PHOTOSYNTHETIC PROPERTIES OF SOME GRASSES IN EASTERN SOUTH DAKOTA

Larry L. Tieszen
Department of Biology
Augustana College, Sioux Falls, S. Dak. 57102

INTRODUCTION

It has recently become quite apparent that the Gramineae is composed of two groups of grasses, one of which is essentially tropical in origin and the other which is primarily temperate in origin and distribution (Downton and Tregunna, 1968). The tropical grasses, representatives of which belong to the chloridoid, eragrastoid, and panicoid lines, are common in tropical regions whereas the remaining groups are represented in the cooler temperate zone. Thus, the frequency with which temperate zone grasses occur increases with an increase in latitude; and, presumably, at the extreme latitudes of arctic tundra regions tropical representatives are excluded. The temperate zones, however, contain a mixture of these two elements as is evident when one compares the number of genera in the tribes which are represented in South Dakota (compiled from van Bruggen). Of the 65 genera, 46 belong to temperate tribes (Agrostideae, 13; Aveneae, 6; Festuceae, 17; Hordeae, 8; and Phalarideae, 2) and 17 are in tribes with strong tropical affinities (Andropogoneae, 3; Chlorideae, 9; and Paniceae, 5). The remaining two genera belong in tribes (Oryzeae and Zizaneae) with poorly defined taxonomic and evolutionary relationships, although Downton and Tregunna (1968) describe the evidence which suggests a closer relationship of the Oryzeae with the temperate groups than with those from the tropics.

Among the cytological, physiological, and biochemical differences between these two groups are some distinct features (Table 1) associated with the photosynthetic process (see Downton and Tregunna, 1968). Tropical grasses produce C_4 dicarboxylic acids as the early products of photosynthesis, do not evolve CO_2 by photorespiration (have CO_2 compensation concentrations below 5 ppm), possess high PEP (phosphoenolpyruvate) carboxylase activity, and possess extensively developed parenchyma bundle sheath cells which contain starch laden chloroplasts. This contrasts with the temperate grasses which synthesize typical Calvin cycle intermediates, possess an active photorespiratory system (CO_2 compensation is around 50 ppm), exhibit low PEP activity and an absence of sheath cells containing specialized plastids. Associated with these features appear to be generally high photosynthetic rates in tropical grasses and higher temperature optima for photosynthesis which appear to parallel the differences in the temperature optima of the two carboxylating enzymes (Treharne and Cooper, 1969).

Table 1. Comparison of Two Photosynthetic Systems

	Calvin System (Temperate)	Hatch and Slack System (Tropical)
Early Products	Phosphoglyceric Acid	C_4 Acids
Photorespiration	Present	Absent
CO_2 Compensation	30-50 ppm	<5 ppm
Carboxylation	RUDr	PEP
Sheath Cells	Poorly developed	Extensively developed
Chloroplasts		Starch laden
Translocation	Slow	Rapid
O_2 Inhibition	Present	Absent
Maximum Photosynthetic Rate	20 mg $CO_2/dm^2/hr$	>20 mg $CO_2/dm^2/hr$
Light Saturation	Low	High
Temperature Optima	Low	High

This project was undertaken to determine some of the photosynthetic properties of local and naturally occurring grasses and to compare pigment contents, photosynthetic rates, and carboxylation activities.

MATERIALS AND METHODS

All species were sampled in the native prairie and forest area in Cactus Hills about two miles northeast of Sioux Falls, South Dakota. The mixed grass prairie consists of *Andropogon scoparius* dominated vegetation on the south-facing slopes, and is separated from the oak forest in the valleys by *Andropogon gerardi, Panicum virgatum,* and other tall grass representatives. Collections were made early in the mornings (generally before 7:30 A.M.) between June 17 and June 29, 1968.

Samples for analyses were cut near ground level, recut beneath water, and transported to the laboratory. They were maintained at about 10° C until the analyses were made (within 5 hours). Humidity was maintained at 70% and light intensity was kept at 100 ftc. Young, fully-expanded leaves were removed, weighed, measured, and used for pigment and dry weight determinations. Chlorophylls were estimated in 80% acetone by the method of Arnon (1949). Voucher specimens of the species studies (Table 2) have been deposited in the herbarium of Augustana College. Ten replicates were usually run for each determination.

Photosynthesis and respiration were measured using a standard open system with an infrared gas analyser. The removal or addition of CO_2 from an airstream of known composition (313± 0.2 ppm CO_2) was monitored by a Beckman differential analyzer. The flow rate was varied during photosynthesis to maintain the CO_2 concentration leaving the chamber above 290 ppm. The air was constantly mixed in the small chambers by means of an electric fan. Relative humidity was maintained at 55% by the saturated salt method of Winston and Bates (1960) and H_2O was removed from the air stream by passage through a drying tube of Drierite. Air temperature in the chamber was monitored by a thermistor which controlled a water bath used to cool the air and water for the water jacket. Temperatures were maintained at 20 ± 0.5° C. During illumination leaf temperatures increased as much as 3-4° C. The light source from a G-E Cool Beam incandescent bulb was filtered through two inches of water to remove infrared radiation. Intensity was monitored with a Weston Photocell or a thermopile and was reduced by inserting neutral filters or by a slight reduction in line voltage.

Young, fully-expanded leaves attached to the plant were enclosed in the photosynthesis chamber which consisted of a clear plexiglass top and a water-jacketed copper bottom. Air tight seals were attained with the use of modeling clay. Initial and terminal dark respiration readings were taken, and an increasing light intensity series was used to determine light intensity curves. Usually the leaves attained a steady photosynthetic rate in 15 minutes although this was extremely variable; and each species appeared to possess its own "induction" period.

Enzyme assays were carried out similar to the methods of Rabin and Trown (1964) for ribulose-1, 5-disphospate carboxylase and Slack and Hatch (1967) for phosphoenolpyruvate carboxylase. Known amounts (usually 300 mg) and areas of leaves were ground in a morter and pestle with an extraction medium of 80 mM Tris-Cl buffer (pH 7.8), 10 mM $MgCl_2$, 0.25 mM EDTA, and 5 mM reduced gluthathione. The supernatant of a 10,000 x g centrifugation was made to 4.0 ml and assayed immediately. Reactions were run in sealed vials at 25° C for 3 minutes after which a small amount of acetic acid was injected to stop the reaction and remove any CO_2 which had not been fixed. Aliquots of the reactions were placed in planchets, dried, and counted in a thin-window Nuclear Chicago Counter. The reaction mixture for RUDP carboxylase contained 8 umoles Tris-Cl (7.8), 1 umole $MgCl_2$, 0.025 umoles EDTA, 0.5 umoles gluthathione, and 0.276 umoles RUDP. The reaction was initiated by the injection of a known volume of enzyme extract. The reaction mixture for the PEP carboxylase assay was as above except PEP replaced RUDP. Protein was assayed by the phenol method.

RESULTS AND DISCUSSION

In this study area there are apparent differences in the ecological requirements of the species belonging to the temperate and tropical groups (Table 2). Grasses belonging to the temperate tribes tend to be found on north-facing slopes, near the hill bottoms or in the understory of the oak forest. The only species in this group which is consistently found on the fully exposed south-facing slope is *Koeleria cristata.* In contrast, all of the grasses in the tropical tribes are found on either the south-facing hillside or the hill bottom. At no time were plants belonging to these tribes found in the understory.

The grasses possessed a wide range of values for leaf density and thickness (Table 3). The two species, *Dactylis glomerata* and *Leersia virginica,* with the highest fresh to dry ratios occupied very dark habitats within the understory of the forest. These species and *Hystrix patula,* another understory species, also possessed the lowest dry weights per leaf area. Although the overall mean leaf thickness value (0.30 mm) is the same as that previously found for arctic and alpine tundra grasses (Tieszen, 1970), the tundra grasses possessed more fresh weight per unit leaf area and less dry weight than these local grasses. A similar difference is seen in the comparison of chlorophyll concentrations where these grasses possessed much less chlorophyll (Table 4) than those from the tundra,

especially when based on dry weight. Among the local grasses, chlorophyll was generally more concentrated in the plants occupying closed or semi-closed habitats although this relationship is not quite as apparent when expressed on a leaf area basis since concentration is also related to leaf thickness.

Mean rates of photosynthesis (Table 5) and respiration (Table 6) are also very variable from species to species. All species except *Sporobolus cryptandrus* showed very active photosynthesis. *Leersia virginica* is exceptional because of its very high rates of respiration and photosynthesis on a dry weight basis. There is an apparent relationship such that the grasses with high fresh to dry ratios generally possess active photosynthetic and respiratory processes when based on leaf dry weight. When based on leaf area the relationship breaks down because most species with high fresh to dry ratios possessed low density thickness values. All species belonging to the temperate tribes had maximal photosynthetic rates below 20 mg CO_2/dm^2 .hr whereas four of the grasses from the tropical tribes had maximal rates above 20 mg CO_2/dm^2.hr. These light saturated rates of photosynthesis were highly correlated with leaf thickness ($r = +.78$) but were not correlated with total *in vitro* carboxylation activity (Table 7). The contribution to the total carboxylation activity by PEP carboxylase is shown by the ratio of RUDP/PEP and indicates that most of the species belonging to the tropical tribes possessed considerable PEP carboxylation activity whereas most grasses from the temperate tribes did not. It should be noted that species with high total carboxylation activity typically possessed high RUDP/PEP ratios.

The grasses were differentiated into two groups on the basis of their light intensity curves. Representatives of the tropical tribes possessed light intensity curves similar in shape to those shown in figure 1 for *Andropogon gerardi, Panicum virgatum* and *Spartina pectinata.* These grasses exhibit the high maximal rates of CO_2 uptake as well as a high light intensity requirement (5000 ftc or more) for saturation. This is in marked contrast to the representative curves (Fig. 2) for the grasses from the temperate tribes. In these species the light saturated rates are lower, and the intensity required for saturation is in some cases as low as 1000 ftc. The efficiency of uptake in the light limited portion on the curve was also generally greater in the temperate representatives than in those from the tropical tribes. Grasses belonging to the temperate tribes possessed the same general curves regardless whether they were found in dense shade or in open habitats as is exemplified by *Bromus inermis, Elymus canadensis,* and *Koeleria cristata.* The factors responsible for determining maximal photosynthetic rates are not entirely understood, but recent studies by Bjorkman (1968) have indicated that in some species there is a high correlation between light saturated rates and carboxylation activity. Thus, this suggests that CO_2 uptake can be limited by carboxylation activity. In this study the only clear relationship with respect to carboxylation activity was that the species with the highest rates on a leaf area basis were also species which possessed significant

PEP carboxylase activity and belonged to the tropical tribes. Resistance to the diffusion of CO_2 from the air to the chloroplast frequently limits CO_2 uptake (Gaastra, 1959), and in these species may be a more important controlling factor than total carboxylation activity.

The relationship between the habitats of the species in this study area and their taxonomic positions suggests an ecological importance for the two photosynthetic systems. Apparently the tropical representatives perform better in open and warmer (also drier) habitats and the temperate representatives are better adapted to the cooler and more shaded areas. The early spring grasses are typically representatives of the temperate tribes which in the prairie become replaced at midsummer by species of the tropical tribes. In fall, temperate grasses again initiate vigorous growth. Thus, this suggests that in early spring photosynthesis would occur primarily by the Calvin system and would be partially replaced as the season progresses by the Hatch and Slack pathway only to become more active again in the fall. This represents an exceedingly intriguing example of ecosystem adaption at the biochemical and physiological levels and provides an excellent system for the study of community diversity, efficiency in production, and integration. An integrated year-long study at the community, population, individual, and biochemical levels should be undertaken.

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Table 2. Tribes of Graminae and Species Used in This Study
(General habitat is indicated in parentheses)

Agrostideae
- Calamovilfa longifolia (hill bottom)
- Stipa comata (N-facing hillside)
- Stipa viridula (N-facing hillside)
- Sporobolus cryptandrus (edge of forest)

Festuceae
- Dactylis glomerata (forest understory)
- Bromus inermis (hill bottom)

Aveneae
- Koeleria cristata (S-facing hillside)

Hordeae
- Elymus canadensis (hill bottom)
- Hystrix patula (forest understory)

Oryzeae
- Leersia virginica (forest understory)

Chlorideae
- Bouteloua curtipedula (S-facing hillside)
- Bouteloua hirsuta (S-facing hillside)
- Spartina pectinata (wet hill bottom)

Andropogoneae
- Andropogon gerardi (hill bottom)
- Andropogon scoparius (S-facing hillside)

Paniceae
- Panicum virgatum (hill bottom)

Table 3. Leaf Weight and Size Characteristics of Grasses Sampled

	Leaf Characteristics				
Species	fr:dry	g fr wt/dm	g dry wt/dm	Thickness (mm)	Width (mm)
Calamovilfa longifolia	2.53	1.75	0.69	0.30	5.8
Stipa comata	2.42	2.50	1.03	0.40	2.9
Stipa viridula	1.99	2.33	1.17	0.34	4.7
Sporobolus cryptandrus	2.81	1.84	0.65	0.24	3.9
Dactylis glomerata	4.43	1.74	0.39	0.42	6.6
Bromus inermis	2.63	1.68	0.64	0.34	8.4
Koeleria cristata	—	2.77	—	0.32	1.7
Elymus canadensis	2.70	1.31	0.48	0.22	9.0
Hystrix patula	2.61	0.87	0.34	0.16	7.5
Leersia virginica	3.22	0.51	0.16	0.10	11.6
Unidentified understory sp.	2.79	2.06	0.74	0.32	2.4
Bouteloua curtipendula	2.42	1.73	0.71	0.22	4.1
Bouteloua hirsuta	2.45	2.12	0.86	0.24	1.4
Spartina pectinata	2.37	2.18	0.92	0.45	8.7
Andropogon gerardi	3.11	1.38	0.44	0.36	6.9
Andropogon scoparius	2.78	1.56	0.56	0.28	3.0
Panicum virgatum	3.05	1.80	0.59	0.34	10.0
Mean value	2.77	1.77	0.65	0.30	5.8
Mean for Arctic (Tieszen, 1970)	3.73	1.90	0.51	0.30	3.43
Mean for Alpine (Tieszen, 1970)	3.48	1.82	0.53	0.30	3.23

Table 4. Total Chlorophyll Content of Young Fully-Expanded Leaves (X ± S.E.)

	Chlorophyll		
Species	mg/g fr wt.	mg/g dry wt.	mg/dm^2
Calamovilfa longifolia	1.32 ± .16	3.34	2.34 ± .33
Stipa comata	1.15 ± .04	2.77	2.86 ± .16
Stipa viridula	0.74 ± .07	1.48	1.73 ± .16
Sporobolus cryptandrus	1.97 ± .10	5.55	3.61 ± .21
Dactylis glomerata	1.76 ± .09	7.81	3.06 ± .22
Bromus inermis	3.16 ± .08	8.31	5.27 ± .27
Koeleria cristata	1.33 ± .15	--	3.69 ± .49
Elymus canadensis	1.76 ± .16	4.76	2.27 ± .22
Hystrix patula	2.42 ± .31	6.32	2.12 ± .29
Leersia virginica	3.29 ± .24	10.60	1.68 ± .13
Unidentified understory sp.	0.57 ± .02	1.59	1.17 ± .04
Bouteloua curtipendula	0.98 ± .06	2.37	1.69 ± .37
Bouteloua hirsuta	0.96 ± .04	2.35	2.05 ± .12
Spartina pectinata	1.79 ± .19	4.27	3.92 ± .41
Andropogon gerardi	1.58 ± .13	4.92	2.15 ± .12
Andropogon scoparius	1.05 ± .26	2.93	1.66 ± .44
Panicum virgatum	1.99 ± .13	6.09	3.55 ± .20
Mean value	1.64	4.72	2.64
Mean for Arctic (Tieszen, 1970)	2.06	7.69	3.74
Mean for Alpine (Tieszen, 1970)	2.04	6.97	3.68

Table 5. Mean Net Rates of Photosynthesis at 5000 Footcandles and 20° C

	Photosynthetic Rate		
Species	mg CO_2/g fr wt.hr	mg CO_2/g dry wt.hr	mg CO_2/dm^2.hr
Calamovilfa longifolia	9.41	23.8	16.7
Stipa Comata	7.14	17.3	17.9
Stipa viridula	6.24	12.4	14.8
Sporobolus cryptandrus	-0.65	-1.84	-1.19
Dactylis glomerata	8.45	37.5	14.5
Bromus inermis	7.29	18.9	12.4
Koeleria cristata	3.02	--	8.38
Elymus canadensis	10.4	28.2	13.5
Hystrix patula	10.2	26.7	8.96
Leersia virginica	14.8	47.7	7.61
Unidentified understory sp.	9.00	25.1	18.5
Bouteloua curtipendula	14.9	36.1	26.9
Bouteloua hirsuta	4.04	9.91	8.45
Spartina pectinata	12.6	29.8	28.4
Andropogon gerardi	15.0	46.7	20.5
Andropogon scoparius	8.90	24.7	13.6
Panicum virgatum	13.3	40.5	23.7

Table 6. Mean Rates of Respiration

	Respiration Rate		
Species	mgCO_2/g fr wt.hr	mgCO_2/g dry wt.hr	mgCO_2/dm^2.hr
Calamovilfa longifolia	-0.55	-1.39	-0.96
Stipa comata	-0.69	-1.68	-1.76
Stipa viridula	-0.58	-1.16	-1.35
Sporobolus cryptandrus	-0.29	-0.81	-0.53
Dactylis glomerata	-0.47	-2.06	-0.81
Bromus inermis	-0.62	-1.65	-1.04
Koeleria cristata	-0.33	--	-0.88
Elymus canadensis	-0.78	-2.10	-0.100
Hystrix patula	-0.23	-0.59	-0.20
Leersia virginica	-1.88	-6.06	-0.95
Unidentified understory sp.	-0.30	-0.85	-0.61
Bouteloua curtipendula	-0.90	-2.18	-1.55
Bouteloua hirsuta	-1.09	-2.66	-2.32
Spartina pectinata	-0.23	-0.55	-0.47
Andropogon gerardi	-0.86	-2.69	-1.18
Andropogon scoparius	-0.70	-1.94	-1.09
Panicum virgatum	-0.64	-1.95	-1.16

Table 7. *In vitro* activity of Ribulose-1, 5-diphosphate and Phosphoenolpyruvate Carboxylase

	mg CO_2/dm^{2}hr	
	Total	RUDP/PEP
Calamovilfa longifolia	8.9	1.4
Stipa comata	22.4	43.0
Stipa viridula	15.0	45.7
Sporobolus cryptandrus	8.9	0.5
Dactylis glomerate	2.9	3.2
Bromus inermis	17.2	46.6
Koeleria cristata	13.7	18.6
Elymus canadensis	22.7	29.2
Hystrix patula	4.9	8.0
Leersia virginica	2.9	24.7
Unidentified understory sp.	7.4	13.7
Bouteloua curtipendula	3.6	6.1
Bouteloua hirsuta	3.7	3.4
Spartina pectinata	9.5	1.1
Andropogon gerardi	2.6	3.2
Andropogon scoparius	0.8	2.1
Panicum virgatum	7.2	0.7

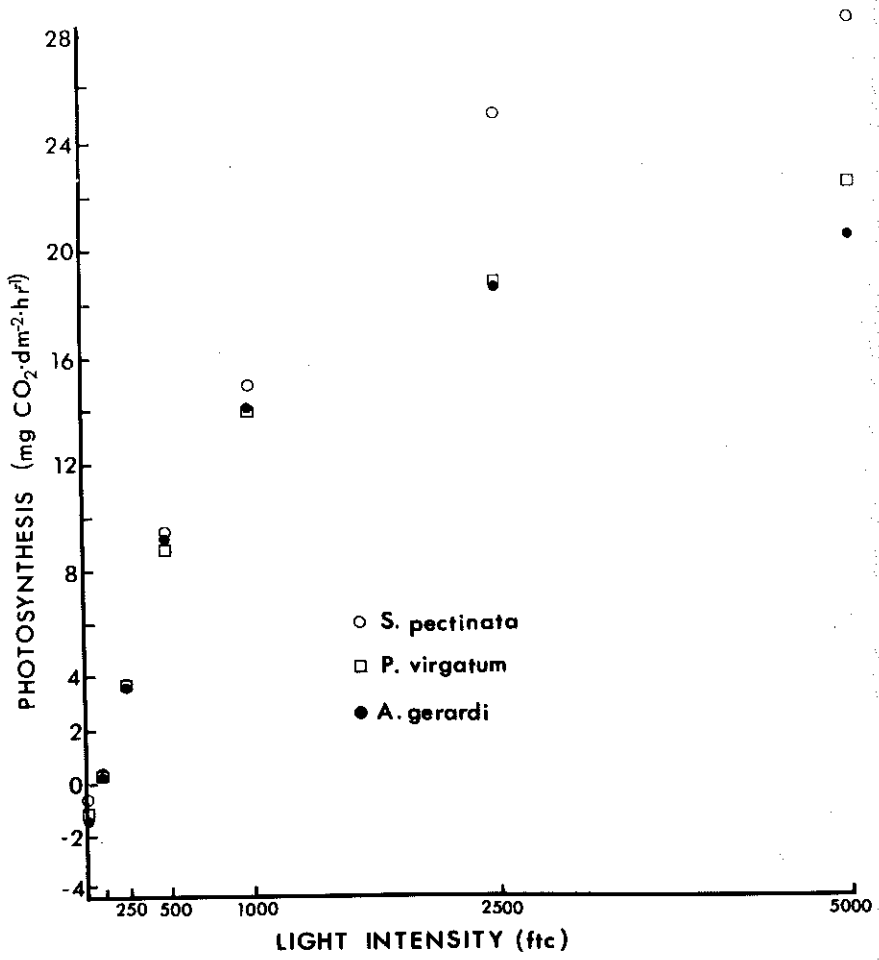

Figure 1. Photosynthetic light intensity curves representative of the tropical tribes. Each point represents the mean of 6 to 10 plants.

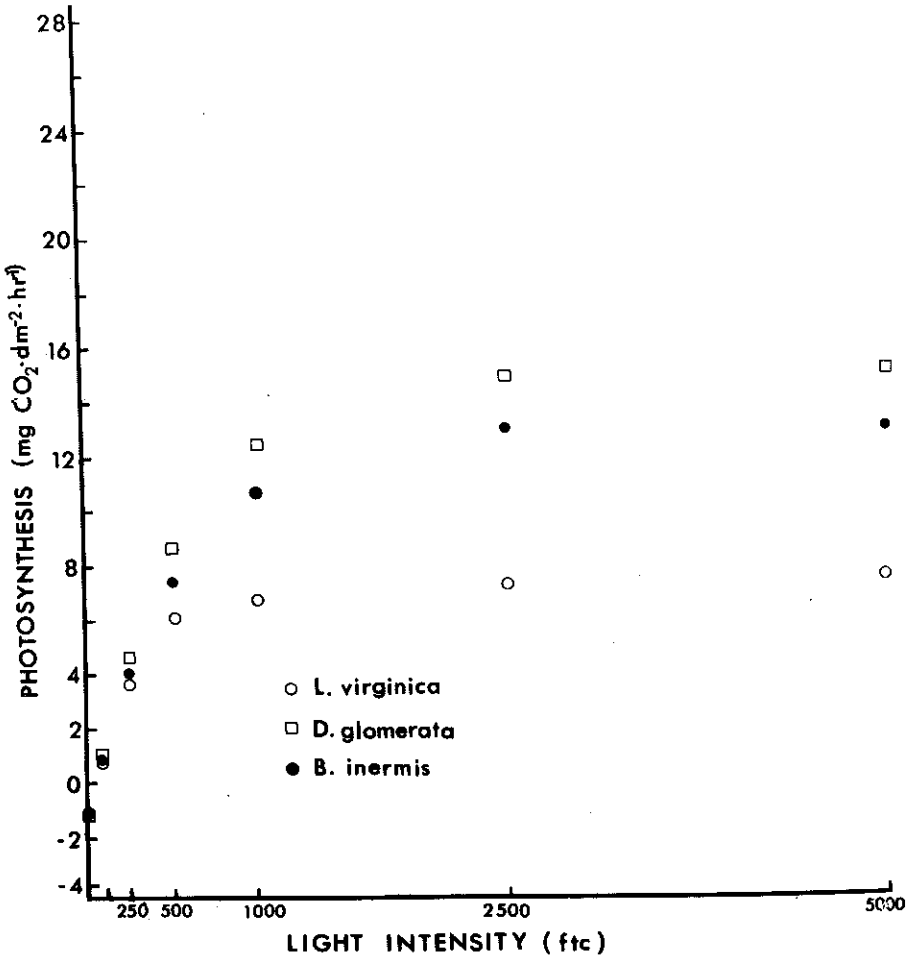

Figure 2. Photosynthetic light intensity curves representative of the temperate tribes. Each point represents the mean of 10 plants.