Diurnal Variations of Nonstructural Carbohydrates in the Individual Parts of Switchgrass Shoots at Anthesis

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Highlight: *Switchgrass (*Panicum virgatum *L.) was harvested at early anthesis in the field at Madison, Wisconsin, during 1972. Shoots were separated into the inflorescence, individual green leaf blades, green leaf sheaths, and internodes at 6 am, 12 noon, 6 pm, and 12 midnight during 3 days. All tissues were analyzed for percentages (dry wt) of reducing and nonreducing sugars, total sugars, starch, and total nonstructural carbohydrates (TNC). Diurnal trends were clearest in the inflorescence, leaf blades, and the upper sheaths and internodes, but they were not always statistically significant. The trend was an increase of nonreducing sugar, total sugar, and starch percentages from 6 am to 6 pm and then a decrease to 12 midnight. Diurnal change in reducing sugar percentage was small in all plant parts. Basal sheaths and internodes tended to increase in percentage of starch and TNC from 6 am to 12 midnight. These are storage parts, and presumably carbohydrates were being translocated continuously from upper parts to these lower sinks for storage, especially after 6 pm. These data indicate that pasturing in the evening might provide advantages insofar as energy concentration in herbage is concerned. The highest content of energy occurred in the inflorescence of all the individual shoot parts. Diurnal trends of elemental concentrations in the shoot parts also were determined and were found to be largely nonsignificant.*

Diurnal changes in nonstructural carbohydrates in the herbage of grasses have been studied most often for the introduced, cool-season (temperate-origin) species, where fructosan is the principal nonstructural polysaccharide accumulated (Smith, 1973). Few studies have been conducted on the diurnal changes in warm-season (tropical-origin) grasses, where starch is the principal nonstructural polysaccharide, and these were made on annuals. Studies of corn (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L.) Moench) leaf blades by Miller (1924) in Kansas showed that total sugar percentages began to increase between 4 and 6 am, reached a maximum sometime between noon and 5 pm, and then decreased until daylight the next morning. Variation was due mostly to sucrose, since reducing sugar percentages remained fairly constant. Starch reached maximum percentages later in the day than did total sugars, underwent little change to midnight, and then decreased rapidly until daylight. In contrast, Eisele (1938) in Iowa found that both reducing sugars and sucrose (mg/g, dry wt) increased between 4 am and 4 pm in corn leaf blades, but changes in starch were not significant. Recently, Lechtenberg et al. (1973) found virtually no diurnal change in free glucose or fructose concentrations in sudangrass (*Sorghum sudanense* Stapf.) herbage, but sucrose increased from 1.8% (dry wt), and starch from 6.6%, at 6 am to 5.8 and 8.8%, respectively, at 6 pm. Half the daily increase in carbohydrate disappeared between 6 pm and midnight. Trends in leaves and total herbage were similar. No data was found for warm-season perennial grasses, such as switchgrass (*Panicum virgatum* L.) and other native species.

The current work was initiated to ascertain the diurnal fluctuations of nonstructural carbohydrate fractions in each individual part of switchgrass shoots. Shoots were sampled during 1972 when they were at early anthesis.

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Materials and Methods

The switchgrass used was a clonal line that had been transplanted in rows 45 cm apart with 30 cm between plants in the row. The clone was an ecotype native to a river-bank area near Madison, Wisc. The Miami silt loam at date of tissue collection in 1972 had a pH of 6.6, and contained 112 kg/ha of available P and 304 kg/ha of exchangeable K. Nitrogen, as ammonium nitrate, was applied

Table 1. Temperature (°C) and net radiation at times of tissue sampling during 1972.*

Date	Time of day	Air temperature	Wm^{-2}**	fc**	Climatic description
July 29	6 am	17.0	40	800	20% cloudy all day
	12 noon	25.0	360	10,000	
	6 pm	23.0	40	800	
	12 midnight	20.0	0	0	
July 30	6 am	18.5	36	700	20 to 40% cloudy during afternoon
	12 noon	27.0	425	11,800	
	6 pm	27.5	84	1,600	
	12 midnight	19.0	0	0	
Aug. 9	6 am	9.5	30	540	Cloudy (40%) during late afternoon
	12 noon	19.5	416	11,600	
	6 pm	21.0	45	950	
	12 midnight	14.0	0	0	

*Readings made at 80 cm above soil surface.
**Wm^{-2} ** = Watts/square meter; fc = footcandles.

broadcast in early spring at 138 kg/ha.

Shoots of switchgrass were collected when they began to flower (early anthesis) during three sunny days on July 29, 30, and August 9, 1972. Shoots were collected at 6 am, 12 noon (N), 6 pm, and 12 midnight (MN) of each day. Light intensity and air temperature measurements at the field site were recorded during each sampling time at 80 cm above soil level (Table 1). The days selected for sampling were warm and sunny. Adequate soil moisture prevailed during the period of sampling.

Table 2. Dry weights (mg) of shoot parts and the inflorescence and stem lengths (cm) of switchgrass.

Shoot part[b]		Weight[a]	Length[a]
Inflorescence		640.6	26.3
Internode	1	138.7	20.5
	2	177.4	15.4
	3	168.9	10.1
	4	169.0	7.1
	5	127.5	4.2
	All	781.5	57.3
	LSD[c]	22.1	4.1
Leaf blade	1	95.2	–
	2	140.8	–
	3	133.0	–
	4	110.9	–
	5	81.5	–
	All	561.6	–
	LSD	21.4	–
Leaf sheath	1	91.2	–
	2	108.8	–
	3	97.0	–
	4	71.8	–
	5	51.3	–
	All	420.1	–
	LSD	15.0	–
Total shoot		2403.8	83.6

[a]Statistical analyses based on days as replicates with each day the mean of 4 sampling times.
[b]Numbered from top to bottom of the shoot.
[c]LSD 0.05 among individual internodes, leaf blades, and leaf sheaths.

At each sampling, approximately 35 shoots of switchgrass of uniform height and development were selected. Shoots were severed at soil level and immediately taken to the laboratory.

Each shoot was separated into inflorescence and each individual green leaf blade, green leaf sheath, and internode. Yellow and dead leaf blades and/or sheaths were discarded, but these were mainly the oldest, basal ones. Length of each internode and the inflorescence was recorded. The separated tissues for each sampling time were dried 48 hours at 70° C and weighed. Tissues were then ground to 40-mesh size, bottled, redried for 12 hours at 70° C, and the bottles sealed.

Tissues were analyzed for reducing and total sugars after extraction with 80% ethanol. Nonreducing sugar values were obtained by subtracting reducing sugar values from those for total sugars. The ethanol residues were treated with takadiastase enzyme solution for starch as described by Smith (1969). Total nonstructural carbohydrate values were obtained by addition of the total sugar and starch values. Reducing power for the different fractions was determined by copper reduction-iodine titration, and the results expressed as glucose (70° C).

Elemental analyses were made on the inflorescence, and the first (top), third, and fifth leaf blade, leaf sheath, and internode. Nitrogen (N) was analyzed by Kjeldahl to include both the organic and inorganic forms. The other elements (P, K, Ca, Mg, Cu, Fe, Zn, B, Mn, and Al) were determined by direct-reading emission spectroscopy.

The data were analyzed statistically as a randomized complete block design, using sampling times as treatments and days as replicates. Significant diurnal trends were calculated at the 0.05 level using orthogonal polynomial coefficients.

Results

Shoot Part Weights and Internode Lengths

Average length of the inflorescence was 26.3 cm, while the uppermost internode was the longest internode: 20.5 cm (Table 2). Internodes became progressively shorter down the shoot. The second leaf blade, leaf sheath, and internode (numbered top to bottom) were heaviest among these respective tissues. The inflorescence made up

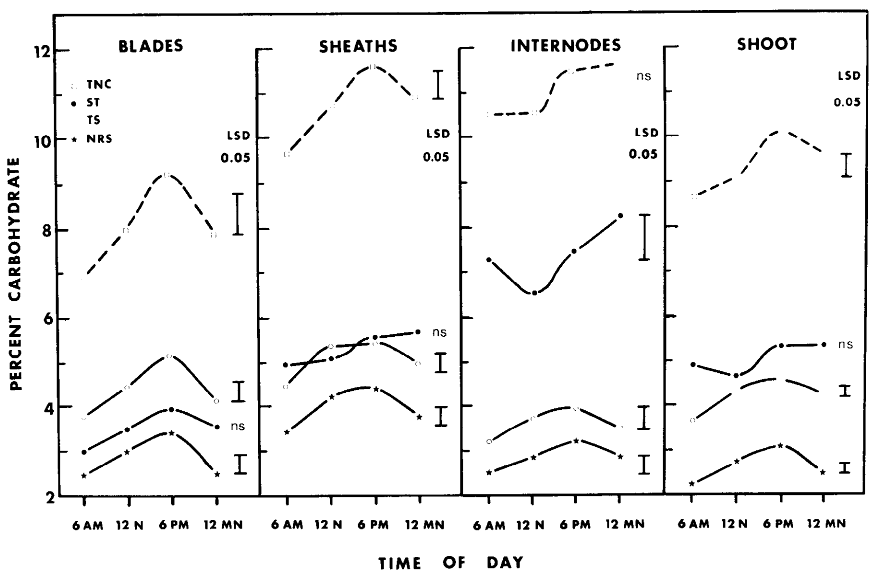

Fig. 1. *Diurnal changes in percentages (dry wt) of nonreducing sugars (NRS), total sugars (TS), starch (ST), and total nonstructural carbohydrates (TNC) in the total leaf blades, leaf sheaths, internodes, and shoot of switchgrass.*

27%, leaf blades 23%, leaf sheaths 17%, and internodes 33% of the total shoot dry weight.

Carbohydrate Concentrations

Concentrations in each individual shoot part are shown in Table 3, and for all leaf blades and sheaths, internodes, and the whole shoot in Figure 1. Diurnal changes in reducing sugar (RS) percentages were very small, and they were not significant (0.05 level) in the inflorescence or leaf sheaths (Table 3), except for the top leaf sheath. Changes in the internodes were significant for internodes 1, 2, and 4, and for the total internodes; increasing from 6 am to 12 noon, plateauing to 6 pm, and then decreasing. The most consistent diurnal changes in RS occurred in the leaf blades. Percentages in all blades, except blade 4, increased significantly from 6 am to 6 pm and then decreased.

Diurnal changes in total sugar (TS) percentages mostly reflected those of nonreducing sugars (NRS). Changes in both sugar fractions were significant at the 0.05 level, except for those of NRS in internodes 2, 3, and 4, and of TS in the inflorescence and internode 2. Percentages increased from 6 am to 6 pm, and then decreased, for all shoot parts, except that percentages were similar in the lower three internodes at 12 noon, 6 pm, and 12 midnight.

Diurnal changes in starch percentage were not significant at the 0.05 level, except for the top leaf blade and leaf sheath and the top and third internode (Table 3), and in the total internodes (Fig. 1). Even so, starch percentages increased from 6 am to 6 pm, and then decreased in the inflorescence, all leaf blades, and the top leaf sheath and internode. The second and third sheaths had similar starch percentages at 6 pm and 12 midnight, but highest percentages in the bottom two sheaths, total sheaths, and all internodes, except the top internode, occurred at 12 midnight.

Diurnal changes in total nonstructural carbohydrates (TNC) were significant at the 0.05 level for all leaf blades and sheaths (Table 3), except for the fourth blade and lower two sheaths. Percentages of TNC increased from 6 am to 6 pm and then decreased to 12 midnight. Diurnal changes in TNC in the inflorescence and internodes were not significant (0.05 level), except for the top internode. Even so, the highest TNC percentages in the inflorescence and top two internodes occurred at 6 pm, but the lower three internodes (Table 3), and total internodes (Fig. 1), were highest at 12 midnight.

Percentages of NRS, TS, and TNC in the total shoot increased from 6 am to 6 pm and then decreased to 12 midnight (Fig. 1). There was no significant diurnal change in starch percentage, but it did reach a high at 6 pm and remained at this level until 12 midnight. No diurnal change in RS percentages was detectable in the total shoot.

Carbohydrate Content

The inflorescence, total leaf blades, total leaf sheaths, total internodes, and total shoot of switchgrass contained the highest content (mg/part) of each carbohydrate fraction at 6 pm. Leaf blade and leaf sheath 3 yielded the

Table 3. Diurnal changes in percentages (dry wt) of nonstructural carbohydrates in the individual shoot parts of switchgrass.*

Time of day	Inflo- rescence	Leaf blades					Leaf sheaths					Internodes				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
							Reducing sugars									
6 am	2.8	1.2	1.2	1.5	1.4	1.6	0.9	1.0	1.5	1.4	1.5	0.8	0.9	0.9	0.5	0.4
12 noon	2.6	1.4	1.4	1.8	1.6	1.8	1.2	1.2	1.5	1.3	1.5	1.1	1.1	1.0	0.6	0.4
6 pm	2.6	1.4	1.5	1.9	1.7	1.9	1.3	1.2	1.6	1.4	1.5	1.1	1.1	0.9	0.6	0.4
12 midnight	3.0	1.3	1.4	1.8	1.6	1.8	1.1	1.1	1.5	1.2	1.3	0.8	0.7	0.8	0.5	0.4
LSD 0.05	n.s.	0.2	0.1	0.1	n.s.	0.1	0.3	n.s.	n.s.	n.s.	n.s.	0.3	0.2	n.s.	0.1	n.s.
							Nonreducing sugars									
6 am	1.1	2.0	2.3	2.5	2.7	2.6	2.4	3.7	3.6	4.3	3.2	1.4	2.9	3.2	2.8	1.7
12 noon	1.5	2.7	3.0	3.0	3.1	2.9	2.8	4.7	4.6	5.0	3.6	1.9	3.2	3.7	3.1	2.0
6 pm	1.9	3.2	3.5	3.7	3.6	3.3	3.6	4.7	4.8	5.4	4.1	2.0	4.0	3.8	3.3	2.1
12 midnight	1.4	2.3	2.6	2.8	2.6	2.6	2.5	4.1	4.0	4.7	3.8	1.4	3.6	3.8	3.2	2.0
LSD 0.05	0.2	0.3	0.4	0.5	0.6	0.4	0.4	0.7	0.5	0.6	0.5	0.7	n.s.	n.s.	n.s.	0.2
							Total sugars									
6 am	3.9	3.2	3.4	4.1	4.1	4.2	3.3	4.7	5.0	5.6	4.7	2.2	3.8	4.1	3.3	2.0
12 noon	4.0	4.1	4.4	4.8	4.7	4.7	4.1	5.9	6.0	6.3	5.1	3.1	4.3	4.6	3.6	2.4
6 pm	4.5	4.6	5.0	5.6	5.4	5.2	4.9	5.9	6.4	6.8	5.6	3.2	5.1	4.7	3.9	2.5
12 midnight	4.4	3.6	4.0	4.5	4.2	4.4	3.6	5.2	5.5	6.0	5.1	2.2	4.3	4.6	3.6	2.4
LSD 0.05	n.s.	0.4	0.5	0.5	0.4	0.4	0.4	0.9	0.6	0.4	0.4	0.8	n.s.	0.5	0.4	0.2
							Starch									
6 am	3.4	2.8	2.6	3.4	3.3	3.5	4.4	4.8	5.8	5.3	5.1	3.9	4.5	8.0	9.5	9.8
12 noon	3.2	3.2	3.1	4.0	3.5	3.7	4.7	4.8	5.9	5.4	5.3	3.8	4.8	8.0	9.1	8.9
6 pm	3.6	4.4	3.8	4.6	3.2	4.1	5.2	5.5	6.9	5.5	5.4	4.4	4.9	8.7	9.8	10.0
12 midnight	3.3	3.6	3.6	4.1	3.5	3.4	4.9	5.5	6.8	6.0	5.8	3.7	5.3	9.1	11.2	11.4
LSD 0.05	n.s.	0.7	n.s.	n.s.	n.s.	n.s.	0.4	n.s.	n.s.	n.s.	n.s.	0.3	n.s.	0.3	n.s.	n.s.
							TNC									
6 am	7.3	6.0	6.0	7.5	7.4	8.3	7.7	9.5	10.8	10.9	9.8	6.0	8.3	12.1	12.9	12.0
12 noon	7.2	7.3	7.5	8.8	8.2	9.5	8.8	10.7	12.0	11.7	10.4	6.9	9.2	12.4	12.7	11.3
6 pm	8.1	9.1	8.8	10.2	8.6	10.1	10.1	11.4	13.3	12.3	11.1	7.6	10.0	13.4	13.7	12.6
12 midnight	7.7	7.2	7.6	8.6	7.7	9.0	8.6	10.7	12.4	12.0	10.9	5.8	9.6	13.7	14.8	13.7
LSD 0.05	n.s.	0.7	0.6	1.1	n.s.	1.0	0.7	0.8	0.9	n.s.	n.s.	0.7	n.s.	n.s.	n.s.	n.s.

*Numbered top to bottom of shoot.

highest content of each carbohydrate fraction among these respective parts, except that leaf blade and leaf sheath 2 were slightly higher than blade or sheath 3 in content of RS. Internodes 2 and 3 were highest, and similar, in content of RS, NRS, and TS among the internodes. However, internode 4 was highest in content of starch and TNC.

Elemental Concentrations

The inflorescence, and the first (top), third, and fifth leaf blade, leaf sheath, and internode were analyzed for concentrations of N, P, K, Ca, Mg, Cu, Fe, Zn, B, Mn, and Al. Diurnal trends of these elements were statistically nonsignificant (0.05 level), except for N in leaf blade 3, Al in leaf blade and leaf sheath 5, and Ca in internode 1. Concentrations in these significant cases increased from 6 am to 12 noon and then decreased to 12 midnight, but Al in leaf sheath 5 was highest at 6 pm. Diurnal trends of elemental concentrations also were nonsignificant for the most part even when calculated at the 0.10 level of probability.

Discussion

Diurnal trends of nonstructural carbohydrates in the total shoot and total leaf blade tissues of switchgrass were similar to those that have been noted in annual grasses, i.e. corn, sorghum, and sudangrass (Miller, 1924; Eisele, 1938; Lechtenberg, et al., 1973). Percentages of total and nonreducing sugars, starch, and TNC increased from early morning to late in the afternoon and then decreased. Diurnal changes in reducing sugar percentage were small. These data support a conclusion made by Lechtenberg et al. (1973) with sudangrass that pasturing in the evening might provide nutritional advantages, at least from the viewpoint of energy concentration in herbage.

Use of only the total shoot, total leaf blades, total leaf sheaths, or clum masked many of the diurnal changes in carbohydrates that occurred in the parts that make up each of these parameters. Although diurnal changes in reducing sugar percentages were small in all shoot parts, the changes were significant in the total leaf blades and total internodes, while they were not in the total shoot. Even so, diurnal changes in reducing sugar percentages were not significant in leaf blade 4 nor in internodes 3 and 5.

Diurnal changes in total sugar percentages were due primarily to fluctuations in nonreducing sugars (sucrose). With percentage of nonreducing sugars, diurnal changes were not only significant in the total shoot, but also in all of its parts, except in internodes 2, 3, and 4. On the other hand, diurnal changes in starch percentage were not significant in the total shoot, total leaf blades, and total sheaths, but this masked the significant changes that occurred in the top leaf blade and sheath. In contrast, diurnal changes in starch percentage were significant in the total internodes, but were significant only in individual internodes 1 and 3.

Percentages of TNC showed the changes in the sum of the nonstructural carbohydrate fractions (sugars plus starch). Diurnal trends in TNC percentage were significant in the total shoot, but this masked the fact that TNC trends were not significant in the inflorescence or total internodes, nor in each individual internode except the top one. Diurnal changes in TNC were significant in the total leaf blades and sheaths, but not in leaf blade 4 nor in the bottom two leaf sheaths.

This investigation indicated that diurnal changes in sugar percentages were due primarily to changes in nonreducing sugars, as also noted by others (Miller, 1924; Lechtenberg et al., 1973; Smith, 1973), and that significant diurnal changes in nonreducing sugar percentage occurred in all plant parts except in some individual internodes. Percentages increased from 6 am to 6 pm and then decreased to 12 midnight. In contrast, significant diurnal changes in starch percentage, the predominant nonstructural polysaccharide, were limited primarily to the upper leaf blade, leaf sheath, and internode, where the highest level also occurred at 6 pm. Although not statistically significant, the diurnal trend of starch percentage in the lowest sheaths and internodes was for a continuous increase from 6 am to 12 midnight. These bottom parts of the switchgrass shoot are storage organs, so that sugars probably were being translocated continuously from the upper leaves for storage as starch in the bottom leaf sheaths and internodes, especially after 6 pm.

Certain plant parts were analyzed by emission spectroscopy for elemental concentrations and by Kjeldahl for total nitrogen. Diurnal changes in concentrations of these elements for the most part were not statistically significant.

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