Variations in the Polarized Leaf Reflectance of *Sorghum bicolor**

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The polarized reflectance factor R_q of sorghum (*Sorghum bicolor, L.*) leaves from field-grown plants was measured *in situ* in the summers of 1983 and 1984. In 1983, three leaves of two randomly selected plants were measured at 2-week intervals. The value of R_q varied, depending on leaf and day of measurement. Measured values of R_q for the adaxial leaf surface ranged from 16 to 53; for the abaxial leaf surface the values ranged from 28 to 69. In 1984, measurements consisted of repeated observations made on the same leaf at biweekly intervals. The values of R_q from the adaxial leaf surface ranged from 26 to 38. Values of R_q from the abaxial leaf surface increased throughout the season, from 16 to 45. Differences in R_q were attributed to changes in surface details of the leaf.

Introduction

Polarized reflectance from a leaf is a surface phenomenon emanating from light scattered at the air–cuticle interface, the first refractive discontinuity encountered by incident radiation. Most previous studies of leaf reflectance have measured total reflectance, which includes both the reflectance from the surface and the reflectance from the internal structure of the leaf. Measuring the polarized leaf reflectance allows separation of the surface component of reflectance from light reflected from the bulk of the leaf tissue. Two light scattering mechanisms can contribute to this polarized reflectance. These are specular reflectance and particle scattering.

The relative contribution of these mechanisms to reflectance will depend on the dimensions of surface features relative to incident wavelengths (Fung, 1983). Large-scale features of the leaf surface, those with dimensions much greater than the wavelength of incident light, reflect light specularly. The angle of specular reflection is equal to the angle of incidence. When the angle of incidence is the Brewster angle, the specularly reflected light is completely polarized; at other angles of incidence, the reflected flux is partially polarized. Surface features with dimensions comparable or smaller than the wavelengths of incident light scatter light. The reflected flux scattered by particles of comparable dimensions to incident wavelengths will be partially polarized and randomly directed. Particles smaller than the wavelengths of incident light preferentially scatter shorter wavelengths; if sparsely distributed, these particles may act as Rayleigh scatterers. The reflected flux directed at 90° to the incident angle will be completely polarized and, at other angles, partially polarized. If small particles are densely distributed, the criteria for Rayleigh

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scattering may not be met; in such a case the reflected flux will still be enhanced in the blue but nonpolarized.

The surfaces of leaves are rough, containing features varying in scale relative to the incident wavelengths. When a rough surface contains both features which are large and small relative to the incident wavelengths, the large-scale roughness dominates scattering in the specular direction, and the small-scale roughness controls scattering away from the specular direction. As a result, the polarized reflectance measured at any one angle is largely dependent upon the features of the leaf which create surface roughness.

Leaves are covered with an extracellular, protective layer called the cuticle whose outermost wax layer provides surface detail on a microscopic and optical level. The morphology of the epicuticular wax may be amorphous, semicrystalline, or crystalline. Details of the epicuticular wax range in optical size from crystalline particles with dimensions less than the wavelengths of sunlight to areas of amorphous wax with dimensions far greater than these wavelengths.

The wax morphology, the shape and size of the epicuticular wax, is dependent on the chemical composition of the wax (Hallam, 1982), which is genetically determined and appears to be characteristic of specific organs of individual species. The chemical composition (Tulloch, 1973; Atkin and Hamilton, 1982a), and, consequently, the wax morphology (Atkin and Hamilton, 1982b); Baker, 1982) have been observed to vary during the ontogeny of plant tissue. The chemical composition and wax morphology can be modified by the prevailing environmental conditions, i.e., radiant energy, temperature, relative humidity, and soil moisture during the period of wax deposition (Baker, 1982). The variations in wax morphology are manifest primarily in variation in wax deposition, particularly in the size and distribution of wax particles. Once the epicuticular wax is deposited, the wax layer may be modified further by disturbances of the leaf surface, i.e., abrasion by wind and rubbing, deposition of atmospheric particulates, disease infestations and insect damage (Juniper and Jeffree, 1983). These factors may effect the distribution of wax particles and contribute their particular optical properties to the features of the leaf surface. Thus the surface detail of the leaf is dynamic, changing in time with age of the leaf and environmental conditions.

Since the features of the leaf surface change with age and conditions under which the plant is grown, the polarized reflectance from the surface may be expected to change concomitantly. Thus variation in the polarized reflectance from leaves is expected among leaves and throughout the growing season.

In this paper the variation in polarized reflectance from leaves of *Sorghum bicolor*, *L.* is studied. Sorghum leaves have been reported to be strong specular reflectors (Grant et al., 1983); and the epicuticular waxes which create a glaucous "bloom" on the stem and leaf surfaces have been studied (Atkin and Hamilton, 1982a,b). This epicuticular wax load is reported to vary with environmental conditions (Jordan et al., 1983). Thus this species appears to be an ideal specimen for polarized reflectance studies.

Methods and Materials

The polarized reflectance from leaves of *Sorghum bicolor*, *L.* planted at the Purdue Agronomy Farm, West Lafayette,

TABLE 1 Polarized reflectance R_q from the Adaxial Surface of *Sorghum Bicoloar* Leaves Measured at the Brewster Angle

Leaf	Date						
No.	25 July	8 Aug.	22 Aug.	5 Sep.	19 Sep.	29 Sep.	Mean
7	24 A[a]						24
8	30 Aa	18 Aa					24
9	53 Ba	27 Bb	16 Ac				28
10		31 Ba	33 Ba	53 Ab			38
11			27 Ba	39 Bb	39 Ab		34
12				53 Aa	39 Ab	24 Ac	37
13					38 Aa	37 Ba	38
14						35 B	35
Mean	35	24	26	48	39	33	

[a] Values represent the mean of four observations on two leaves. Within columns, means followed by the same uppercase letters are not significantly different using Duncan's Multiple Range Test, $\alpha = 0.05$. Within rows, means followed by the same lowercase letters are not significantly different using Duncan's Multiple Range Test α, $\alpha = 0.05$.

IN was measured in the summers of 1983 and 1984. In 1983, hybrid grain sorghum, Savannah V (Northrup-King) was planted on 29 June. Three weeks after emergence, measurements of leaf reflectance were begun. On 25 July, two measurements of the polarized leaf reflectance from both the adaxial and abaxial surfaces of leaves 7,8,9 from two plants were made. At biweekly intervals, subsequent measurements of polarized reflectance were made on the leaves of two randomly selected plants (Table 1). Final measurements of polarized reflectance were taken only from the adaxial surfaces of leaves 13, 14, and 15 on 29 September. This day followed a light frost. The effect of the frost on sorghum was apparent in the bronzing of the sorghum foliage.

In 1984, hybrid grain sorghum, Savannah III (Northrup-King) was planted on 28 June. Approximately 3 weeks following emergence, on 25 July, two measurements of polarized leaf reflectance from both the adaxial and abaxial surfaces were made on leaf 8, the most recently expanded leaf, of four plants. Subsequent measurements of polarized leaf reflectance were taken on the same leaves on a bi-weekly basis. Final measurements of polarized leaf reflectance were made on 23 August.

Reflectance measurements were taken with a portable polarization photometer (Vanderbilt and Grant, 1986), which allows nondestructive measurements of leaf reflectance at an approximation of the Brewster angle (55° from normal) in six wavelength bands. These wavelength bands include five wavelengths in the visible and near-infrared regions of the spectrum centered at 450, 500, 550, 650, and 730 nm with half-power bandwidth of 70 nm, and a sixth wavelength band which includes the visible wavelength region (450–700 nm).

The measure of reflectance of each sample, V_{sample}, was calibrated to 1) a painted $BaSO_4$ standard V_{BaSO_4} of known reflectance R_{BaSO_4} and 2) the dark level of the instrument, V_{dark}. The bidirectional reflectance factor of the sample, the ratio of the flux from the sample to the flux from an ideal, white, diffuse surface similarly illuminated and viewed, is then

$$R = \left[\left(V_{sample} - V_{dark}\right) / \left(V_{BaSO_4} - V_{dark}\right)\right] \times R_{BaSO_4} \times 100$$

Each observation consisted of a pair of measured reflectance factors, R_{max} and R_{min}, representing, respectively, the maximum and minimum amount of radiation transmitted by the polarization analyzer. From theses calculated values, the variable R_q was calculated:

$$R_q = (R_{max} - R_{min})/2.0,$$

where R_q represents the polarized component of the reflectance factor.

Results and Discussion

The results of the measurements of polarized leaf reflectance clearly show there is no one constant value for the polarized reflectance of *Sorghum bicolor*. The value of polarized reflectance varied, depending upon the leaf and from day to day.

The results of the measurements of polarized reflectance R_q, measured in 1983, from the adaxial and abaxial surfaces of *Sorghum bicolor* are presented in Tables 1 and 2, respectively. R_q from both the adaxial and abaxial surfaces varied with the day on which the measurements were made. These differences in the values of polarized reflectance cannot be attributed to variations in the measurements system since all measurements were calibrated with a painted $BaSO_4$ reference surface. Tables 1 and 2 also show that on any one day throughout the growing season there may be variation in the polarized reflectance among leaves.

The epicuticular waxes of leaves of *Sorghum bicolor* change with the ontogenic development of the plant. Atkin and Hamilton (1982b) used a scanning electron microscope to examine the surfaces of leaves of two varieties of *Sorghum bicolor* grown under controlled environmental conditions. Leaves were observed 14, 28, and 52 days following emergence; at these times, the development of the plants corresponded to Vanderlip's development stages 1 (4 leaves), 2 (8 leaves), and 3 (12 leaves), respectively (Atkin and Hamilton, 1982a). The adaxial surface of both varieties had clusters of wax flakes, 100–160 nm thick and 210–1580 nm in diameter, when ob-

TABLE 2 Polarized Reflectance R_q from the Abaxial Surface of *Sorghum Bicolor* Leaves Measured at the Brewster Angle

Leaf No.	Date: 25 July	4 Aug.	22 Aug.	5 Sep.	19 Sep.	Mean
7	42 A[a]					42
8	38 Aa	28 ABb				33
9	65 Ba	38 Ab	47 Ab			49
10		18 Ba	54 Ab	40 Ac		41
11			69 Ba	44 Ab	44 Ab	52
12				66 Ba	33 Bb	50
13					47 A	47
Mean	46	28	56	49	42	

[a] Values represent the mean of four observations on two leaves. Within columns, means followed by the same uppercase letter are not significantly different using Duncan's Multiple Range Test, $\alpha = 0.05$. Within rows, means followed by the same lowercase letter are not significantly different using Duncan's Multiple Range Test, $\alpha = 0.05$.

served 14 days following emergence. One variety retained these wax particles throughout the study. The other variety had lost the clusters of wax flakes by the 28th day. The abaxial surface contained wax filiaments, 500–1250 nm in diameter and 14,000 nm long. For one variety, these wax filiaments appeared first on the 28-day-old sample, but were not present on young leaves of 52-day-old plants.

Surface features, in particular, the size and distribution of epicuticular waxes, can be modified by the prevailing environmental conditions at the time of wax deposition (Baker, 1982). Since leaves formed at different stages in the development of the plant are subjected to differing environmental conditions, i.e., light intensity, temperature, relative humidity, and soil moisture when grown under natural conditions, variation in the distribution and size of epicuticular wax particles among leaves is expected.

Leaves differing in age are subjected to differing environmental effects such as wind, rain, and dust during their lifetime. Their variation in life history may be manifest by different patterns of abrasion on the surface of the leaves.

This discussion of the differences in surface details among leaves and possible changes in surface details created by the changing environment give reasonable expectations that the surfaces of the measured leaves will vary with leaf age. Since the polarized reflectance from the leaves is dependent on characteristics of the leaf surface, the measured differences in R_q may be attributed to variation in the surface features of the sorghum leaves.

The results of the repeated measurements of the polarized reflectance throughout the life of the eighth leaf are contained in Table 3. The polarized reflectance R_q of leaf 8 changed throughout the study. R_q from the adaxial surface increased in the 2-week period between the initial measurement and the second measurement. Subsequent measurements of R_q from the adaxial surface demonstrated a decrease in the polarized reflectance. R_q from the abaxial surface increased with each repeated measurement.

These differences in the measured values of R_q cannot be attributed to variation in the measurement system since all measurements were calibrated with a painted $BaSO_4$ reference surface. However, since repeated measurements were made on the same leaves throughout the study, the possibility that artifacts were introduced by the repeated measurements cannot be discounted.

The polarization photometer is designed to measure reflectance in the specular direction. An increase in the value of

TABLE 3 Polarized Reflectance R_q from the Surfaces of the Eighth Leaf of *Sorghum Bicolor* Measured at the Brewster Angle

Date	Adaxial Surface	Abaxial Surface
25 July	38 a[a]	16 a
31 July	52 b	21 b
8 August	32 ac	34 c
23 August	26 c	45 d

[a] Values represent the mean of eight observations on four leaves. Within columns, means followed by the same letters are not significantly different using Duncan's Multiple Range Test, $\alpha = 0.05$.

R_q from one measurement period to the next suggests an increase in the area or number of large-scale features capable of specularly reflecting light. Such an increase also could occur if small, light-scattering, epicuticular wax particles were removed from the leaf surface. This would be similar to the observed appearance of a shiny surface when the wax bloom of the sorghum leaf sheath is wiped. A less drastic but similar disturbance of the wax filiaments of the abaxial surface of the sorghum leaf may occur each time the leaf is positioned in the polarization photometer. Such a disturbance would account for the increase in the value of R_q with each subsequent measurement.

Changes in the value of R_q from the adaxial surface are not so readily assigned to artifactual disturbance by the measurement process. The values of R_q from the adaxial surface of *Sorghum bicolor* increased in the 2-week period between the initial measurement of polarized reflectance and the second measurement. This increase suggests an increase in the area or number of large-scale features capable of specularly reflecting light. Such an increase could occur if clusters of wax particles, similar to those observed by Atkin and Hamilton (1982b), were removed from the leaf surface. Wax particles might be removed by the measurement process or by the abrasive action of wind and rain.

Subsequent measurements of the polarized reflectance from the adaxial surface declined in value. This decrease in the value of R_q suggests that the adaxial surface of the sorghum leaves became rougher, with fewer areas of large-scale features capable of specularly reflecting light. This could occur with the deposition of dust particles on the leaf surface. The presence of such small-sized particles would decrease the area capable of specularly reflecting light and increase the scattering of light away from the specular direction.

Conclusions

In summary, the polarized reflectance from the leaves of *Sorghum bicolor* is not a constant value. Polarized reflectance varies, among leaves of the plant canopy and throughout the growing season. These differences in the polarized reflectance of leaves may be attributed to variations in the details of the features of the leaf surface.

These findings suggest that care should be taken in interpreting changes or variations in total reflectance. Such changes may reflect disturbances of the leaf surface or may be manifestations of physiological perturbations within the internal leaf structure. Total reflectance measurements alone cannot separate the source of reflectance into separate physical entities. Polarized reflectance measurements can; thus measurements of polarized reflectance should allow greater confidence in assigning causal effects to changes in leaf reflectance.

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