Remote Sensing Brief

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Leaf Reflectance-Nitrogen-Chlorophyll Relations in Buffelgrass

It appears that both leaf and canopy reflectance are sensitive to different chlorophyll concentrations in plant leaves.

Abstract: *The relation of leaf reflectance with leaf nitrogen (N) content and chlorophyll (chl) concentration was investigated using buffelgrass (*Cenchrus ciliaris*) from field plots having five levels of N fertilizer. Six wavelengths (WL) were studied: 0.50, 0.55, 0.60, 0.65, 0.70, and 0.75 μm. Leaves from nonfertilized buffelgrass plants and those from plants that received 56 kg N/ha had significantly higher ($p = 0.05$) reflectance at the 0.50-, 0.55-, and 0.60-μm WL's than did leaves from plants that received 112, 168, and 224 kg N/ha. Leaf reflectance values at the 0.65-, 0.70-, and 0.75-μm WL's also differed significantly, but differences were not as great as those at the 0.50-, 0.55-, and 0.60-μm WL's. Leaves from buffelgrass plants fertilized with 112, 168, and 224 kg N/ha had significantly higher ($p = 0.05$) levels of N and chl concentrations than those from plants that received no fertilizer or 56 kg N/ha. Asymptotic regression models showed that the 0.50- and 0.55-μm WL's best described the inverse relationship of leaf reflectance with leaf chl concentration and leaf N content. The correlation coefficients were highly significant ($p = 0.01$) for all four equations. These results indicated that leaf reflectance measurements may be useful to estimate N content of buffelgrass.*

Introduction

Light reflectance by leaves within the 0.40- to 0.75-μm waveband (WB) of the spectrum is influenced primarily by chlorophyll (chl) pigments (Benedict and Swindler, 1961; Birth and McVey, 1968; Thomas and Gausman, 1977). Reflectance can be used to evaluate turf color (Birth and McVey, 1968), estimate the nitrogen (N) status of sweet pepper (*Capsicum annuum*) leaves (Thomas and Oerther, 1972), measure amounts of biomass production or vegetation density (Tucker *et al.*, 1973; Wiegand *et al.*, 1974), and demonstrate that leaf spectral and color-infrared (CIR) film image differences exist among shrubs and woody plant species (Driscoll and Coleman, 1974; Gausman *et al.*, 1976, 1977).

Richardson *et al.* (1983) used radiometric field plant canopy measurements to estimate the biomass and N content of Alicia grass (*Cynodon* spp.), a forage species found in pastures in south Texas. A lack of basic knowledge of the relations among light reflectance factors, N content, and chl concentration of range grasses prompted us to further investigate these parameters on buffelgrass (*Cenchrus ciliaris*). Buffelgrass is the most important forage species found on south Texas rangelands. In this study, a range of N contents of buffelgrass was produced in field plots. Our objective was to determine

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the relation of leaf reflectance measurements from buffelgrass in the various plots with N content and chl concentration.

Materials and Methods

The experimental site was a 0.45-ha, fenced buffelgrass pasture located on the 2,800-ha H. Yturria Ranch near La Joya in Hidalgo County, Texas. The 0.45 ha of buffelgrass were arranged in a randomized complete block design with three replications of five fertility treatments: 0, 56, 112, 168, and 224 kg elemental N/ha from ammonium sulfate that was applied by broadcasting on 26 February 1982. The grass responded slowly to the fertilizer because the area had received only minimal rainfall until early May 1982, when about 20 cm of rain was recorded over a 10-day period. On 1 June 1982 the buffelgrass plants were actively growing and in flush foliage development.

On 3 June 1982 one recently matured, fully expanded leaf was collected from five different buffelgrass plants in each plot. The detached leaves were enclosed immediately in air-tight plastic bags and stored on ice to minimize dehydration during transport to the laboratory.

Reflectance measurements and total chl concentration were determined on each of the same five leaves from each plot. Total diffuse reflectance of upper (adaxial) surface of single leaves over the 0.50 to 0.75-μm visible WB was measured with a Beckman* Model DK-2A spectrophotometer equipped with a reflectance attachment. Data were corrected for decay of the barium sulfate standard to give absolute radiometric data (Allen and Richardson 1971). Six wavelengths (WL) were selected for study: 0.50, 0.55, 0.60, 0.65, 0.70, and 0.75 μm. Total chl was extracted from each leaf with 85 percent acetone, and the amount of chl was determined colorimetrically using methods of the Association of Official Agricultural Chemists (Horwitz, 1965). The mean reflectance and chl concentration of the five leaves were used to represent each plot.

Grass samples were taken on 4 June 1982 for N assay by hand clipping composite samples of recently matured leaves from 15 or more buffelgrass plants per plot. All plant samples were oven-dried for 48 hrs at 65°C. Samples for N assays were ground in a Wiley* mill through a 1-mm mesh screen, thoroughly mixed, and stored in sealed jars. Total N was determined on three subsamples from each composited sample using the Kjeldahl method (Peech *et al.*, 1947). The mean N content for three subsamples was used to represent each plot.

An analysis of variance was conducted to determine significant differences among leaf reflectance, leaf N content, and leaf total chl concentration (Steel

* Trade names or company names are included only for the benefit of the reader and do not imply endorsement of or preferential treatment by the U.S. Department of Agriculture of the product listed.

Table 1. Effect of N Fertilizer on the Mean Total Chlorophyll Concentration, N content, and Diffuse Reflectance of Single Leaves of Buffelgrass.

N Applied	Reflectance						Chlorophyll	N
	0.50 μm*	0.55 μm	0.60 μm	0.65 μm	0.70 μm	0.75 μm		
kg/ha			%				mg/g	%
0	12.7 a	17.2 a	11.8 a	10.3 a	32.3 a	43.0 c	1.25 c	1.02 b
56	11.9 a	15.9 a	11.0 a	10.0 ab	31.6 ab	43.1 c	1.43 c	1.23 b
112	10.2 b	13.3 b	9.8 b	9.4 bc	30.6 bc	45.9 a	2.38 b	1.49 a
168	9.9 b	12.9 b	9.6 b	9.3 bc	30.1 c	45.6 a	2.69 a	1.57 a
224	9.8 b	12.9 b	9.4 b	9.0 c	29.7 c	44.5 b	2.41 b	1.64 a

* Values within each column followed by the same letter do not differ significantly at the 5% probability level, according to Duncan's multiple range test.

TABLE 2. COEFFICIENTS OF DETERMINATION (r^2) FOR ASYMPTOTIC REGRESSION MODELS RELATING SINGLE LEAF REFLECTANCE OF BUFFELGRASS TO CHLOROPHYLL AND NITROGEN OVER THE 0.50- TO 0.75-μm WAVEBANDS. THE DETAILED EQUATION COEFFICIENTS ARE GIVEN FOR THE 0.50- AND 0.55-μm WAVELENGTHS. THE STANDARD ERROR OF ESTIMATE (SYX) FOR THE REGRESSION EQUATIONS ARE ALSO LISTED. THE DEGREES OF FREEDOM WAS 13 FOR THIS STUDY (df = $n - 2$). CORRELATIONS AT ALL WAVELENGTHS, EXCEPT THE 0.75-μm, WERE NEGATIVE.

Wavelength (μm)	Nitrogen (N, %)	Chlorophyll (chl, mg/g)
0.50	−0.766**	−0.939**
0.55	−0.805**	−0.931**
0.60	−0.769**	−0.908**
0.65	−0.630*	−0.834**
0.70	−0.689**	−0.846**
0.75	0.490	0.766**

Wavelength (μm)	Equation
0.50	$\frac{1}{chl} = 2.27 - \frac{18.5}{R}$, $r^2 = 0.939^{**}$, Syx = 0.05
0.50	$\frac{1}{N} = 1.93 - \frac{12.8}{N}$, $r^2 = 0.766^{**}$, Syx = 0.08
0.55	$\frac{1}{chl} = 2.08 - \frac{21.8}{R}$, $r^2 = 0.931^{**}$, Syx = 0.05
0.55	$\frac{1}{N} = 1.83 - \frac{15.4}{R}$, $r^2 = 0.805^{**}$, Syx = 0.07

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.

and Torrie, 1960). Duncan's multiple range test was used to test differences among means at the 5 percent probability level. An asymptotic regression model was used to describe the relation of single leaf reflectance of buffelgrass with its leaf N content and chl concentration.

RESULTS AND DISCUSSION

Spectrophotometrically measured mean leaf reflectance values for fertilized and nonfertilized buffelgrass at the six WL's selected for study are given in Table 1. Leaves from nonfertilized buffelgrass and those from plants fertilized with 56 kg N/ha had significantly higher ($p = 0.05$) reflectance at the 0.50-, 0.55-, and 0.60-μm WL's than did leaves from plants that received 112, 168, and 224 kg N/ha. At the 0.65- and 0.70-μm WL's, leaf reflectance values differed significantly among the five treatments, but differences were not as great as those at the 0.50-, 0.55-, and 0.60-μm WL's. Leaf reflectance value differences among the five treatments was significant at the 0.75-μm WL, but means were inversely ranked as compared to the other WL's. Leaves from plants receiving 112, 168, and 224 kg N/ha had significantly higher reflectance at the 0.75-μm WL than did leaves from nonfertilized plants or those from plants fertilized with 56 kg N/ha, and leaves from plants receiving 112 and 168 kg N/ha had significantly higher reflectance than those from plants fertilized with 224 kg N/ha. Because the near-infrared region of the spectrum commences at about the 0.75-μm WL, differences in leaf reflecance at this WL may have been contributed to by differences in internal leaf structure (Gausman, 1974). Conversely, differences in leaf reflectance values among the five treatments at the five visible WL's (0.50 to 0.70 μm) were primarily influenced by leaf pigmentation (Thomas and Gausman, 1977).

The N treatments also affected the total chl concentration and N content of the leaves (Table 1). Leaves from buffelgrass plants fertilized with 112, 168, and 224 kg N/ha had significantly higher ($p = 0.05$) total chl concentrations than those from plants fertilized with 56 kg N/ha or those from nonfertilized plants. The chl concentration of leaves from plants fertilized with 168 kg N/ha was significantly higher than those from plants fertilized with either 112 or 224 kg N/ha. Leaves from plants fertilized with 112, 168, and 224 kg N/ha had significantly higher levels of N than those from plants fertilized with 56 kg N/ha or nonfertilized plants.

Single leaf reflectance values for the 0.50- to 0.70-μm WL's were inversely related to leaf chl concentration and N content of buffelgrass, whereas reflectance values at the 0.75-μm WL were directly related to these parameters (Table 2). These relations were best described by an asymptotic regression model similar to that used by Thomas and Oerther (1972) for leaves from greenhouse-grown sweet peppers. Thomas and Gausman (1977) have shown the 0.55-μm WL to be superior for individually relating leaf pigments, particularly chl, to leaf reflectance. The correlations of chl and N with leaf reflectance for the six WL's studied indicated that the 0.50- and 0.55-μm WL's were best for this study. The coefficients of determination (r^2) were highly significant for 10 out of 12 regressions. Thus, it ap-

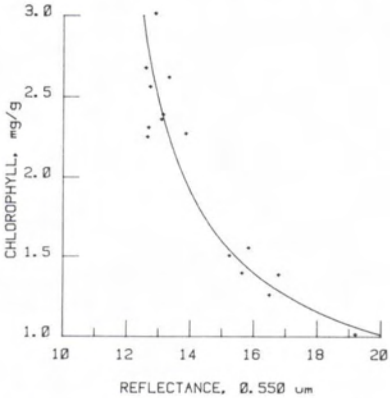

FIG. 1. Agreement of asymptotic regression model (solid line) to the relation between single leaf reflectance (R) of buffelgrass and chlorophyll (chl) concentration (+). Equation of regression model is

$$\frac{1}{\text{chl}} = 2.08 - \frac{21.8}{R},\ r^2 = 0.931.$$

pears that leaf reflectance is significantly related to leaf total chl concentration and N content.

Figure 1 shows the inverse correlation of chl to reflectance at the 0.55-μm WL. There is a 7.5 percent range in reflectance (12.5 to 20.0 percent) for a 2 mg/g range of chl (1 to 3 mg/g) that equates to a 0.27 mg/g per 1 percent chl to reflectance sensitivity. This significant chl to reflectance sensitivity in the visible band has been detected in field studies, such as Richardson (1984), between maturing and senescing cotton (*Gossypium hirsutum* L.) canopies using hand held radiometers. Because buffelgrass is structurally a more uniform growing plant than cotton, the sensitivity effect between chl and reflectance should be easier to detect in the field.

The reflectance sensitivity to chl concentration in leaves has also been demonstrated by single-leaf laboratory reflectance measurements of cotton (Gausman *et al.*, 1969-1970). Single-leaf near-infrared reflectance was directly related and visible reflectance was inversely related to increased chl content in the leaves of Cycocel-treated cotton plants. (Cycocel is a chemical plant growth regulator.) Moreover, Cycocel-treated plants (higher leaf chl content) in the field were darker red on CIR aerial photographs than were non-treated plants (lower leaf chl content). Thus, it appears that both leaf and canopy reflectance are sensitive to different chl concentrations in plant leaves.

Data presented here indicated that leaf reflectance measurements should be useful to estimate the N content of grasses. This information may be helpful to range resource managers in judging when to apply N during the growing season.

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