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Color Photos, Cotton Leaves and Soil Salinity

Photographic imagery of cotton leaves in relation to their age, chlorophyll content, and stem position from plants grown in soil areas of low and high salinity.

Purpose of Study

The interpretation of false color imagery to detect spectral reflectance differences between plant genera or species is often a difficult and empirical procedure. Changes in the tonal response of Kodak Ektachrome Infrared Aero Film 8443 (EIR)†, caused by changes in magenta dye concentration, have been often incorrectly attributed to changes in infrared light reflectance of the subject; light- and dark-red tones have been interpreted as indicating high and low infrared light reflectance, respectively. This study was conducted to show that changes in visible light reflectance, caused by variations in plant leaf chlorophyll content, influence the red tonal response of EIR transparencies and prints.

Procedures

Flame- and acid-delinted cotton seeds, variety Texas Planting Seed Association 7A, were planted in a field near Raymondville, Texas on March 24, 1969. Areas were selected in the field that had relatively low and high levels of salinity. The low- and high-salinity areas had average soil saturation extract readings of 1.23 ± 0.14 (standard deviation) and 3.20 ± 1.00 mmho/cm at planting time; at leaf harvest time (July 14) the readings were 1.18 ± 0.35 and 3.13 ± 1.00 mmho/cm, respectively. Rainfall was 11.5 cm. between time of planting and harvest; 7.9 cm. of the rain fell in May.

Cotyledonary leaves began to emerge on April 3 and 5 in the low- and high-salt areas, respectively. Twenty cotton plants were randomly selected in each area, and dates of true leaf emergence (unfolding) were recorded for each node on every plant. Leaf petioles were tagged later with the appropriate date.

Leaves were harvested from uniformly tagged plants on July 7 for leaf thickness, moisture, and chlorophyll determinations, and between 1300 to 1400 hour DST on July 14 for photographic imagery. Photographs were taken on July 7 also, but they were unsatisfactory; and there were insufficient plants remaining July 14 for moisture, thickness and chlorophyll determinations. Little leaf growth occurred on plants, however, between July 7 and July 14. For example, during this interim the leaf on node 21 (3rd down from the apex) of low-salt plants expanded from an area of 17 to 38 $cm.^2$; leaves of high-salt plants had essentially not grown since June 20. On July 14, average plant heights were 94.7 ± 2.2 (standard deviation) and 64.8 ± 2.1 cm. for the low and high salinity areas, respectively. Plants in both areas had open bolls on their bottom branches.

Representative plants to be photographed were pulled from the soil and immediately taken inside a building to provide shade and thus reduce moisture loss. Leaves were re-

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† Trade names are included for information only and do not constitute endorsement by the U. S. Department of Agriculture.

moved from the plants and placed in order from the plant apex to the base (basipetally) on satin-finished black cloth with a 1/4-inch plywood backing. This was moved to sunlight for photography. Approximately 1.5 hours elapsed between removal of plants from the soil and film exposure.

Overhead photographs were taken with Kodak Ektachrome Infrared Aero 8443 (EIR), and Kodak Ektachrome ER 5257 (E) films in a Minolta SRT 101 camera (58 mm. lens) from a height of 30 feet with a "cherry picker", between 1145 and 1215 hours DST under clear sky conditions. A Minolta O 54 orange filter with an approximate absorption edge of 100 percent at 500 nm was used over the camera lens with the EIR film to withhold blue radiation. Exposures were *f*10 at 1/500 sec. and *f*8 at 1/250 sec. for EIR and E films, respectively.

Density readings (180 per 2 mm. film distance) were made across leaf images on the EIR and E color transparencies with a Joyce, Loebl recording microdensitometer using a white band pass filter.

Total chlorophyll (chlorophyll a + chlorophyll b) was determined using procedures of Hall and Hacskaylo (1963) and Horwitz (1965). Total chlorophyll was calculated as:

$$7.12 \log_{10} \frac{I_0}{I} \text{ (at 660 nm)} + 16.8 \log_{10} \frac{I_0}{I} \text{ (at 642.5 nm)},$$

where I_o and I are transmittance values for the solvent and unknown solutions, respectively.

Leaf thicknesses were determined with a linear displacement transducer and digital voltmeter (Heilman *et al.*, 1968).

Statistical procedures used are given by Steel and Torrie (1960).

Results and Discussion

Plate 1 depicts E (left) and EIR (right) combined photographs of leaves of cotton plants grown in high (shortest columns of leaves) and low (longest columns of leaves) salinity areas in the same field. These leaves will be referred to as high- and low-salt leaves, respectively. Leaves are arranged in columns basipetally (from the youngest and smallest at the top to the oldest and largest at the bottom). The numbers adjacent to each of the four columns of leaves designate the node (number to left of hyphen) and the corresponding chronological age in days after leaf emergence (number to right of hyphen).

ABSTRACT: *Cotton plant leaves from a relatively high-saline soil area appeared higher in chlorophyll content on photographs than leaves from a low-saline soil area using Kodak Ektachrome ER Film 5257. Immature leaves near plant apexes were approximately 1.5 times higher in chemically determined chlorophyll contents than more mature, lower leaves. The white-light densities of leaf images on film transparencies increased as leaf chlorophyll contents increased. Leaves with higher chlorophyll concentration induced a darker red tone on Kodak Etkachrome Infrared Aero Film 8443 than leaves with low chlorophyll because of the reduced visible light reflectance. A lower reflectance over the approximate wavelength interval 600 to 700 nm resulted in greater saturation of the magenta positive image which predominates in the viewer's subjective impression of lightness or darkness in the Kodak Etkachrome Infrared Aero transparency or print.*

High-salt leaves compared with low-salt leaves, Plate 1, gave a significantly darker tonal response or appearance (unpaired t test, $p = .01$) regardless of differences in chronological age for both E and EIR photographs. As discussed more fully later, this was caused mainly by the higher chlorophyll content of high-salt leaves, attested to by: (1) their darker green color on the E photograph in Plate 1, and (2) by results of other studies (Thomas, Wiegand, and Myers, 1967; Gausman, Allen, Myers, and Cardenas, 1969).

Variations in tonal responses occur between leaf images within the columns of low-salt leaves for both EIR and E photographs, Plate 1, but they are particularly evident for the EIR photograph. A darker red saturation or appearance occurs at nodes 21 to 23; and a lighter, relatively constant red saturation is evident at nodes 10 to 20, although there is a slightly darker tone for nodes 10 and 11 compared with nodes 12 to 20.

Total chlorophyll (chlorophyll a + chlorophyll *b*) was determined on low salt leaves. Data presented are averages of two and three determinations for leaves representing node 21 and nodes 11, 13, 15, 17, and 19, respectively. Average chlorophyll concentrations of leaves for nodes 19 and 21, Plate 1, were 21.7 and 34.2 mg./liter, respectively. Values ranged between 21.3 and 28.0 mg./liter with a mean of 22.0 mg./liter for nodes 11, 13, 15, and 17. The mean of all chlorophyll determinations was 24.2 ± 5.8 (standard deviation) mg./liter.

Densities of leaf images on film transparencies, determined with a microdensitometer with a white light band-pass filter, were associated with leaf chlorophyll contents, which can be best shown by comparing results for nodes 19 and 21, Plate 1. Leaves representing node 21, compared with node 19, had approximately 1.5 times more chlorophyll and a 16% denser image to white light transmittance for both EIR and E transparencies.

Besides having a high chlorophyll content, immature leaves have mesophylls with few intercellular spaces or a compact cellular structure (Gausman, Allen, Cardenas, and Richardson, 1969), whereas mature leaves have lower pigmentation and lacunose mesophylls (many intercellular spaces). The reflectance of visible, 400 to 750 nm, and near-infrared, 750 to 1350 nm, radiation increases as leaf pigmentation and mesophyll compactness decrease. Since EIR film is sensitive to visible radiation and to infrared radiation (IR) over the wavelength range 700 to 900 nm (Fritz, 1967), it seems feasible that either high pigmentation and/or compact mesophyll structure could cause the darker red tone of the leaves at nodes 22, 23, and 21 compared with nodes 10 to 20 for low-salt leaves, Plate 1, and of leaves of all nodes for the high-salt leaves. Arguments that chlorophyll content had more influence than cellular structure are: (1) High salt leaves of approximately the same chronological age compared with low-salt leaves (nodes 18 *vs.* 21, 16 *vs.* 18, 10 *vs.* 12), and therefore closely alike in cellular structure and near-IR light reflectance (Gausman *et al.*, 1969), were darker in tone. (2) There was little difference in water content and thickness of leaves of the same chronological age; for example, the high-salt leaves representing node 18 and low-salt leaves for node 21 had water contents of 72.0 and 74.0 percent and thicknesses of 0.167 and 0.161 mm, respectively. (3) A greater contrast was observed between leaves on the EIR photograph when they were viewed through a green compared with blue or red filters, indicating that the magenta tonal image predominated, which is affected by the red radiation in the visible spectrum. (4) Documentation discussed below indicates that the red light-induced magenta image usually predominates in false-image photography of healthy vegetation.

The Ektachrome Infrared film (EIR) has three image layers individually sensitized to green, 500 to 600 nm; red, 600 to 700 nm; and infrared radiation, 700-900 nm, instead of to blue, green, and red radiation for E film (Fritz, 1967). A yellow filter is used on the camera to absorb the blue radiation, to which these layers are also sensitive. Upon processing, yellow, magenta, and cyan positive images are formed in the green-, red-, and IR-sensitive layers, respectively. The overall impression to an observer viewing the finished print or transparency will depend upon which of the positive images in the dye layers predominates with respect to visual appearance. Because the eye sensitivity peaks in the green, the magenta layer generally contributes most to the subjective impression of lightness or darkness in a color print or transparency‡. For example, healthy leaves, with high IR compared with low IR reflectance for unhealthy leaves, record red because a light-toned cyan image (less dense or less saturated) results, which allows the transmittance of more red radiation in the viewing.

In Plate 1, the magenta image shows the greatest tonal contrast, particularly between low-salt leaves on the EIR photograph. This was verified by viewing the leaf images through a green filter. The difference between leaves in red reflectance was caused by variation in leaf chlorophyll contents. Chlorophyll *a* and *b* have absorption maxima at 675 and 650 nm, respectively (Clayton, 1965), which fall within the 600 to 700 nm red light wavelength range to which the magenta dye layer of the EIR film is sensitive. Thus relatively, Table 1, high chlorophyll (nodes 21 to 23) increased red light absorptance, decreased its reflectance (less red radiation impinging on the film), and caused a more saturated image in the magenta dye layer which allowed less green light transmittance, and thus produced a darker appearance. Less chlorophyll (nodes 10 to 20) caused higher red light reflectance, less magenta dye, and a lighter appearance.

‡ Private communique, Norman L. Fritz, Eastman Kodak Company, Rochester, New York. July 1, 1969.

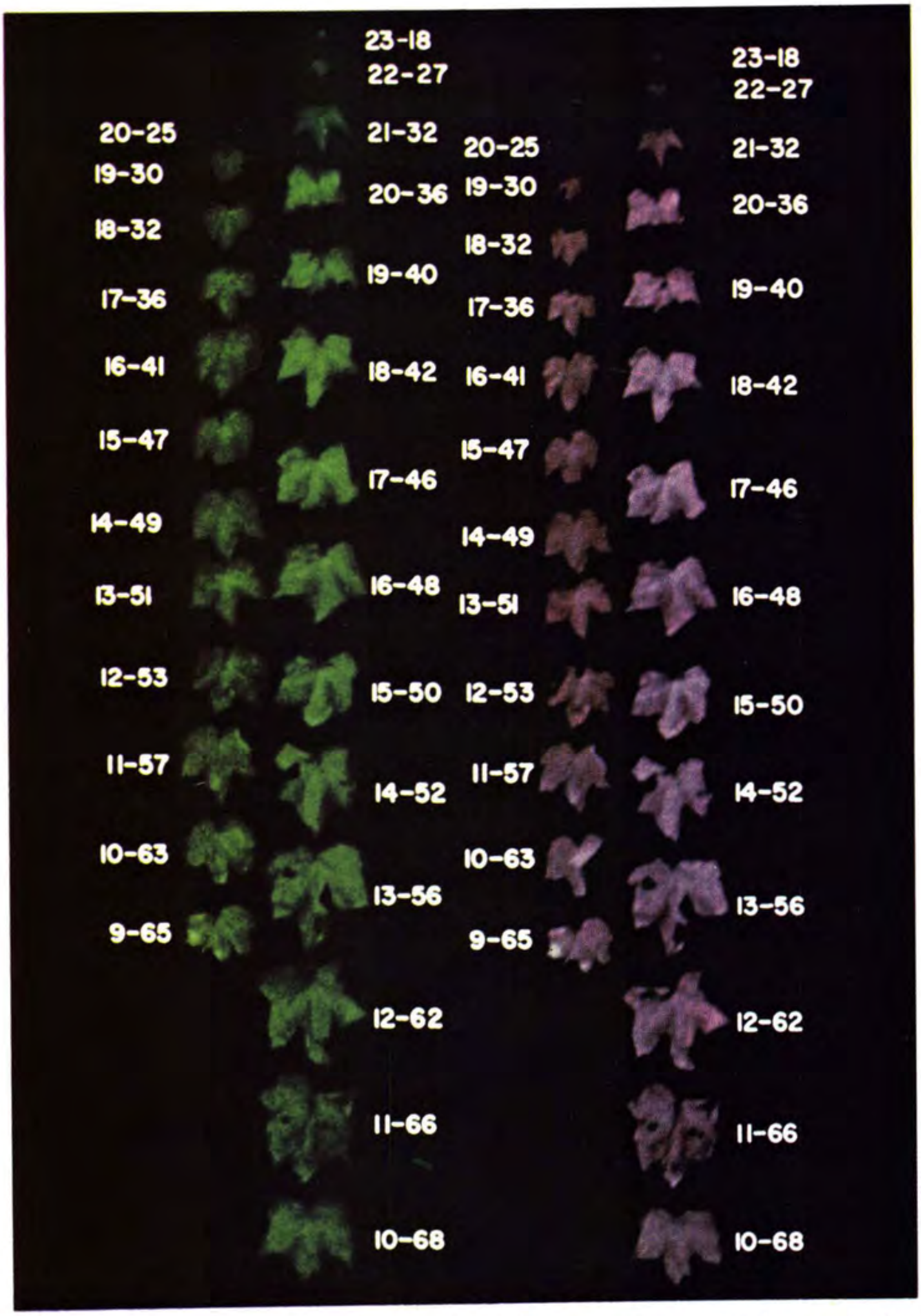

PLATE 1. Ektachrome (E) (left two columns) and EIR (right two columns) color photographs of cotton plant leaves grown in high (shortest columns of leaves) and low (longest columns of leaves) saline soil areas, respectively. Leaves are arranged in columns basipetally (from the youngest and smallest at the top to the oldest and largest at the bottom). The numbers adjacent to each of the four columns of leaves designate the node (number to left of hyphen) and the corresponding chronological age in days after leaf emergence (number to right of hyphen.)

TABLE 1. COMPARISON OF VISIBLE AND NEAR-INFRARED (IR) LIGHT REFLECTANCE OF IMMATURE (NODES 21–23) AND MATURE (NODES 10–20) LOW-SALT LEAVES, PLATE 1, IN RELATION TO THE SATURATION OF THE EIR FILM DYE LAYERS AND THE LEAF IMAGE APPEARANCE ON THE EIR PHOTOGRAPH

Leaves (nodes), Plate 1	Chlorophyll content	Mesophyll structure	Light reflectance		Positive image densities			Red color saturation	Appearance (red tone)
			Visible	IR	Yellow	Magenta	Cyan		
21 to 23	Higher	Compact	Lower	Lower	Higher	Higher	Slightly higher	Higher	Darker
10 to 20	Lower	Lacunose[a]	Higher	Higher[b]	Lower	Lower	Lower	Lower	Lighter

[a] Many intercellular spaces.
[b] Leaf from node 21 was only about 2% higher in IR reflectance than leaf from node 20 (Gausman, Allen, Cardenas, and Richardson, 1969).

SUMMARY

Immature leaves near the top of a representative cotton plant, compared with lower mature leaves, photographed to give a darker green and red appearance for E and EIR films, respectively, The darker tonal response was caused by the higher chlorophyll content of immature compared with mature leaves, which caused them to absorb more of the red portion of the visible radiation and thereby reduced its reflectance. A lower amount of red radiation, over the approximate wavelength range 600 to 700 nm, gave a dark-red tone which was caused by a high saturation of the magenta positive image, which contributed most to the viewer's subjective impression of darkness in an EIR transparency or color print.

Leaves from plants grown in saline soil had a higher chlorophyll content than low-salt leaves, and they correspondingly gave a darker red tone on EIR film, because they reflected less of the visible red radiation.

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Articles for Next Month

A. P. Colvocoresses, ERTS—A satellite imagery.
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L. A. Woodward, Survey project planning.
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