with fewer organelles in bundle sheath cells and more widely spaced veins than are normally observed in C_4 plants (4, 16). Quebedeaux and Chollet (22) reported an intermediate response of growth parameters of *P. milioides* to changes in O_2 and CO_2 concentration when compared to *P. bisulcatum* Thunb. (C_3) and *P. miliaceum* L. (C_4). Reduced photorespiration in *P. milioides* may be ascribed in large part to slightly elevated activities of P-enolpyruvate carboxylase detected in this species (10, 17, 18, 21) in combination with relatively low activities of ribulose bisP oxygenase (10, 18) and glycolate oxidase (21).

Recent information suggests that compartmentation of P-enolpyruvate carboxylase and ribulose bisP carboxylase in the mesophyll and bundle sheath cells, respectively, may be an important factor in the reduced photorespiration in *P. milioides* (23), although no such compartmentation was detected in an earlier study (21). *Panicum hians* Ell. is a North American species which is considered conspecific with *P. milioides* (b) and is included in some of the studies referred to above (10, 21).

In the following study, photosynthetic O_2 responses, Γ and leaf anatomical characteristics of 33 species of grasses were investigated in an attempt to locate additional grasses with characteristics intermediate to those normally observed in C_3 and C_4 grasses.

MATERIALS AND METHODS

Plant Material. Plants exhibiting C_4 photosynthesis have higher $^{13}C/^{12}C$ ratios in their tissues than do C_3 plants (7, 25) and these ratios reflect the CO_2 assimilation pathways of the respective plant types. Since we were looking for species with characteristics intermediate to C_3 and C_4 photosynthetic types, grasses were considered for this study if their reported $^{13}C/^{12}C$ values were near the upper limits of the range for C_3 species or near the lower limits of the range for C_4 plants. Furthermore, some plants were chosen from habitats that are not usually associated with their CO_2 fixation cycle, for instance, C_4 grasses adapted to temperate latitudes.

Most of the plants investigated were grown from seed obtained from USDA Plant Introduction Stations. *Panicum hylaeicum*, *P. laxum*, *P. prionitis*, *P. rivulare*, and *P. schenckii* were collected in South America and included in this study because they have been assigned to a taxonomic group (*Laxa*) in the genus which includes *P. milioides* (15). *Paspalum repens* was also collected in South America. Specimens of *Paspalum urvillei*, *F. arundiancea*, *Glyceria striata*, and an unidentified *Panicum* species were collected locally.

EXPERIMENT 1

Apparent Photosynthesis at 21 and 2% O_2. Plants were potted in field soil and grown in the greenhouse under an automatic

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[1] This research was supported by state and Hatch funds allocated to the Georgia Agricultural Experiment Stations.

[2] Abbreviations: AP: apparent photosynthesis; Γ: CO_2 compensation concentration; PPFD: photosynthetic photon flux density.

sprinkler irrigation system. Commercial fertilizer containing 5, 4.4, and 12.4% N, P, and K, respectively, was applied about every 10 days after seedlings were established. When the leaves of the plants were almost large enough for photosynthesis measurements, the grasses were transferred to a growth chamber and remained there 7 to 14 days before measurements were taken. Chamber temperature and PPFD at plant height were maintained at 27 C and 1045 μE m^{-2} s^{-1} respectively, during the 12-h day. Night temperature was 22 C.

Apparent photosynthesis measurements were made in an open system with an IR gas analyzer. The assimilation chamber was 9.5 cm long, 4.5 cm wide, and 1.5 cm deep, made of Plexiglas, and equipped with a small fan. Photosynthesis was measured on the youngest, fully expanded leaves of each plant at 2 and 21% O_2. There were three replicates of each measurement. Gas containing 335 μl l^{-1} of CO_2 and either 2 or 21% O_2 was bubbled through water at a constant temperature to raise the dew point to approximately 16 C. Leaf temperature was maintained at 25 C and monitored by a thermocouple touching the bottom side of the leaf. A PPFD of 1,600 μE m^{-2} s^{-1} was provided from a 1,500-w multivapor lamp. Three photosynthesis chambers were connected to the gas supply in parallel, so that leaves from three different plants could be equilibrating at the same time. The exhaust from the chambers was switched to the gas analyzer when CO_2 measurement was made.

CO_2 Compensation Concentration. Measurements of Γ were conducted according to the method of Goldsworthy and Day (11) on the same plants used in the previous experiment following several weeks of growth in the greenhouse. Two dishes of sections from young fully expanded leaves were inserted into a Mylar bag (20 × 45 cm) which was then inflated with air containing 21% O_2 and about 30 μl l^{-1} CO_2. The bags were placed inside a growth chamber maintained at 25 ± 2 C and with a PPFD of 318 μE m^{-2} s^{-1}. Approximately 3 h later the CO_2 concentration in the Mylar bag was determined with a gas analyzer. Three separate determinations were made for each plant.

Leaf Anatomy. Leaves used in the photosynthesis study were cut into 1- to 5-mm sections, fixed in a formalin-acetic acid-ethanol mixture, dehydrated in a tertiarybutyl alcohyl series, embedded in paraffin, and sectioned with a rotary microtome. The sections were stained with safranin and fast green.

Interveinal distance (center to center of vascular bundles) and maximum lateral cell count (number of mesophyll cells separating bundle sheaths) (14) were recorded for each plant from the average of 10 measurements of several cross-sections.

EXPERIMENT 2

Species which have been placed in the *Laxa* group were studied further in experiment 2. The *Laxa* species investigated were *P. milioides*, *P. schenckii*, *P. decipiens*, *P. prionitis*, *P. rivulare*, *P. laxum*, and *P. hylaeicum*. *F. arundinacea* and *P. maximum* were also included in this study and served as C_3 and C_4 controls, respectively.

Three cuttings for each species were transplanted into three separate pots. The pots were arranged in a randomized complete block design on a greenhouse bench. The nine grass species were present in each of three blocks. Supplemental lighting was provided 14 h per day by three multivapor lamps. The grasses were illuminated with approximately 1,000 to 1,200 μE m^{-2} s^{-1} at plant height. They were fertilized with half-strength Hoagland solution modified with $(NH_4)_2SO_4$ as the N source. NH_4NO_3 was also applied every 7 days for a few weeks prior to and during the photosynthesis and Γ measurements.

Leaf photosynthesis measurements were performed in the same manner as described for experiment 1. Determinations of Γ were conducted as described for experiment 1, except the temperature and PPFD during the measurements were 27.5 C and 914 μE m^{-2} s^{-1}, respectively.

Leaf cross-sections were prepared as described in experiment 1 for measurements of interveinal distance and maximum lateral cell count. In addition, leaf sections of 1 to 2 mm^2 were fixed in glutaraldehyde in phosphate buffer (pH 7.4). The tissue was washed for 4 h in several changes of phosphate buffer containing 2% sucrose and postfixed in 1% OsO_4 (pH 7.4 in phosphate-sucrose buffer) for 12 h. The tissue was dehydrated in an ethanol series and embedded in plastic. Sections of 1 or 2 μm were cut with a glass knife, mounted on microscope slides, and stained with toluidine blue.

RESULTS

EXPERIMENT 1

Gas Exchange Characteristics. Two basic patterns emerged from the CO_2 exchange measurements. An O_2-insensitive group of plants included species from the Aeluropideae, Chlorideae, Eragrosteae and Paniceae tribes. Apparent photosynthesis rates of these plants were generally high (average at 21% O_2 = 42.8 ± 6.1 mg dm^{-2} h^{-1}) and unaffected by the change in O_2 concentration (Table I). This response is typical for C_4 plants (1, 9). Apparent photosynthesis of *Setaria italica* was unaffected by O_2 concentration but was relatively low (28.3 ± 3.9 mg dm^{-2} h^{-1} at 21% O_2) for a C_4 species. However, the leaves were noticeably chlorotic when AP was measured. The O_2-sensitive group of grasses included species from the Festuceae, Paniceae, Phalarideae, and Stipeae tribes. For this group, AP increased as the O_2 level was reduced from 21 to 2%, and at 21% O_2 AP was generally lower than observed in the O_2-insensitive group.

The percentage change in AP at 2% O_2 relative to rates attained at 21% O_2 is an estimate of the relative magnitude of both photorespiration and O_2 inhibition of photosynthesis (8). This value for the group referred to as O_2-insensitive is approximately zero (Table I) and indicates a lack of detectable photorespiration and/or O_2 inhibition of photosynthesis in these species. The range in O_2 responses exhibited by the O_2-sensitive grasses excluding *P. schenckii* was from 37.3% for *P. hylaeicum* to 62.8% for *Stipa tenuis*. The unusually low O_2 response of *P. schenckii*, 25.1 ± 10.1, was similar to values previously obtained for *P. milioides* (4), a closely related species.

With one exception, all of the O_2-insensitive species displayed Γ values of less than 4 μl l^{-1} (Table I). The value for *P. prionitis*, was 6 ± 5 μl l^{-1}. The O_2-sensitive grasses, except *P. schenckii*, has Γ values ranging from 45 to 55 μl l^{-1}. *P. schenckii* exhibited a Γ of 10 ± 3 μl l^{-1}. The Γ value for *P. schenckii* was less than those estimated previously for *P. milioides* (4, 18, 20, 22).

Leaf Anatomy. Interveinal distances for the O_2-insensitive species ranged from 0.07 to 0.19 mm with a mean of 0.12 ± 0.04 mm (Table II). While the interveinal distances in the O_2-sensitive plants were generally greater (0.25 ± 0.10 mm), the range of values for these species (0.12–0.46 mm) overlapped with those of the O_2-insensitive grasses. No more than three cells separated the bundle sheaths in the O_2-insensitive grasses with the exception of *P. prionitis* which had an average of 3.2 cells between adjacent bundle sheaths (Table II). At least four cells and an average of 9.1 ± 2.7 mesophyll cells separated the bundle sheaths in the O_2-sensitive species. The bundle sheath cells of O_2-sensitive species contained consistently fewer organelles than were observed in the more developed bundle sheath cells of the O_2-insensitive grasses.

EXPERIMENT 2

Gas Exchange Characteristics. Gas exchange studies resulted in a classification of these grasses into three groups (Table III). *Panicum prionitis* and *P. maximum* had high AP rates at 21% O_2 which were unaffected by O_2 concentration. The O_2 responses of *P. rivulare*, *P. hylaeicum*, and *P. laxum* were either similar to or slightly greater than that of *F. arundinacea* with AP ranging from 38 to 53.5% higher at 2% than at 21% O_2.

Table I. *Apparent Photosynthesis at 2 and 21% O_2, O_2 Response, and CO_2 Compensation Concentrations (Γ) of Various Gramineae Species*

Tribe, species	Apparent photosynthesis[1]		O_2 response[2]	Γ[1]
	2% O_2	21% O_2		
	$mg\ dm^{-2}\ h^{-1}$		*%*	*$\mu l\ l^{-1}$*
Oxygen-insensitive Species				
Aeluropideae				
Vaseyochloa multinervosa (Vasey) Hitchc.	43.8 ± 1.3[3]	44.3 ± 0.5	−1.2 ± 1.7	3 ± 1
Chlorideae				
Chloris cucullata Bisch.	35.2 ± 6.7[4]	36.6 ± 4.8[4]	−4.2 ± 5.6[4]	—
Chloris distichophylla Lag.	47.5 ± 6.6[4]	47.6 ± 4.0[4]	−0.4 ± 5.4[4]	—
Chloris gayana Kunth. (2)[5]	45.6 ± 7.0	45.4 ± 6.9	0.5 ± 1.6	2 ± 1
Eragrosteae				
Eragrostis curvula (Schrad.) Nees	42.5 ± 5.8	42.1 ± 9.6	2.8 ± 11.6	2 ± 1
Paniceae				
Echinochloa frumentacea Roxb.	32.6 ± 3.3[4]	33.5 ± 3.0[4]	−3.0 ± 1.3	2 ± 1
Echinochloa crusgalli (L.) Beauv.	44.8 ± 4.6	45.3 ± 5.4	−0.9 ± 1.5	—
Panicum antidotale Retz.	46.5 ± 4.4	45.2 ± 6.1	0.6 ± 3.5	1 ± 1
Panicum miliaceum L.	44.7 ± 2.0	45.5 ± 1.9	−1.8 ± 0.5	1 ± 0
Panicum prionitis Griseb.	39.4 ± 15.3	39.9 ± 15.2	−1.5 ± 1.0	6 ± 5
Panicum virgatum L. (2)	50.9 ± 7.8	50.4 ± 7.9	1.0 ± 3.6	3 ± 2
Panicum sp.	50.0 ± 5.5	50.6 ± 4.6	−1.3 ± 2.1	1 ± 1
Paspalum repens Berg.	42.2 ± 3.1	42.6 ± 3.8	−1.0 ± 2.9	1 ± 1
Paspalum urvillei Steud.	45.9 ± 4.3	44.1 ± 2.8	4.1 ± 3.1[4]	—
Setaria italica (L.) Beauv.	28.5 ± 4.2	28.3 ± 3.9	2.2 ± 2.1	2 ± 0
Mean and SD of O_2-insensitive species	42.7 ± 6.3	42.8 ± 6.1	−0.3 ± 2.2	2 ± 2
Oxygen-sensitive species				
Festuceae				
Dactylis aschersoniana	33.1 ± 5.1	23.3 ± 3.8	42.0 ± 2.8	—
Dactylis glomerata L. (6)	32.3 ± 9.5	22.5 ± 6.6	44.1 ± 7.8	47 ± 3
Festuca arundinacea Schreb. (3)	36.2 ± 6.6	25.6 ± 4.6	41.4 ± 5.3	47 ± 3
Glyceria striata (Lam.) Hitch.	24.7 ± 4.6	20.7 ± 2.8	43.6 ± 3.7	47 ± 0
Paniceae				
Panicum hylaeicum Mez. (3)	28.4 ± 6.7	20.8 ± 5.3	37.3 ± 5.2	55 ± 14
Panicum rivulare Trin.	36.3 ± 4.3	26.0 ± 2.6	39.3 ± 3.4	53 ± 2
Panicum schenckii Hack.	36.7 ± 5.1	29.2 ± 1.8	25.1 ± 10.1	10 ± 3
Phalarideae				
Phalaris arundinacea L. (6)	32.3 ± 7.4	23.3 ± 5.3	45.0 ± 8.3	48 ± 3
Stipeae				
Stipa columbiana Macoun.	47.0 ± 7.3	30.1 ± 1.7	55.6 ± 14.7	46 ± 2
Stipa occidentalis Thurb.	32.4 ± 7.4	22.7 ± 5.1	42.8 ± 8.5	45 ± 1
Stipa speciosa Trin. and Rupr.	50.6 ± 1.9	34.1 ± 2.6	48.5 ± 6.8	54 ± 4
Stipa splendens Trin.	34.4 ± 3.2	23.2 ± 1.4	48.2 ± 6.2	53 ± 3
Stipa tenacissima L.	48.2 ± 8.3	32.5 ± 7.1	48.9 ± 6.5	53 ± 2
Stipa tenuis Willd. ex Steud.	58.6 ± 3.1	36.2 ± 4.4	62.8 ± 12.6	55 ± 9
Mean and SD of O_2-sensitive species	38.3 ± 9.1	26.4 ± 5.1	44.6 ± 8.7	47 ± 12

[1] Apparent photosynthesis and Γ were measured at 25 C.

[2] Percentage increase or decrease (−) of apparent photosynthesis at 2% O_2 compared to rates attained at 21% O_2.

[3] Mean and standard deviation of three measurements unless otherwise indicated.

[4] Mean and standard deviation of two measurements.

[5] Numbers in parentheses refer to number of introductions or different plant specimens examined per species.

The O_2 responses of *P. schenckii*, *P. decipiens*, and *P. milioides* were similar and were significantly different from those of *P. maximum* and *F. arundinacea*. Whereas these three *Panicums* showed reduced photorespiration and O_2 effects on photosynthesis, their AP rates at 21% O_2 were slightly less than that of *F. arundinacea* and the other three O_2-sensitive *Panicums*.

Differences in Γ among the species followed the same pattern as O_2 response. That is, Γ was low in *P. maximum* and *P. prionitis*, intermediate in *P. decipiens*, *P. milioides*, and *P. schenckii*, and high in *F. arundinacea*, *P. hylaeicum*, *P. laxum*, and *P. rivulare*. The intermediate Γ values for *P. milioides*, *P. schenckii*, and *P. decipiens* were significantly different from those observed in the other grasses.

Table II. *Leaf Anatomical Characteristics of Various Gramineae Species*

Tribe, species	Interveinal distance[1]	Max. lateral cell count[1]
	mm	
Oxygen-insensitive species		
Aeluropideae		
Vaseyochloa multinervosa (Vasey) Hitchc.	0.19 ± 0.01	3.0 ± 0.5
Chlorideae		
Chloris cucullata Bisch.	0.09 ± 0.01	2.0 ± 0
Chloris distichophylla Lag.	0.15 ± 0.01	2.7 ± 0.5
Chloris gayana Kunth.	0.10 ± 0.03	2.1 ± 0.3
Eragrosteae		
Eragrostis curvula (Schrad.) Nees	0.12 ± 0.01	2.2 ± 0.4
Paniceae		
Echinochloa frumentacea Roxb.	0.10 ± 0.01	2.6 ± 0.5
Echinochloa crusgalli (L.) Beauv.	0.07 ± 0	2.0 ± 0
Panicum antidotale Retz.	0.07 ± 0	2.0 ± 0
Panicum miliaceum L.	0.18 ± 0.01	2.8 ± 0.4
Panicum prionitis Griseb.	0.17 ± 0.02	3.2 ± 0.9
Panicum virgatum L. (2)[2]	0.15 ± 0.02	2.0 ± 0
Panicum sp.	0.17 ± 0.02	2.6 ± 0.5
Paspalum repens Berg.	0.08 ± 0.01	2.2 ± 0.4
Paspalum urvillei Steud.	0.10 ± 0.01	2.4 ± 0.5
Setaria italica (L.) Beauv.	0.11 ± 0.01	3.0 ± 0
Mean and SD of O_2-insensitive species	0.12 ± 0.04	2.5 ± 0.4
Oxygen-sensitive species		
Festuceae		
Dactylis aschersoniana	0.23 ± 0.06	6.1 ± 2.3
Dactylis glomerata L. (6)	0.19 ± 0.05	6.0 ± 1.4
Festuca arundinacea Schreb. (3)	0.38 ± 0.07	11.0 ± 1.8
Glyceria striata (Lam.) Hitch.	0.12 ± 0.01	4.2 ± 0.6
Paniceae		
Panicum hylaeicum Mez. (2)	0.29 ± 0.09	8.9 ± 2.0
Panicum rivulare Trin.	0.21 ± 0.02	7.9 ± 1.2
Panicum schenckii Hack.	0.20 ± 0.02	5.5 ± 1.0
Phlarideae		
Phalaris arundinacea L. (6)	0.25 ± 0.03	9.8 ± 1.5
Stipeae		
Stipa columbiana Macoun.	0.46 ± 0.04	11.8 ± 1.5
Stipa occidentalis Thurb.	0.17 ± 0.02	8.0 ± 1.2
Stipa speciosa Trin. and Rupr.	0.28 ± 0.02	12.4 ± 2.6
Stipa splendens Trin.	0.23 ± 0.02	13.0 ± 1.4
Stipa tenacissima L.	0.18 ± 0.01	8.6 ± 1.0
Stipa tenuis Willd. ex Steud.	0.18 ± 0.01	8.0 ± 1.3
Mean and SD of O_2-sensitive species	0.25 ± 0.10	9.1 ± 2.7

[1] Mean and standard deviation of 10 measurements.

[2] Numbers in parentheses refer to number of introductions or different plant specimens examined per species.

Leaf Anatomy. Interveinal distance and maximum lateral cell count (Table IV) were least in *P. Maximum* (0.11 ± 0.01 mm and 2.0 ± 0 cells, respectively) and greatest in *F. arundinacea* (0.46 ± 0.02 mm and 10.8 ± 1.2 cells, respectively). Intermediate values for these two parameters were observed in the remaining *Panicum* species.

The leaf anatomy of *P. maximum* (Fig. 1A) is characteristic of the C_4 or Kranz leaf anatomy. There is close proximity of adjacent bundle sheaths and numerous chloroplasts and other organelles

Table III. *Apparent Photosynthesis at 2 and 21% O_2, O_2 Response, and CO_2 Compensation Concentrations (Γ) of Nine Gramineae Species*

Species	Apparent Photosynthesis[1]		O_2 Response[2]	Γ[1]
	2% O_2	21% O_2		
	mg dm^{-2} h^{-1}		*%*	*μl l^{-1}*
Panicum maximum Jacq.	54.4 ± 8.5[3]	54.4 ± 9.5	0.2 ± 4.9	5 ± 4
Panicum prionitis Griseb.	42.7 ± 14.2	41.9 ± 13.8	2.0 ± 0.9	7 ± 6
Panicum decipiens Nees ex Trin.	29.8 ± 3.9	22.9 ± 3.0	30.7 ± 2.3	23 ± 8
Panicum milioides Nees ex Trin.	31.4 ± 1.6	24.6 ± 0.6	27.2 ± 3.5	17 ± 9
Panicum schenckii Hack.	33.8 ± 4.2	26.5 ± 3.0	27.3 ± 4.5	14 ± 6
Festuca arundinacea Schreb.	43.1 ± 8.7	30.1 ± 6.9	43.6 ± 3.5	69 ± 11
Panicum hylaeicum Mez	39.4 ± 4.0	28.5 ± 2.1	38.0 ± 4.5	59 ± 5
Panicum laxum Sw.	49.5 ± 2.8	35.5 ± 1.4	39.2 ± 3.9	57 ± 12
Panicum rivulare Trin.	49.0 ± 20.8	31.0 ± 13.8	53.5 ± 3.9	62 ± 1
L.S.D. .05			6.7	10

[1] Apparent photosynthesis and Γ measured at 25 and 27.5 C, respectively.

[2] Percentage increase of apparent photosynthesis at 2% compared to rate attained at 21% O_2.

[3] Mean and standard deviation of three measurements.

Table IV. *Leaf Anatomical Characteristics of Nine Gramineae Species*

Species	Interveinal Distance	Max. Lateral Cell Count[1]
	mm	
Panicum maximum Jacq.	0.11 ± 0.01	2.0 ± 0
Panicum prionitis Griseb.	0.15 ± 0.03	4.1 ± 1.7
Panicum decipiens Nees ex Trin.	0.28 ± 0.03	7.8 ± 1.2
Panicum milioides Nees ex Trin.	0.18 ± 0.01	6.4 ± 1.8
Panicum schenckii Hack.	0.20 ± 0.01	5.2 ± 1.6
Festuca arundinacea Schreb.	0.46 ± 0.02	10.8 ± 1.2
Panicum hylaeicum Mez	0.25 ± 0.02	7.9 ± 0.9
Panicum laxum Sw.	0.20 ± 0.01	6.4 ± 0.7
Panicum rivulare Trin.	0.28 ± 0.03	7.5 ± 1.1

[1] Mean and standard deviation of 10 measurements of several cross-sections.

within the bundle sheath cells. Outside the well developed vascular bundle sheath is a radial arrangement of thin walled mesophyll cells.

An unusual variation of Kranz anatomy is seen in the leaf cross-section of *P. prionitis* (Fig. 1B). The vascular tissue of large veins is surrounded by two bundle sheaths. The outer bundle sheath is composed of empty parenchyma cells whereas the inner sheath is composed of chloroplast-containing cells. This inner "Kranz" sheath has very thick walled cells and occupies a position which is occupied by the mestome sheath in most other grass species. Data for interveinal distance and maximum lateral cell count of *P. prionitis* in experiments 1 and 2 were obtained from measurements between such "fully developed" bundle sheaths. These distances were slightly greater than those observed in *P. maximum* and all of the O_2-insensitive grasses in experiment 1. Numerous smaller veins without the complete double sheath were scattered throughout the leaf of *P. prionitis*, and inclusion of these smaller vascular bundles in the measurements of interveinal distances would have resulted in values similar to those for other C_4 grasses.

Cross-sections of two *Panicum* species with apparent C_3 metabolism are presented in Figure 1, C and D. The empty bundle sheath cells (Fig. 1D) and relatively distant spacing of the bundle sheaths in *P. rivulare* (Table IV) are characteristic of C_3 leaf anatomy. A peculiar trait of this *Panicum* species is the possession of large air spaces adjacent to many of the bundle sheaths. Leaf anatomy in *P. laxum* leaves (Fig. 1C) is similar to that observed in other C_3 grasses investigated in this study.

Leaf cross-sections of *P. schenckii* (Fig. 1E), *P. decipiens* and *P. milioides* (Fig. 1F) display both Kranz and non-Kranz characteristics. Although the distance and the number of mesophyll cells between veins were intermediate between the values obtained for *P. maximum* and *F. arundinacea* (Table IV), they were not different from those values obtained for *Dactylis* or *Stipa* (Table II) nor from those *Panicum* species with greater O_2 response and higher Γ (Table III). However, the clustering of chloroplasts and other organelles on the centripetal walls of the bundle sheath cells is similar to that found in NAD-malic enzyme type species with C_4 photosynthetic metabolism (12, 13). The proportion of the bundle sheath cell volume which is occupied by chloroplasts and other organelles appears to be considerably less in *P. schenckii*, *P. decipiens*, and *P. milioides* (Fig. 1, E and F) as compared to *P. maximum* (Fig. 1A) and other O_2-insensitive grasses investigated in this study.

DISCUSSION

The classification of grasses in this study as O_2-sensitive or O_2-insensitive, except for three species in the *Laxa* group of *Panicum*, is very similar to the classification of C_3 and C_4 species by others (6, 9, 19). Those characterized as O_2-insensitive had increases in AP of 4% or less at 2% O_2 relative to 21% O_2 while O_2-sensitive species exhibited increases of about 40% or more. The Γ values were greater than 44 μl l^{-1} for the O_2-sensitive species and generally less than 4 μl l^{-1} for the O_2-insensitive ones.

The large range in O_2 response for O_2-sensitive species excluding the intermediate *Panicums*, from 37.3 to 62.8%, is similar to that reported by Downes and Hesketh (9). Since the percentage increase in AP at 2% O_2 is greater at low CO_2 concentrations, variation in stomatal resistance among species may influence intercellular CO_2 concentration and thereby O_2 response. Diffusive resistance was not estimated in these experiments, so the possibility of stomatal resistance differences accounting for the variability in O_2 effects cannot be evaluated. Other experiments (5) have shown that *P. milioides* and *P. schenckii* have stomatal resistances similar to the C_3 species *F. arundinacea* under conditions similar to those in this study. The variation in O_2 response of AP in most of the O_2-sensitive species is probably not a reflection of variation in photorespiration, since Γ was not correlated with O_2 response. The reduced O_2 response in *P. milioides*, *P. schenckii*, and *P. decipiens* was associated with lower Γ than observed in the other O_2-sensitive species and therefore probably represents a reduced photorespiration rate.

Anatomical characteristics usually associated with C_4 grasses (1, 6) were observed for those species which did not respond to O_2. In general, the O_2-sensitive grasses possessed leaf anatomical characteristics of C_3 species, but there was considerable variability in interveinal distance, which overlapped the distances for O_2-insensitive species. For example, leaves of *G. striata*, an O_2-sensitive species, had an interveinal distance of 0.12 mm, which was the same as the mean for all of the O_2-insensitive species. Its leaves also had only four mesophyll cells between veins compared to three for the O_2-insensitive species, *Vaseyochloa multinervosa* and *S. italica*. *Panicum milioides*, *P. schenckii* and *P. decipiens* which had reduced O_2-responses compared to the other O_2-sensitive plants, had interveinal distances and maximum lateral cell counts similar to the two species of *Dactylis* and greater than *G. striata*. *Dactylis* and *G. striata* exhibited AP responses to O_2 and Γ values typical of C_3 plants. Therefore, close spacing of veins may not be indicative of intermediacy between C_3 and C_4 photosynthetic types as suggested by Kanai and Kashiwagi (16).

Among species of the *Laxa* group of *Panicum*, *P. prionitis* did not respond to O_2, *P. laxum*, *P. hylaeicum*, and *P. rivulare* responded similar to most C_3 species and in *P. milioides*, *P. schenckii*, and *P. decipiens* AP was stimulated only about 60% as much by low O_2 as was the case for other O_2-sensitive species. Values of Γ

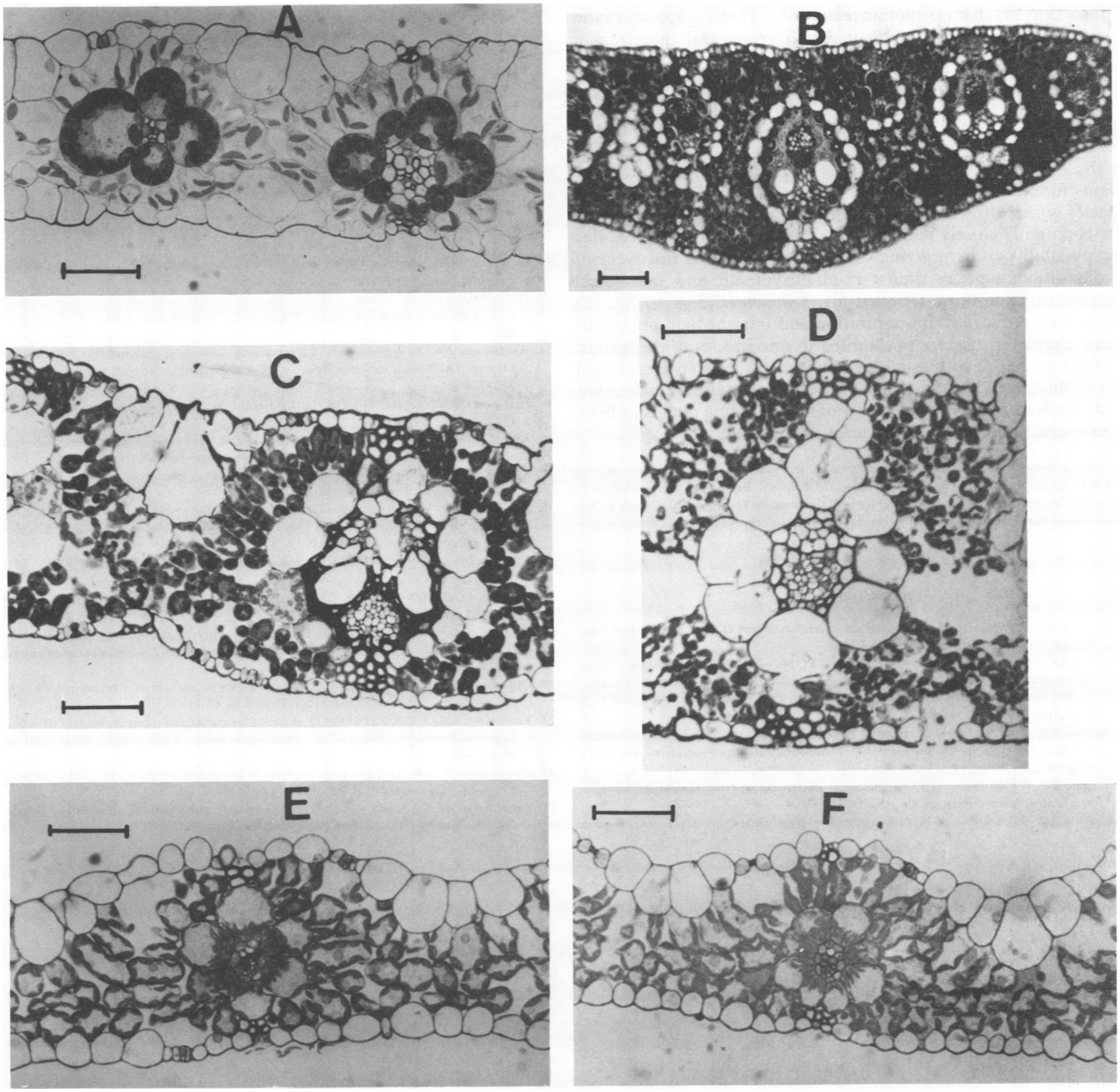

FIG. 1. Leaf cross-sections of *P. maximum* (A), *P. prionitis* (B), *P. laxum* (C), *P. rivulare* (D), *P. schenckii* (E), and *P. milioides* (F). All sections were plastic-embedded, except *P. prionitis* which was paraffin-embedded. Horizontal scale for each species represents 50 μm.

varied among the *Laxa* species in the same way as O_2 response. Differences among these species also occur in leaf anatomy (Fig. 1) and activities of enzymes involved in CO_2 fixation (Reger, B., unpublished data), indicating C_3, C_4, and intermediate photosynthetic types within the *Laxa* group. The indication in earlier work (2, 3, 10) that *P. laxum* had intermediate Γ values resulted from an error in identification. The plant catalogued by the USDA Plant Introduction Station as *P. laxum* (P. I. No. 310026) was later identified as *P. milioides.*

Differences in physiology and leaf anatomy may indicate that the *Laxa* group, established mainly on the basis of panicle and floret morphology, is not composed of closely related species. In fact, Brown (6) has recently proposed the raising to generic rank (*Steinchisma*) of *P. milioides*, *P. decipiens*, and other species with similar leaf anatomy and floral characters. *P. laxum* was retained in the Laxa group by Brown (6) along with seven other species. *P. hylaeicum* appears closely related to *P. laxum* on the basis of spikelet structure (24) and leaf anatomy so presumably it also belongs in the *Laxa* group. Brown (6) assigned *P. prionitis* and *P. rivulare* to the *Grandia* group of *Panicum* which contains at least four other species, one with Kranz and three with non-Kranz anatomy. Brown proposed that the two Kranz species in this group were recently evolved C_4 plants, with the Kranz cells arising from the mestome sheath, but with empty parenchyma sheath cells persisting. Nearly all other C_4 species in which the Kranz cells evolved from the mestome sheath lack a parenchyma sheath. Although the species we have examined which were originally assigned to the *Laxa* group differ physiologically, they all have a basic chromosome number of 10 (6; Bouton, J. H., unpublished) whereas most species of *Panicum* have nine as the basic number.

Thus it appears that taxonomic relationships among species of the *Laxa* group including the *Steinchisma* genus and the *Grandia* group are unclear and require further study.

The occurrence of C_3 and C_4 species in closely related groups is rare in the Gramineae. Except in the *Laxa* group of *Panicum*, no species examined in this study showed gas exchange characteristics intermediate between C_3 and C_4 species. The variability observed in the *Laxa* group, however, makes it a very promising group of plants for study of relationships between C_3 and C_4 grasses. The species which Brown (6) recently assigned to the Grandia group are apparently closely related C_3 and C_4 species, which may offer the possibility of hybridizing C_3 and C_4 plants. The discovery of two additional species similar in photosynthetic and anatomical characteristics to those reported for *P. milioides* (3, 4, 10, 17, 18) shows that reduced photorespiration and intermediate photosynthetic characteristics are not limited to one species in this genus. Further studies of species in this group of *Panicum* species should aid in understanding relationships between C_3 and C_4 photosynthetic pathways and the possibility of manipulating photosynthetic characteristics to improve efficiency of C_3 plants.

Acknowledgments—We would like to acknowledge the following people for their help in collecting species in the *Laxa* group of *Panicum*: Dr. Bernardo Rosengurtt, Universidad de la Republica, Montevideo, Uruguay; Dr. Ismar Barreto, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil; Mr. Camilo Quarin, Universidad Nacional del Nordeste, Corrientes, Argentina.

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