Effect of Rotylenchulus reniformis on Reflectance of Cotton Plant Leaves[1]

H. W. GAUSMAN, C. M. HEALD, JR., and D. E. ESCOBAR[2]

Abstract: Differences between light reflectance from leaves of cotton (*Gossypium hirsutum*) plants grown with a low- or no-nematode (*Rotylenchulus reniformis*) population (nonstressed), and from leaves grown with a high nematode population (stressed) were measured in field and greenhouse experiments. Reflectance was measured spectrophotometrically in the laboratory on single leaves and spectroradiometrically in the field on plant canopies. Nematode-stressed cotton plants were stunted with fewer, smaller, and darker-green leaves than nonstressed plants. Over the 0.5- to 2.5-μm waveband, stressed leaves had lower reflectance than nonstressed leaves of the same chronological age for both field- and greenhouse-grown plants. Reflectance differences between stressed and nonstressed leaves in the visible (0.5 to 0.75 μm), near-infrared (0.75 to 1.35 μm) and infrared water absorption (1.35 to 2.5 μm) regions were primarily caused by differences in leaf chlorophyll concentration, mesophyll structure, and water content, respectively. Results indicate the potential for remotely sensing nematode-infested plants to distinguish them from normal plants. *Key words:* stressed and nonstressed leaves, remote sensing, chlorophyll content.

Interpretation of remotely sensed data from aircraft and spacecraft requires an understanding of reflectance of features on the earth's surface (20, 21). In agriculture, the specific problem is interpreting reflectance produced by vegetation, usually superimposed on a soil background (1). Plant leaves often yield most of the signal measured by remote sensors in aircraft and spacecraft. Therefore, they are of prime interest in characterizing vegetation, and their interaction with electromagnetic radiation must be understood.

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[2]Plant Physiologist, Nematologist, and Biological Technician, respectively, U.S. Department of Agriculture, Weslaco, TX 78596.

Reflectance and transmittance of a plant leaf have been explained on the basis of critical reflection of light at the cell wall-air interface of the spongy mesophyll tissue (4, 9, 12, 13, 19, 22, 23). Sinclair et al. (17) hypothesized that leaf reflectance derives from the diffuse characteristics of plant cell walls. Light reflectance from a leaf is generally reduced over all wavelengths when the leaf is infiltrated with water (15, 16) or an oil mixture (5, 23). Most of the reflectance, therefore, originates internally and is reduced when the cell wall-air interfaces are eliminated. However, reflectance at the 0.68- and 1.95-μm wavelengths is relatively unchanged by infiltrations, so most of it must originate from the cuticle or leaf surface (5, 23). Internal refractive index discontinuities other than cell wall-air interfaces are responsible for some of the near-infrared light (0.75- to 1.35-μm) reflected by a leaf (3, 17, 23).

The spectral reflectance, absorptance, and transmittance and the geometrical and optical parameters (void-area index, index of refraction, scattering coefficient, absorption coefficient, and infinite reflectance) have been determined for 11 plant genera (6) and for 20 crop plants (7). The dispersion curves (index of refraction plotted against wavelength) for all plants were quite similar. Experimental and theoretical determinations of thickness necessary to produce observed leaf absorption were in close agreement. At 1.65 μm, infinite reflectance was shown to be a function of the calculated thickness of the identical compact layers of which a leaf is assumed to be composed. In general, leaves with compact mesophylls had the lowest and leaves with porous mesophylls had the highest reflectance (6, 7). Results indicated that the mesophyll arrangement within leaves comprising plant canopies affected the magnitude of a signal reaching the detector of a remote sensor.

The purpose of this research was to determine if there were differences between the light reflectance for leaves of cotton plants grown with a low- or no-nematode population (nonstressed) and plant leaves grown with a high nematode population (stressed).

MATERIALS AND METHODS

Field experiment: Cotton, *Gossypium hirsutum* L., plants were selected from a nematicide test using two adjacent plots; one treated with a soil fumigant, 1,3-dichloropropene 1,2-dichloropropane (D-D), and the other nontreated. Each plot consisted of four rows 16.4 m long spaced 84 cm apart. The soil was a Hidalgo sandy clay loam naturally infested with the reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira. The fumigant was applied at the rate of 114 liters/ha with two chisels per row approximately 25 cm deep and 13 cm to either side of the row middle. Cotton seed (cultivar Stoneville 7A) were planted 15 March 1974, 6 days after fumigant was applied.

Soil was sampled for nematode analysis on 7 March (prefumigation count) and 14 May 1974; each sample consisted of 100 g, a composite of five samples taken from the middle two rows. Nematodes were separated from the soil by the Baermann funnel technique.

Soil fumigation reduced the nematode population from 800/100 g to 40/100 g soil as determined in the May count. Prefumigation March counts were 240 and 333 nematodes/100 g soil for nonfumigated and fumigated, respectively.

Day lengths during the 73 days from plant emergence to leaf collections were 13.2 to 13.6 h. Mean daily temperature and relative humidity ranged from 22.6 to 31.8 C and from 63.2 to 87.1%, respectively. The field was irrigated once with approximately 15 cm of water, and 11 cm of rain fell.

Greenhouse experiment: Thirty 15-cm diam metal pots were filled with 1,500 g of steam-sterilized Hidalgo sandy clay loam soil, and two Stoneville 7A cotton seeds were planted in each pot. Five days after emergence, plants were thinned to one plant per pot. Nematodes (*R. reniformis*) for inoculation were collected from infested cotton fields and increased on cowpea (*Vigna unguiculata* (L.) Walp. 'Blackeye') roots in the greenhouse. These nematodes were washed five times in sterile distilled water and 45,000 then added to each of 15 pots in 40 ml of water into four 0.6-cm diam holes (10 cm deep) equally spaced 2.5 cm from point of plant location. Leaves for spectral measurements were collected 41 days after planting, and 2 days later the pots were emptied and a composite sample of 100 g of soil was taken from each pot. Nematodes were separated from soil and counted as in the field test. Nematodes recovered from the greenhouse test averaged 2,833/100 g of soil with a range from 900 to 7,320.

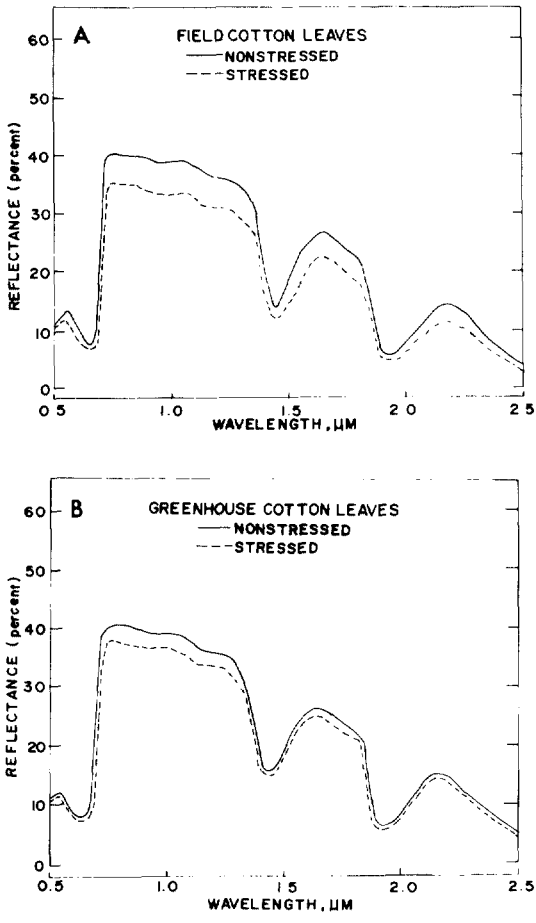

FIG. 1-(A,B). Cotton leaf reflectance spectrum changes in plants under stress from high populations of the reniform nematode, *Rotylenchulus reniformis*. Total diffuse light reflectance spectra over the 0.5- to 2.5-µm waveband of nonstressed and stressed cotton leaves from plants grown **A**) in the field, and **B**) in the greenhouse.

Leaf collection and measurements: For the field experiment, one leaf was sampled from each of 32 randomly selected plants for each treatment. Seven leaves were saved for chlorophyll analyses and 25 leaves were used for spectral measurements. When leaves became macroscopically visible, the dates were recorded, and as leaves became large enough, they were tagged. Thus, leaves of the same chronological age were used for both treatments. All leaves were 12 days old when spectral measurements were made. However, since leaves of stressed nonfumigated plants grew slower and were stunted, they were from different nodes than leaves of nonstressed fumigated plants. Nonstressed leaves were sampled from the 16th node (counting up from plant bottom); stressed leaves were sampled from the 13th and 14th nodes. Plant heights on leaf collecting day were 49.3 and 61.7 cm for stressed and nonstressed plants, respectively.

For the greenhouse experiment, leaves from both nonstressed (fumigated) and stressed (nonfumigated) plants were sampled from the 9th and 10th nodes. Leaves of the same chronological age (11 days old) were used for both treatments. Plant heights on leaf sampling day were 36.5 cm and 32.6 cm for the nonstressed and stressed plants, respectively. Only 14 leaves from each treatment were used for spectral measurements. Leaves were halved lengthwise; one half was used for spectrophotometric measurements, and the other half was used for chlorophyll analyses. Immediately after harvest, leaves were wrapped in polyethylene to minimize dehydration and then stored on ice.

Measurements of thickness, diffuse reflectance, transmittance, and fixation of tissue were completed within 7 h after leaves were collected. Leaf thickness was measured with a linear-displacement transducer and digital voltmeter (8). Leaf area was determined with a planimeter. Water content of leaves was determined on a dry-weight basis; leaves were oven-dried at 68 C for 48 h and cooled in a desiccator before weighing.

Tissue pieces sampled from the center of leaves were fixed in formalin-acetic acid-alcohol, dehydrated with a tertiary butanol series, embedded in paraffin, stained with the safranin fast-green combination, and transversally microtomed at 12-µm thickness (11).

A Beckman® Model DK-2A spectrophotometer, equipped with a reflectance attachment, was used to measure total diffuse reflectance on upper (adaxial) surfaces of single leaves over the 0.5- to 2.5-µm waveband. Data were corrected for decay of the barium sulfate standard (2) to give absolute radiometric data.

To reduce the enormous amount of spectrophotometrically generated data and facilitate interpretation, seven wavelengths were selected from the 41 wavelengths measured at 0.05-µm increments over the 0.50- to 2.50-µm waveband. Wavelengths selected were 0.55, 0.65, 0.85, 1.45, 1.65, 1.95, and 2.20 µm; representing, respectively, the green reflectance peak, chlorophyll absorption band, a wavelength on the near-

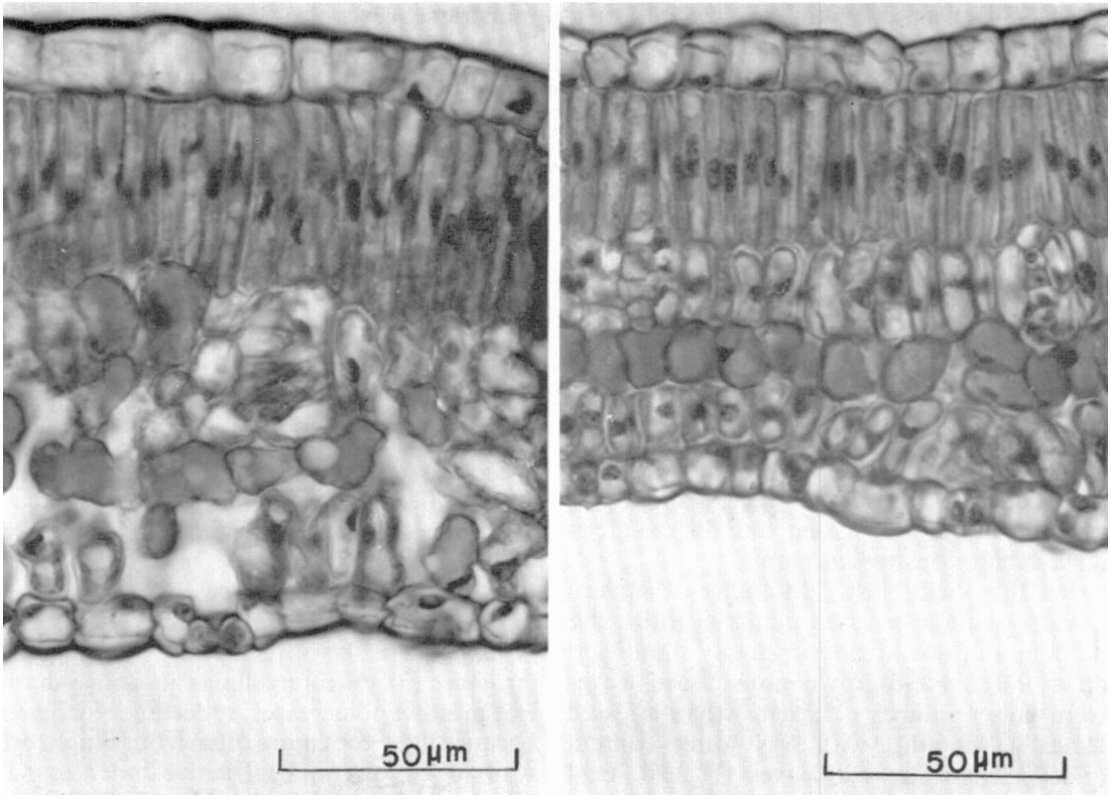

FIG. 2-(A, B). Cotton leaf structural changes in plants under stress from high populations of the reniform nematode, *Rotylenchulus reniformis*. Photomicrographs of internal structure of **A)** nonstressed, and **B)** stressed cotton leaves from plants grown in the field.

infrared plateau, the 1.45-μm water-absorption band, the 1.65-μm peak following the 1.45-μm water-absorption band, the 1.95-μm water-absorption band, and the 2.2-μm peak following the 1.95-μm water-absorption band.

The *t*-test (18) was used to test statistically the differences between means of stressed and nonstressed leaves for reflectance data at each of the seven wavelengths. For each treatment and wavelength, reflectances were averaged for 14 and 25 leaves for the greenhouse and field experiments, respectively. Total chlorophyll was determined by a routine method (10) on leaf samples stored 10 days at -15 C $\pm$ 5 C.

Spectroradiometric field measurements were made to support the spectrophotometrically measured greenhouse light reflectance results of plant leaves from the field and greenhouse experiments. Cotton plants (134 days old) from each of two other separate plots from the same nematode-infested field

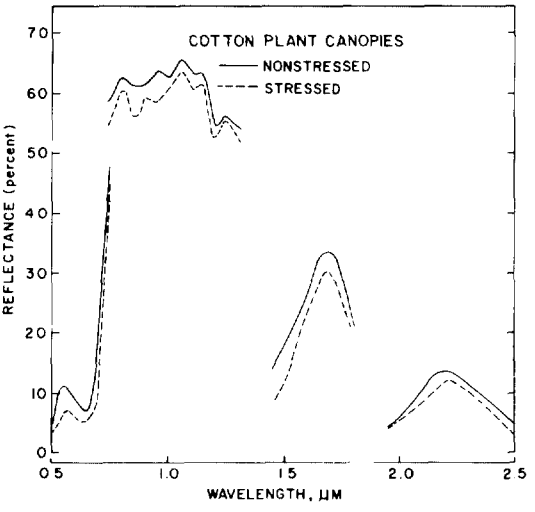

FIG. 3. Cotton field plant canopy reflectance spectra changes when plants are stressed by high populations of the reniform nematode, *Rotylenchulus reniformis*. Spectroradiometric reflectance spectra of single nonstressed and stressed field cotton plant canopies over the 0.5- to 2.5-μm waveband.

were selected for spectroradiometric measurements. An Exotech® Model 20 spectroradiometer (14) was used to measure reflected radiation from nonstressed and stressed single plant canopies over the 0.5- to 2.5-μm waveband. Measurements were made with sensors with a 15-degree field view (0.2 m^2) placed 1.5 m above each plant canopy. Nonstressed plants were 107.0-cm high and had more foliage with larger and lighter green leaves than the 81.0-cm high stressed plants. The foliage for both treatments was somewhat blemished and perforated by insects.

RESULTS AND DISCUSSION

Plant growth: Stressed cotton plants from the field and greenhouse experiments were essentially alike in appearance; plants were stunted with fewer, smaller, and darker-green leaves than nonstressed plants.

In the field test, differences between nonstressed and stressed plants in leaf water content and leaf area were highly significant ($P = 0.01$). Field-grown nonstressed leaves were larger in area (25.5 cm^2) than stressed leaves (17.3 cm^2) with less water content (77.5%) than stressed leaves (78.3%). Leaf thicknesses of nonstressed (.16 mm) and stressed (.15 mm) leaves were statistically alike.

For the greenhouse plants, the difference between stressed and nonstressed leaves for water content, leaf thickness, and leaf area was not significant. Leaf water content, thickness, and area were 70.1%, 0.18 mm, and 25.4 cm^2 for nonstressed leaves and 73.1%, 0.18 mm, and 22.5 cm^2 for stressed leaves, respectively.

Reflectance spectra: The 0.5- to 2.5-μm waveband can be characterized by three categories: (i) the visible region (0.5- to 0.75-μm) dominated by pigment absorption of light; (ii) the near-infrared region (0.75- to 1.35-μm) of high reflectance affected by leaf structure; and (iii) the infrared water absorption region (1.35- to 2.5-μm) greatly influenced by the amount of water in the leaf tissue with strong water absorption bands occurring at 1.45- and 1.95-μm wavelengths.

In both field- (Fig. 1-A) and greenhouse-grown plants (Fig. 1-B) stressed leaves had lower reflectance than nonstressed leaves over the entire 0.5- to 2.5-μm waveband. Thus, greenhouse results supported field results.

Within the visible spectral region, at the 0.55- and 0.65-μm wavelengths, stressed field-grown leaves had lower reflectance than nonstressed leaves ($P = 0.01$), apparently because stressed leaves contained more chlorophyll (4.3 mg/g) than nonstressed leaves (4.1 mg/g). Leaves with high chlorophyll concentration have more light absorptance and, consequently, less reflectance than leaves with low chlorophyll concentration. No significant difference was noted between the light reflectances of greenhouse-grown stressed and nonstressed leaves at the 0.55- and 0.65-μm wavelengths; although like field-grown leaves, their chlorophyll concentration was higher for stressed (4.0 mg/g) than for nonstressed (3.7 mg/g) leaves. Within the near-infrared spectral region at the 0.85-μm wavelength, stressed leaves had lower reflectance than nonstressed leaves ($P = 0.01$) for both greenhouse-grown (2.6%) and field-grown (4.9%) plants.

Reflectance in the near-infrared waveband is known to be affected by internal leaf structure (3). As intercellular air spaces in the leaf mesophyll increases, reflectance increases because light goes more often from a high (cell wall) to a low (air) refractive index and is scattered. Stressed leaves (Fig. 2-A) showed no evidence of abnormal cells, but they had a compact cellular arrangement in the mesophyll with few intercellular spaces, whereas nonstressed leaves (Fig. 2-B) had a loosely arranged (spongy) mesophyll with many intercellular spaces. Thus, the lower reflectance of stressed leaves is associated with a compact mesophyll, and the higher reflectance of nonstressed leaves is associated with a spongy mesophyll. The near-infrared reflectance difference between nonstressed and stressed leaves apparently was caused by differences in internal cellular structure of the leaf mesophyll. Since results for greenhouse- and field-grown leaves were the same, this negates the premise that the field-applied nematicide may have chemically induced the compactness of stressed leaves.

Over the 1.35- to 2.5-μm waveband, stressed leaves had less reflectance than nonstressed leaves (Fig. 1), but only differences for field-grown plants were statistically significant ($P = 0.01$). Average reflectance differences between stressed and nonstressed leaves for field-grown plants were 2.1, 4.0, 0.6, and 2.6% at the 1.45-, 1.65-, 1.95-, and 2.2-μm wavelengths, respectively. The

lower reflectance of stressed leaves, compared with nonstressed leaves, within the 1.35- to 2.5-μm waveband was apparently caused by water accumulation and/or absorption. Both field- and greenhouse-stressed leaves had higher water contents than did nonstressed leaves.

Spectroradiometric field data: Both stressed and nonstressed plant canopies completely obscured the soil within the field of view that measurements were made. Stressed plants had a lower reflectance than nonstressed plants over the entire 0.5- to 2.5-μm waveband. The lower reflectance of the stunted stressed plants (Fig. 3) compared with nonstressed plants in the visible, near-infrared, and infrared water absorption regions was primarily caused by their darker-green foliage, smaller leaves with a more compact internal structure, and more succulent foliage, respectively. Hence, spectroradiometric field data supported the spectrophotometric laboratory reflectance measurements on leaves collected from field- and greenhouse-grown plants.

Our data show that leaves of nematode-stressed cotton plants have less reflectance than leaves of nonstressed plants over the entire 0.5- to 2.5-μm waveband. The reflected spectral responses of leaves from plants grown under different stress conditions such as nematode infestation, salinity stress, water stress, nutrient deficiencies, insect infestations, and diseases must be known. In remote sensing, an awareness of these reflectance characteristics should facilitate detecting stressed plants and distinguishing them from normal plants. Such studies should be encouraged so that a better understanding can be acquired about the reflectance produced by stressed plant leaves. Our results indicate that remote sensing has much potential for distinguishing nematode-infected plants from noninfected plants.

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