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Plant Size, etc., and Aerial Films*

Chlorophyll concentration of chlorotic leaves, expressed as milligrams of chlorophyll per cubic centimeter of leaf volume, was positively correlated to film density.

Abstract: *Sites with normal and chlorotic sorghum* (Sorghum bicolor (L.) Moench) *plants were aerially photographed at an altitude of 152 m on Kodak infrared color film. Field and laboratory measurements were studied to determine the relation of plant size (total leaf area and volume and leaf area index) and chlorophyll concentration to film response. Leaf reflectance was measured in the laboratory with a spectrophotometer, and canopy reflectance was measured in the field with a spectroradiometer. Normal plants had larger total leaf areas and volumes and leaf area indexes than chlorotic plants and 6 to 10 times larger chlorophyll concentrations. Chlorotic leaves had deformed cellular structure and higher reflectance in the visible region compared with normal leaves. Chlorophyll concentration of chlorotic leaves, expressed as milligrams of chlorophyll per cubic centimeter of leaf volume, was positively correlated to film density readings. Images on the film became darker as chlorophyll concentration increased. Plant size was not related to density readings.*

Introduction

Grain sorghum (*Sorghum bicolor* (L.) Moench) is one of the annual crops most sensitive to iron deficiency (Krantz and Melsted, 1964). The deficiency symptom is easily identified; leaves are yellow with dark-green veins (Amador, *et al.*, 1970). The interveinal chlorosis (striping) extends the full length of blades. Sometimes leaves are yellow with white tips. If iron deficiency is severe, plants become white and die.

The correlation of leaf area index (LAI) for sorghum with grain yield may be poorer for iron-deficient plants than for normal plants. Leaf area index is defined as area of leaf per unit area land surface. Chlorophyll content may be a better indicator of yield potential than LAI because of its relation with the photosynthetic capacity of plants.

The objectives of this study were (1) to determine the effect of iron deficiency (chlorosis) on the spectral response of sorghum and (2) to measure the physical and chemical properties that would account for differences in spectral response.

Materials and Methods

Two 16.5- by 24.4-m areas were selected within a commercial grain sorghum (*Sorghum bicolor* (L). Moench) field near Linn, Texas. One area had green appearing (normal) sorghum plants, and the other area had chlorotic (yellowish, iron deficient) sorghum plants. Sorghum plants were in the pre-boot stage of growth. The iron deficiency was moderately severe because essentially all leaves of affected plants were yellow. Each area was subdivided into 12 plots, each 5.5 by 6.1 m. Sorghum rows were 0.9 m apart. Plant populations were approximately equivalent to

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36,500 and 48,200 plants/ha for chlorotic and normal sorghum plots, respectively. One plant was randomly selected within each plot for chlorophyll assay, LAI determination, and leaf size and spectrophotometric measurements.

The two areas were photographed from an aircraft on Kodak Aerochrome Infrared (AIR) film* type 2443, 9½-inch format, at 152 m. A Zeiss RMK-A-15/23 camera was used with KLF_{36} (glass and anti-vignetting plates) and a 15 G (yellow) filter. Infrared film was used because it is sensitive to infrared light reflected by vegetation, and chlorotic plants reflect less infrared light than normal plants. The exposure was 1/225 sec at $f/5.6$ with a scale of 1:1000. After the aerial photography was completed, the leaf area of one plant randomly selected from each plot was determined by the method of Slickter, *et al.* (1961). Then plants were pulled from the soil, wrapped immediately in plastic wrap, stored on ice to minimize moisture loss, and transported to the laboratory. In the laboratory (1 hr. after plant removal from the soil), six leaves (nodes 4 to 9) were excised from the plants for physical measurements and water content and chlorophyll determinations. (A node is the part of the stem at which a leaf is attached.) Leaves from nodes 1 to 3 were not used because they were dehydrated.

A Beckman Model DK-2A spectrophotometer, equipped with a reflectance attachment, was used to measure total diffuse reflectance and transmittance over the 500- to 2,500-nm wavelength interval (WLI) on upper (adaxial) surfaces of leaves from the eighth node. Data were corrected to give absolute radiometric values (Allen and Richardson, 1971), and absorptance was calculated as: *Absorptance = 1 − [reflectance + transmittance].*

A ground based, Exotech Model 20 Spectroradiometer described by Leamer, *et al.* (1973) was used to measure reflected radiation from chlorotic and normal plants in the field over the 500- to 740-nm WLI. Measurements were made 6.1 to 7.6 m above the plants, with a 15° field of view.

Tissue pieces, taken near the center of leaves (node 8) approximately one-half inch on either side of the midrib, were fixed in formalin-acetic acid-alcohol (FAA), dehydrated with a tertiary butyl alcohol series, embedded in paraffin, stained with the safranin-fast green combination (Jensen, 1962) and transversally microtomed at 14-μm thicknesses. The relatively thick transections were used to accentuate the intercellular spaces. Photomicrographs were obtained with a Zeiss Standard Universal Photomicroscope.

Leaves from each plant (nodes 4 to 9) were minced, composited, and thoroughly mixed. Two-gram samples were used for water content determinations, and two-gram samples were stored in a freezer at $-15 \pm 5°C$ for chlorophyll analyses. Water content of leaves was determined on a dry-weight basis *(fresh weight − oven dry weight/fresh weight)*; leaves were oven-dried at 68°C for 48 hr. and cooled in a desiccator before weighing. Leaf thickness was determined with a linear displacement transducer and digital voltmeter (Heilman, *et al.*, 1968). Total chlorophyll content of leaves was determined by the method of Horwitz (1965) on leaf samples stored in a freezer at −15° for 65 days.

Optical count readings were made on aerial infrared (AIR) film with a Joyce, Loebl automatic recording microdensitometer using tungsten light (no filter), and red (Wratten 92; 615 to 700 nm), green (Wratten 93; 510 to 585 nm), and blue (Wratten 94; 410 to 495 nm) bandpass filters in the light beam. The microdensitometer output is in optical counts (reciprocal of transmission) that are punched onto paper tape (Cardenas, *et al.*, 1971). The optical count is related to optical density (O.D.) by the relation:

O.D. = [(Optical counts − base reading) (wedge factor)] + (step wedge density)

The paper tape output consists of a base-line count plus added counts that depend on the position of a graduated step wedge after it travels a sufficient distance to balance the intensity of the light beam coming through itself with the intensity of the light beam coming through the film. The base-line count is manually set to correspond to the standard optical density of the first step of a calibrated step wedge. One row of each sorghum plot was used for obtaining optical count readings. The rows were solid with foliage. One scan line was run for each plot. There were 32 readings (data bits) for each scan line on the film. The area of a data bit was about 0.1 mm^2.

Data, except correlation coefficients, are given as means of 12 plants. Data were subjected to conventional statistical analyses (Snedecor, 1956).

* Trade and company names are used for the convenience of the reader and do not imply endorsement or preferential treatment by the U.S. Department of Agriculture.

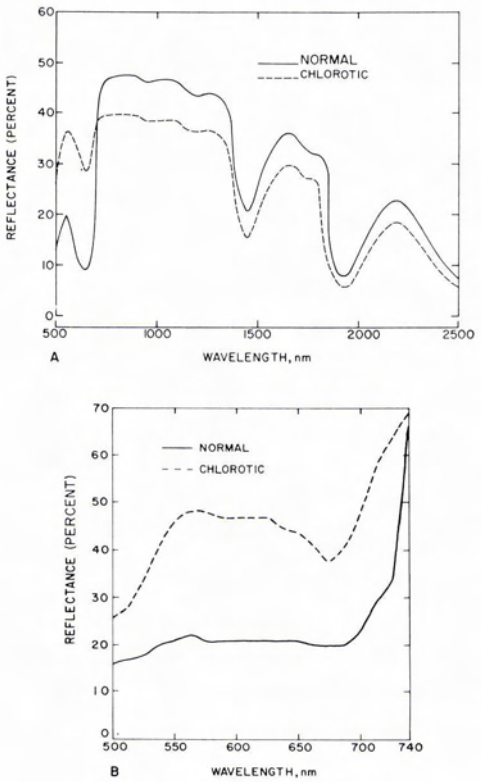

FIG. 1. Effect of normal and chlorotic sorghum leaves on (*top*) reflectance spectrophotometrically measured in the laboratory over the 500- to 2,500-nm wavelength interval; and on (*bottom*) reflectance of normal and chlorotic canopies measured in the field with a spectroradiometer over the 500- to 740-nm wavelength interval.

RESULTS AND DISCUSSION

Figure 1A shows the reflectance spectra of normal and chlorotic sorghum leaves. Chlorotic leaves had significantly higher (p .01) reflectance (36.1 percent at 550 nm) in the visible WLI (500 to 750 nm) than normal leaves (19.6 percent at 550 nm), but chlorotic leaves had significantly lower (p .01) reflectance (38.5 percent at 1,000 nm) than normal leaves (46.4 percent at 1,000 nm) over the 750- to 2,500-nm WLI. Differences in absorptance and transmittance between normal and chlorotic leaves were also statistically significant (p .01). Transmittance spectra (not shown) were the same shape as reflectance spectra. The transmittance of chlorotic leaves at the 550- and 1,000-nm wavelengths (49.3 and 58.8 percent, respectively) was higher than normal leaves at the 550- and 1,000-nm wavelengths (5.8 and 50.6 percent, respectively). Differences in absorptance between normal and chlorotic leaves were mainly within the 500- to 750-nm WLI. Absorptances of normal and chlorotic leaves were 74.6 and 14.6 percent, respectively, at the 550-nm wavelength.

Figure 1B shows results of reflectance measurements made in the field with an Exotech Model 20 spectroradiometer. Reflectance was much higher for chlorotic compared with normal sorghum plants over the 500- to 740-nm WLI. The greatest difference of 26 percent was near the 550-nm wavelength.

Figure 2 shows transections of normal (upper photo) and chlorotic (lower photo) sorghum leaves from node 8. Chlorotic leaves (.015 cm) were significantly thinner at the 1 percent probability level than normal leaves (.019 cm). Chlorotic leaves were more involuted (rolled inward) with smaller epidermal and bulliform cells (enlarged cells in the upper epidermis) than normal leaves. Bundle sheaths in both normal and chlorotic leaves appear typical. The mesophyll is more finely divided (more intercellular air spaces) for normal than for chlorotic leaves. This is confirmed by the higher near-infrared reflectance (750 to 1,350 nm) of normal compared with

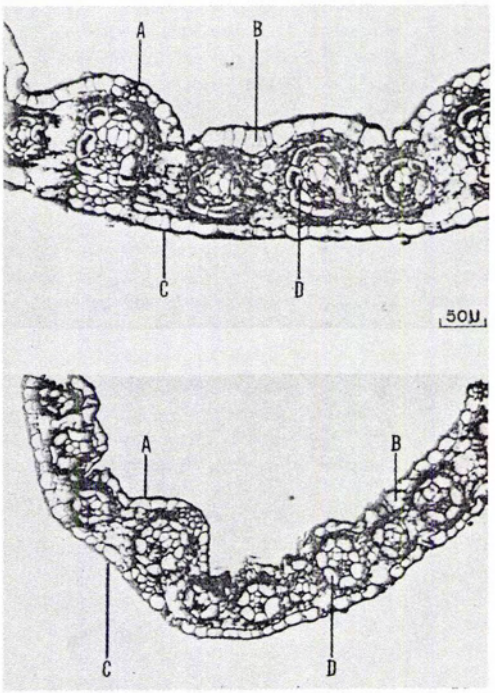

FIG. 2. Transections of normal (upper) and chlorotic (lower) sorghum leaves. Details: *A*, upper epidermis; *B*, bulliform cells; *C*, lower epidermis; *D*, bundle sheath.

TABLE 1. OPTICAL COUNT MEASUREMENTS MADE ON POSITIVE AIR FILM TRANSPARENCIES WITH NO FILTER AND BLUE, GREEN, AND RED FILTERS; AND CHLOROPHYLL CONCENTRATIONS AND PHYSICAL MEASUREMENTS OF NORMAL AND CHLOROTIC SORGHUM PLANTS

	Normal plants	*Chlorotic plants*	*Difference*
Optical counts with:			
Blue filter	87.4	43.1	44.3*
Green filter	124.2	67.3	56.9*
Red filter	130.7	89.6	41.1*
No filter	112.2	57.0	55.2*
Chlorophyll in leaf tissue:			
mg/g	13.44	2.31	11.13*
Total plant, mg	128.19	13.11	115.08*
mg/cm^2	.091	.012	.079*
mg/cm^3	4.96	.77	4.19*
Physical measurements:			
Total leaf area, cm^2	1415.7	1105.8	309.9*
Total leaf volume, cm^3	26.0	16.9	9.1*
Leaf area index (LAI)	4.2	2.5	1.7*

* Significant at 1% probability level.

chlorotic leaves, Figure 1A. Leaves with many air spaces have higher near-infrared reflectance than leaves with few air spaces (Gausman *et al.*, 1970).

Table 1 gives optical counts from densitometric measurements made on an AIR positive transparency (plate 1) with no filter and with blue, green, and red filters. In every instance, optical counts were significantly higher (p .01) for normal plants compared with chlorotic plants. However, the largest numerical difference in optical counts between normal and chlorotic plants occurred with the green filter. Hence, data for the green filter were selected for the correlation studies that are considered later.

Table 1 also presents chlorophyll analyses for normal and chlorotic plants expressed as mg of chlorophyll per g of plant tissue, chlorophyll content of the total plant in mg, mg of chlorophyll per cm^2 of leaf area, and mg of chlorophyll per cm^3 of leaf volume. Chlorophyll concentrations were 6 to 10 times higher (significant, p .01) in normal than in chlorotic tissue.

As shown in Table 1, chlorotic plants were significantly smaller (p .01) in total leaf area (1415.7 vs. 1105.8 cm^2) and leaf volume (26.0 vs. 16.9 cm^3) than normal plants. Leaf area indexes were 4.2 and 2.5 for normal and chlorotic plants, respectively.

Table 2 gives the coefficients for correlations of optical count measurements with chlorophyll assays and with physical measurements. A significant coefficient ($r = 0.583$) was obtained for the correlation of optical

TABLE 2. COEFFICIENTS FOR CORRELATIONS OF OPTICAL COUNT READINGS (GREEN FILTER) ON AIR POSITIVE TRANSPARENCIES WITH CHLOROPHYLL CONCENTRATIONS AND PHYSICAL MEASUREMENTS OF NORMAL AND CHLOROTIC SORGHUM PLANTS

	Normal plants	*Chlorotic plants*
Chlorophyll in leaf tissue:		
mg/g	.422	.360
Total plant	.239	.398
mg/cm^2	.236	.565
mg/cm^3	.422	.583*
Physical measurements:		
Total leaf area, cm^2	.101	−.125
Total leaf volume, cm^3	−.050	.053
Leaf area index (LAI)	.101	−.127

* Significant at 5% probability level.

count readings with chlorophyll concentration of chlorotic plants expressed as mg of chlorophyll per cm^3 of leaf volume. Reflectance has a curvilinear relation with visible light reflectance (Thomas and Oerther, 1972). Above a certain chlorophyll concentration, reflectance changes very little as chlorophyll concentration increases. Below a certain chlorophyll concentration, small changes in chlorophyll concentration may cause large changes in light reflectance. Light reflectance, in turn, affects the optical density of photographic film. Subsequently, normal sorghum with high levels of chlorophyll would be expected to have a poor relation, and chlorotic sorghum with low levels of chlorophyll would have a

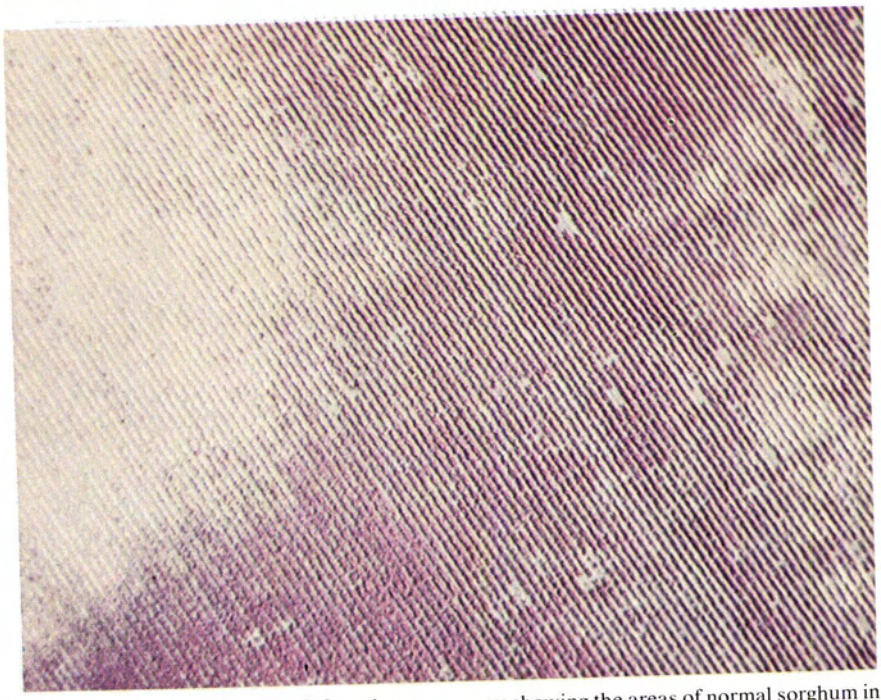

Plate 1. A positive print of an infrared transparency showing the areas of normal sorghum in the center (magenta) and iron-deficient sorghum on the left (white).

good relation with film density measurements. Correlation coefficients for the relation of optical count readings with physical measurements were very low.

Conclusions

Normal plants had larger total leaf areas and volumes and LAI's than chlorotic plants, and 6 to 10 times larger chlorophyll concentrations. Chlorotic leaves had deformed cellular structure and higher reflectance in the visible region compared with normal leaves.

Chlorophyll concentration of chlorotic leaves, expressed as mg of chlorophyll per cm^3 of leaf volume, was positively correlated to film density readings. Images on the film became darker as chlorophyll concentration increased. Plant size was not related to density readings.

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