Photosynthesis and Nutritive Value in Leaves of Three Warm-Season Grasses before and after Defoliation

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ABSTRACT

Forage yields are influenced by plant response to defoliation. We examined the photosynthesis and nutritive value of first (first leaves) and third (third leaves) fully expanded leaves (numbered from the apex) in three warm-season (C4) grasses. Net photosynthetic rates at uniform temperature and light both before and after a 2-wk exposure to full sunlight and the effect of leaf position on nutritive value were determined on vegetative tillers in well-established swards of bermudagrass [*Cynodon dactylon* L. (Per.) cv. Tifton 44], caucasian bluestem [*Bothriochloa caucasica* (Trin.) C.E. Hubb.], and Atlantic coastal panicgrass [*Panicum amarum* var. *amarulum* (Hitchcock and Chase) P.G. Palmer] growing on a Cecil clay loam (clayey, kaolinitic, thermic Typic Hapludults). Bermudagrass had the greatest level of crude protein (CP), followed by panicgrass and bluestem. Fiber was greater in the first leaves than in the third leaves for bermudagrass and panicgrass but not for bluestem. Photosynthetic rates of panicgrass and bluestem third leaves estimated 2 wk after defoliation of the surrounding canopy were less than estimates made before defoliation in the first leaves but were similar to the third leaves before canopy defoliation. The third leaves of bermudagrass 2 wk after defoliation had lesser photosynthetic rates per unit chlorophyll than the first or third leaves before defoliation. Photosynthetic rates were correlated with hemicellulose across leaf classes and species ($r^2 = 0.93$). The photosynthetic decline observed in third leaves of bermudagrass compared with panicgrass and bluestem is evidence of variation in leaf response after defoliation among warm-season grasses.

G RASS CANOPIES contain dynamic populations of leaves with growth and senescence taking place simultaneously. The youngest leaves are often near the top of the canopy and reach their maximum photosynthetic ability by full expansion (Jewiss, 1965; Murchie et al., 2002). Grazing often results in the selective removal of young leaf tissue and grazing systems can be managed to vary the residual leaf area. New leaf tissue is formed in response to defoliation and some studies have reported increased photosynthetic rates in the remaining older leaves (McNaughton, 1983; Wallace et al., 1984; Briske, 1986) but other studies have reported no increase in net photosynthesis after defoliation (Detling and Painter, 1983; Ryle and Powell, 1975). Murchie et al. (2002), in a study with a C₃ grass, suggested that leaf age was dominant in limiting photosynthesis in the upper portions of the canopy while acclimation to light was dominant in the lower portions of the canopy.

Variation in the nutritive value of forages with maturity has been extensively researched (Buxton and Mertens, 1995; Sollenberger and Cherney, 1995) and shifts in proportions of leaf and stem during maturation of the forage crop often explain variation in nutritive value (Buxton and Mertens, 1995). Greater variation in yield than in nutritive value has been associated with environmental factors (Buxton and Casler, 1993). The nutritive value of individual leaves has received less research emphasis but studies of leaf growth and chemistry can contribute to understanding of forage growth (Volenec and Nelson, 1995; Macadam and Nelson, 2002).

The severity of defoliation in a grazing system could be managed to retain a portion of the older leaves lower in the pasture canopy if they remain photosynthetically productive. Variation of nutritive value among canopy leaves may also impact the diet of the grazing animal as defoliation is managed since the uppermost and younger portions of the canopy would be preferentially removed. The objectives of this study were to test for variation in the relative responses found in the photosynthetic and respiration rates of bermudagrass, caucasian bluestem, and Atlantic coastal panicgrass first leaves and third leaves before and after a 2-wk exposure period to ambient sunlight following defoliation of the surrounding canopy. Comparisons of photosynthetic rates among the warm-season (C₄) grasses were of less interest than the relative response to the 2-wk exposure period. Nutritive value of the first and third leaves was estimated as supporting data by measuring leaf IVDMD, CP, and Van Soest fiber fractions.

MATERIALS AND METHODS

Field Methods

Three forage species (bermudagrass, caucasian bluestem, and Atlantic coastal panicgrass) were established a year before the research on a Cecil clay loam near Raleigh, NC. These three treatments were established in a randomized complete block design with two replicates. The plots measured 25.6 by 8.5 m and were sampled over 2 yr. Within these plots, we selected leaves to represent the leaf classes studied.

Plots were burned in late winter and allowed to regrow before sampling. Weeds were controlled using 2,4-D (2,4-dichlorophenoxyacetic acid) and Princep [2-chloro-4,6-bis (ethylamino)-*s*-triazine]. Nitrogen was applied as ammonium nitrate at 90 kg ha^{-1} four times both years during the summer at 3- to 4-wk intervals beginning in early May (total $N = 360$ kg ha^{-1} yr^{-1}). Panicgrass and bluestem were defoliated to a 150-mm stubble and bermudagrass was defoliated to a 100-mm stubble once during the summer before heading.

Data were collected from June through August in both

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Abbreviations: ADF, acid detergent fiber; CP crude protein; IVDMD, in vitro dry matter disappearance; NDF, neutral detergent fiber; PAR, photosynthetically active radiation.

years with plants in a vegetative stage. During data collection in the first year, rainfall averaged 11 mm mo^{-1}, compared with 15 mm mo^{-1} during the second year. Because of poor rainfall distribution, plots were irrigated in July of the second year with 32 mm of water distributed over 3 d to prevent excessive moisture stress. Mean temperatures were 24.5°C in the first year and 25.5°C in the second year for the 3 mo in which data were collected. Overall, observations of photosynthetically active radiation (PAR) in the first year were less that those in the second year (37 vs. 39 mol d^{-1}) but relative humidity (83 vs. 63%) was greater in the first year than in the second year.

Physiological Measurements

Specific leaf weight (g m^{-2}) was estimated by determining the area of a leaf sample of at least 500 cm^2 from each plot (Delta-T Devices LTD, Burwell, Cambridge, UK) and then drying the sample at 75°C for 24 h. Plots were sampled once in the first year and after examining a preliminary analysis of variance and logistics the plots were sampled twice in the second year to estimate specific leaf weight.

The percent transmission of PAR was determined with quantum radiation sensors. Fifty measurements were recorded per plot with sensors set in full sun and adjacent to the third leaf from the apex of a tiller at each measurement site. Percent transmission was estimated twice in the first year and after examining a preliminary analysis of variance and logistics the percent transmission was estimated four times in the second year.

In the second year, chlorophyll content was estimated four times for the first and third leaves, and also for third leaves 2 wk after defoliation of the surrounding canopy by extraction with N,N-dimethylformamide and absorption at 664 nm (chlorophyll a) and 647 nm (chlorophyll b). Chlorophyll a and b concentrations were estimated using Moran's equations (Moran, 1982).

During the summer (mean temperature 25°C), to test for photosynthetic response, two sets of paired first and third leaves were selected and cut from each plot during the morning (before 900 h). Leaves were placed between layers of damp germination paper and transported to the laboratory for photosynthetic estimates. Measurements were completed before 1500 h each day. To test for the effects of 2 wk of re-exposure to full ambient sunlight, two tillers having first and third leaves without visible defects were selected within two areas of each plot. A 1-m^2 area was defoliated to approximately 5 cm around the pair of tillers, and the two remaining tillers were then defoliated to within 20 mm of the third leaf. After the 2-wk period of exposure to ambient sunlight and temperatures, the remaining third leaves were harvested and net photosynthesis and respiration were estimated in vitro. Results were compared with measurements on the first and third leaves from the beginning of the 2-wk period to estimate the relative response to defoliation. Two exposure periods were conducted in the first year and after examining a preliminary analysis of variance and logistics we conducted four exposure periods in the second year.

An in vitro system was used to provide reproducible conditions for estimates of net photosynthesis (Delieu and Walker, 1983; Akhkha et al., 2001). Leaf segments were removed from the center portions of the first, third, and re-exposed third leaves for each of the species. Two samples from each plot were placed in a sealed chamber and dark-adapted for 1 min. The leaf segments were then illuminated with approximately 2000 µmol m^{-2} s^{-1} PAR in a gas mixture containing 300 µmol mol^{-1} CO$_2$ at 25°C for 3 min. The change in oxygen concentra-

tion within the chamber was used to estimate the photosynthetic rate (Hansatech, Kings Lynn, Norfolk, UK). Following photosynthetic estimates, the leaf segments were subjected to 1900 µmol mol^{-1} O$_2$ and 100 µmol mol^{-1} CO$_2$ at 25°C in total darkness for 5 min during which respiration rate was estimated as O$_2$ uptake.

Nutritive Value

Samples of first and third fully expanded leaf lamina were collected from each plot once in the first year and after examining a preliminary analysis of variance and logistics we sampled twice in the second year. Samples were quick-frozen in liquid N, freeze dried, and ground to pass a 1-mm screen. Crude protein (AOAC, 1990), IVDMD (Tilley and Terry, 1963; Burns and Cope, 1974), neutral detergent fiber (NDF), and acid detergent fiber (ADF) (Van Soest and Robertson, 1980) were determined. Hemicellulose was estimated as the difference between NDF and ADF.

Statistical Analysis

The experimental design utilized the three forage species arranged in a randomized complete block. The data were analyzed over years with years as a random effect and forage species and leaf classes as fixed effects (SAS Inst., 1979). Treatment and interaction effects were tested with a least significant difference (LSD) test ($P = 0.10$). The decision to test at a $P = 0.10$ was made a priori based on the small sample size ($n = 16$ for chlorophyll, 24 for photosynthesis and 12 for fiber fractions). Tests were based on expected mean squares and the split-block treatment × year interaction sum of squares were pooled with the default error term when no significance was indicated by an initial F-test. When the split-block interaction with years was significant, synthetic F-tests were performed with estimated degrees of freedom (Satterthwaite, 1946; Gaylor and Hopper, 1969). Linear regression was also used to test for relationships among the variables.

RESULTS AND DISCUSSION

Leaf Characteristics

Specific leaf weights differed among panicgrass (81 g m^{-2}), bluestem (36 g m^{-2}), and bermudagrass (47 g m^{-2}). The species also differed in PAR transmission to the third leaves. Bermudagrass had greater PAR (39% transmission) at the level of the third leaves than the other two species, which were similar (13%). Because of differences in morphology, the third leaves of bermudagrass were generally nearer the top of the canopy than the other two grass species.

A species × leaf interaction for the chlorophyll a/b ratio resulted because of the behavior of bluestem (Table 1). The first leaf chlorophyll ratio of bluestem was high and similar to the third leaves after the 2-wk re-exposure period in contrast to bermudagrass and panicgrass in which first leaves had the greater ratio while third leaves and third leaves after the re-exposure period were similar. After the re-exposure period, the third leaf chlorophyll ratio of bluestem was greater than the value estimated for the third leaves before the 2-wk period and may reflect a reorganization of chlorophyll in response to greater PAR (Sukenik et al., 1990; De la Torre and Burkey, 1990).

Total chlorophyll on a leaf area basis (g m^{-2}) differed

among species (Table 1). Bermudagrass and bluestem were similar, but both had a lower chlorophyll content than panicgrass which was likely a reflection of its greater specific leaf weight. Although there was no plant species × leaf class interaction, leaf classes differed in chlorophyll content with first and re-exposed third leaves similar and lower than third leaves before defoliation of the surrounding canopy. This effect of increased PAR was similar for all three species.

Gas Exchange

Net photosynthesis in bermudagrass was greater than the rates observed for bluestem and panicgrass per unit chlorophyll. An overall leaf class effect indicated that photosynthesis per unit chlorophyll was greatest for the first leaves followed by third leaves and third leaves after 2 wk of re-exposure; however, a species × leaf interaction occurred (Table 2). The interaction was a result of variation in the response of the species to both the 2-wk re-exposure treatment and the magnitude of the reduction in net photosynthesis between the first and third leaves. For example, the decline of photosynthetic rates in the re-exposed third leaves noted for the overall leaf effect only occurred in bermudagrass. Photosynthetic rates before and after re-exposure in the third leaves of bluestem and panicgrass were similar. This may indicate a potentially greater contribution from the third leaves of these two species during regrowth after partial defoliation exposes an older population of leaves.

Photosynthesis main effects existed for species and leaf position expressed on a leaf area basis but no interaction was present. On a leaf area basis, bermudagrass had the greatest net photosynthetic rate followed by panicgrass and bluestem. Because of the high levels of CO_2 (300 μmol mol^{-1}) and PAR (2000 μmol m^{-2} s^{-1}) during measurements of photosynthesis, physiological

Table 1. Chlorophyll *a/b* ratio and total chlorophyll (Year 2) in first, third, and re-exposed third leaves 2 wk after canopy defoliation in bermudagrass, bluestem, and panicgrass.

Species	Leaf class	Chlorophyll Ratio *a/b*	Chlorophyll Total
			g m²
Bermudagrass	first	3.5†	0.29
	third	3.2	0.33
	third re-exposed	3.2	0.29
	avg.	3.3	0.30
Bluestem	first	3.9	0.28
	third	3.3	0.32
	third re-exposed	3.8	0.27
	avg.	3.7	0.29
Panicgrass	first	3.3	0.49
	third	2.8	0.51
	third re-exposed	2.9	0.45
	avg.	3.0	0.48
Avg. leaf	first	3.6	0.35
	third	3.1	0.39
	third re-exposed	3.3	0.34
SE		0.7	0.05
LSD(0.10) for main effects and interaction‡:			
Species		0.2	0.02
Leaf		0.3	0.03
Species × leaf (MP)		0.3	NS
Species × leaf (SP)		0.3	NS

† Each value represents a mean of two field replicates, two subsamples per plot, and four field samples (*n* = 16).
‡ NS = no difference; MP = species comparisons in same or different leaf class; sp. = leaf class comparisons in same species.

rather than morphological differences are assumed to play the major role in the differences between the species. However, comparisons of species must be made with caution when using this methodology of estimating photosynthesis (Akhkha et al., 2001). As was the case when expressed on a chlorophyll basis, leaf photosynthetic rates declined from first to third leaves and also to re-exposed third leaves on an area basis. The lesser photosynthesis of the third leaves may have been the result of physiological adaptation to shading and the

Table 2. Photosynthesis and respiration in first, third, and re-exposed third leaves 2 wk after canopy defoliation in bermudagrass, bluestem, and panicgrass.

Species	Leaf class	Photosynthesis Per unit chlorophyll	Photosynthesis Per unit leaf area	Respiration Per unit leaf area
		μmol g^{-1} s^{-1}	μmol m^{-2} s^{-1}	μmol m^{-2} s^{-1}
Bermudagrass	first	81.5†	23.8	3.6
	third	59.8	20.8	3.1
	third re-exposed	51.6	17.4	4.0
	avg.	64.3	20.7	3.6
Bluestem	first	30.3	8.5	2.3
	third	19.3	6.0	2.8
	third re-exposed	20.5	6.0	3.3
	avg.	23.4	6.8	2.8
Panicgrass	first	29.5	14.1	2.4
	third	21.0	10.1	2.2
	third re-exposed	20.7	9.6	2.7
	avg.	23.7	11.3	2.4
Avg. leaf	first	47.1	15.5	2.8
	third	33.4	12.3	2.7
	third re-exposed	30.9	11.0	3.3
SE		9.1	2.8	0.3
LSD(0.10) for main effects and interaction‡:				
Species		4.3	2.9	0.4
Leaf		2.3	0.3	NS
Species × leaf (MP)		5.8	NS	0.5
Species × leaf (SP)		4.6	NS	0.6

† Each value represents a mean of two field replicates, two subsamples per plot, and two (*n* = 8 in first year) or four (*n* = 16 in second year) field samples.
‡ NS = no difference; MP = comparisons of leaf class within the same species; sp. = comparisons across species.

Table 3. In vitro dry matter disappearance (IVDMD), neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicellulose (HEMI) of leaf lamina in bermudagrass, bluestem, and panicgrass in Year 1 and 2.

Species	Year	IVDMD	NDF	ADF	HEMI
			g kg^{-1}		
Bermudagrass	1	585†	726	334	396
	2	631	684	291	398
	avg.	608	705	313	397
Bluestem	1	600	624	351	278
	2	635	616	308	306
	avg.	618	620	330	292
Panicgrass	1	578	661	340	323
	2	647	648	326	324
	avg.	613	655	333	324
SE		13	12	3	9
LSD(0.10) for main effects and interaction‡:					
Species		NS	7	11	11
Year		6	NS	7	NS
Species × year (MP)		NS	11	12	13
Species × year (SP)		NS	27	8	20

† Each value represents a mean of two field replicates, two leaf positions, and one ($n = 4$ in Year 1) or two ($n = 8$ in Year 2) field samples.
‡ NS = no difference; MP = comparisons of years within the same species; sp. = comparisons across species.

Table 4. Neutral detergent fiber (NDF) and hemicellulose (HEMI) in first and third leaves of bermudagrass, bluestem, and panicgrass.

Species	Leaf class	NDF	HEMI
		g kg^{-1}	
Bermudagrass	first	717†	404
	third	693	390
	avg.	705	397
Bluestem	first	620	290
	third	620	295
	avg.	620	292
Panicgrass	first	672	334
	third	637	313
	avg.	655	324
SE		12	9
LSD(0.10) for main effects and interaction‡:			
Species		7	11
Leaf		NS	NS
Species × leaf (MP)		9	12
Species × leaf (SP)		33	32

† Each value represents a mean of two field replicates and one ($n = 2$ in Year 1) or two ($n = 4$ in Year 2) field samples.
‡ NS = no difference; MP = comparisons of leaf class within the same species; sp. = comparisons across species.

greater photosynthesis of the first leaves may have been the result of the younger tissue and adaptation to a more favorable light environment.

A species × leaf interaction occurred for respiration (Table 2) because bermudagrass and panicgrass leaves showed similar relationships between respiration and leaf position while bluestem responded differently. Bermudagrass and panicgrass had greater respiration rates for first leaves and re-exposed third leaves when compared with third leaves. In bluestem, respiration in third leaves after re-exposure was greater than in first leaves.

Nutritive Value

Bermudagrass had the greatest level of CP (163 g kg^{-1}), followed by panicgrass (140 g kg^{-1}) and bluestem (113 g kg^{-1}), but all three levels were >70 g kg^{-1} and are generally considered adequate for beef production (Buxton and Mertens, 1995) and no interactions occurred for these data (data not shown). The overall IVDMD was lower in the first year (582 g kg^{-1}) than the second year (638 g kg^{-1}). The increased rainfall and irrigation in the second year along with the observed greater PAR may have resulted in this difference. No species or year × species interaction was found (Table 3).

There were species × year interactions for estimates of NDF but the interaction was the result of minor variation from year to year (Table 3). Bermudagrass had the greatest concentration of NDF, and in the first year was 42 g kg^{-1} greater than the second. Both bluestem and panicgrass decreased by approximately 10 g kg^{-1} NDF between years and bluestem had the lowest overall NDF concentration. A species × leaf interaction occurred because NDF declined between the first and third leaves of bermudagrass and panicgrass, whereas the first and third leaves of bluestem were similar (Table 4). This was likely a differential response to the greater moisture and PAR in the second year.

Averaged over years, bermudagrass had less ADF

than bluestem and panicgrass, which were similar (Table 3). First year ADF was greater than second year; however, in the second year ADF decreased 43 g kg^{-1} for bermudagrass and bluestem, and only 14 g kg^{-1} for panicgrass, resulting in an interaction between species and year. This may have been related to environmental variation.

Bermudagrass had the greatest concentration of hemicellulose followed by panicgrass and bluestem in both years (Table 3). Hemicellulose in the second year was greater than in the first year for bluestem, but not for bermudagrass or panicgrass. Bermudagrass hemicellulose was greater in concentration for both first and third leaves followed by panicgrass and bluestem (Table 4). A leaf class × species interaction occurred because the first and third leaves of bluestem had similar concentrations of hemicellulose, while bermudagrass and panicgrass first leaves had greater concentrations of hemicellulose than third leaves.

Estimates of digestibility (IVDMD) did not vary among species and yet fiber concentration (NDF) and composition (ADF and hemicellulose) did. These results infer that a relatively greater proportion of bermudagrass fiber in the leaf lamina was digestible. This was consistent with the lower ADF and greater hemicellulose of bermudagrass.

Association of Photosynthesis and Hemicellulose

A positive relationship was found between photosynthesis and hemicellulose (Fig. 1). It is possible the observed relationship is simply related to the three species selected for the experiment. As indicated in Fig. 1, bermudagrass had the highest photosynthetic rates and hemicellulose concentrations followed by panicgrass and bluestem. Care should be exercised in species to species comparisons when using this method of estimating photosynthetic rates (Akhkha et al., 2001). The strong relationship of hemicellulose to photosynthesis may be associated with the anatomy of these C4 grasses. For example, it is possible that hemicellulose concentration

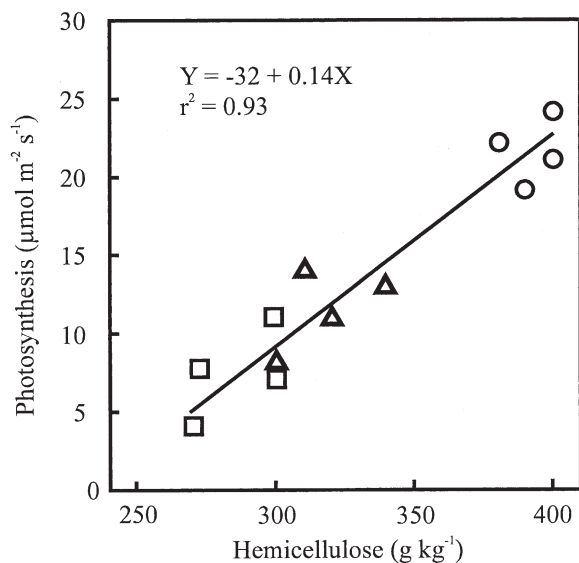$$Y = -32 + 0.14X$$
$$r^2 = 0.93$$

Fig. 1. Relationship between net photosynthesis and hemicellulose concentration for bermudagrass (indicated by circles), caucasian bluestem (indicated by squares), and Atlantic coastal panicgrass (indicated by triangles).

is related to the number of bundle sheaths in the grass lamina with the number of bundle sheaths per unit leaf area then related to the photosynthetic rate. If the effect holds within a species, it may warrant further study as a variable related to both photosynthesis and nutritive value.

CONCLUSIONS

Grazing systems can provide an opportunity to match defoliation to the growth and physiological characteristics of pasture species. Since younger leaves near the top of the canopy are preferentially harvested during grazing, the relative performance of any remaining older leaves can be important for regrowth. We found sufficient variation in the response of the third leaves of these three grass species to time and light environment to warrant further study with additional leaf classes. Based on the continued decline in photosynthetic responses in the third leaves of bermudagrass, even when exposed to full sun, it appears that partial defoliation may be relatively less efficient for production and a more complete defoliation may be relatively more efficient when compared with similar defoliations in bluestem and panicgrass.

The nutritive value of the leaves varied in a complex manner among plant species but the IVDMD was similar among leaf classes. Consequently, leaf/stem ratios and leaf senescence are more likely to be the principle sources of variation in the nutritive value of the grazing animals diet. The strong relationship of hemicellulose to photosynthesis is likely associated with the kranz anatomy of these C4 grasses.

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