# DETECTION OF THE PHOSPHORUS LEVEL IN COTTON LEAVES BY SPECTROPHOTOMETRY

Ву

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#### PREFACE

This study was concerned with the possibility of finding a method whereby reflectance of monochromatic light by plant leaves would indicate the phosphorus concentration in their tissues. Cotton plants were grown hydroponically and reflectance studies were conducted by use of a Beckman DU-2 spectrophotometer.

The author is particularly indebted to his thesis advisor, Dr. Bobby L. Clary, who has assisted and encouraged me during this study.

Appreciation is extended to other members of the Advisory

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#### CHAPTER I

#### INTRODUCTION

#### Problem Definition

Plants incorporate certain pigments within chloroplasts which are capable of changing light energy into chemical energy. All life depends upon this photochemical phenomenon (13) and any factor such as nutrient or water deficiency, or other than optimum environmental conditions hinders the photosynthetic process. If all climatic factors are favorable for plant growth, the chemical status of the plant determines the abnormality or normality of growth (17). In order for an agriculturist to realize maximum production, it is important that the chemical status of a plant be easily, quickly, and accurately determined.

Presently there is not a plant nutrient analysis method that is entirely satisfactory from every aspect. To realize maximum benefit from such an analysis, it should be convenient, rapid, inexpensive, and reliable. It is important to have such an analysis method so each crop's growth can be monitored continuously and its growing conditions promptly modified as required for optimum production.

In an effort to meet the above criteria several methods of plant analyses now exist. They are known as chemical and spectrographic analyses and rapid tissue tests. The advantages and disadvantages of these methods will be discussed in the "Literature Review" section. Each one falls short by not meeting the criteria stated above.

The interplay of light and leaves, being one of nature's most common phenomenon, is affected when a plant, due to unfavorable chemical balance, develops chlorosis, premature yellowing, mottling, necrosis, or other abnormalities. There is a characteristic amount of light reflected, absorbed, and transmitted for a healthy leaf of a given species. Further studies are needed to determine whether or not these characteristic optical values are satisfactorily uniform and detectable for analysis purposes.

Optical properties of plants are now commonly referred to in literature and the usual meaning refers to the amount of light reflected, absorbed and transmitted by leaves. These properties result largely from the reflecting surface of the leaf and the absorption of leaf pigments (43) and pure water (16). Since Coblentz's (9) early work in checking the reflectance of some leaves along with other surfaces in 1908, many investigations have been directed toward finding the factors that affect leaf reflectance.

The selection of phosphorus concentrations in cotton leaves for reflectance studies does not imply a significance that is paramount in plant growth. The criteria for selection were to have a broadleaf plant and a nutrient that were readily accessible, and plant growth conditions and nutrient availability that could be easily controlled. The reasoning as formally stated would be: If different levels of the essential element phosphorus were made available to a cotton plant which resulted

<sup>&</sup>lt;sup>1</sup>The inorganic element phosphorus is normally absorbed by plants as soluble phosphate ions (13) and use of the word phosphorus throughout this study does not suggest otherwise.

in distinguishable leaf reflectance characteristics, then different levels of availability of other essential elements to other plants would likely give analogous results, in that similar reflectance studies could be carried out.

# **Objectives**

The motivation for this study was to find a relatively simple way of determining the chemical balance of a plant by use of light.

Specifically the objectives are:

- (1) To relate light reflectance characteristics of a cotton leaf to its phosphorus concentration.
- (2) To establish a signature reflectance curve in the visual wavelength for a given cotton variety which is grown in a controlled favorable environment without nutritional or moisture stress.
- (3) To determine if leaves of different phosphate concentrations have distinctive colorimetric dominant wavelengths, color purities and lightnesses.
- (4) To investigate the relationship between reflectance and leaf thickness and between reflectance and specific weight.

#### Limitations of the Study

This study was made to determine if the diffuse reflected light from the upper (adaxial) surface of cotton leaves would indicate the phosphorus level in the leaves.

Monochromatic light between 400 and 700 nanometers wavelength as directed by a Beckman Model DU-2 spectrophotometer was reflected from

leaf samples of Westburn  $70^2$  variety of upland cotton. The reflectance measured was relative to the amount of reflectance from a magnesium carbonate (MgCO $_3$ ) standard. Only relative reflectance was used throughout the study.

Leaves were from cotton plants that were grown under uniform conditions except for the amount of phosphorus made available to the plants. All plants were germinated and transplanted on the same dates but were grown in containers of modified Hoagland's solution with four different levels of phosphorus applied. Leaves of the same morphological age were plucked and submitted to analysis.

The terms light, visual range, and visual radiation are used to designate radiant energy between 400 and 700 nanometers of the electromagnetic spectrum unless otherwise defined.

<sup>&</sup>lt;sup>2</sup>A commercial variety of cotton jointly released in 1970 by the Agricultural Experiment Station of Oklahoma State University and the Crops Research Division of the USDA.

#### CHAPTER II

#### LITERATURE REVIEW

An object becomes visible when light reflected from it reaches the eye. Reflected light has tremendous influence on our economy. By it, foods are judged in part as to "goodness," paintings are appraised, and "eye appeal" of clothing and residences are determined. Light is first mentioned in the Bible in Genesis 1:3, and the effect of reflected light is implied in Genesis 3:6 when Eve saw the Tree of Knowledge "was pleasant to the eyes." She made an evaluation—as many plant producers have done—of a plant by its appearance.

#### Plant Analysis

In a general sense, plant analysis is the study of the plant's nutrient content as related to its growth (33). For example, studies of phosphorus deficiency in plants generally conclude that this deficiency results in stunted plants with purple veined, off-color green leaves (17). For specific leaf samples it is desirable to not only note its appearance, but be able to accurately detect amounts of nutrients present. Available analytical methods are classified as either chemical or physical.

The chemical methods of analyses are laboratory tests, and "rapid tissue tests," which are usually carried out in the field. The laboratory chemical analysis of leaves measures the amount of an element in

tissue and in the plant sap whereas, the rapid tissue tests merely measure the amount of nutrients incorporated into the tissue (33). The laboratory method has proven to be reliable though somewhat laborious, in that skilled technicians, elaborate equipment, laboratory quality chemicals, and relatively long periods of time are required. The field analyses methods appeal to the producer due to its simplicity and apparent indication as to what is promptly required to improve chemical balance in the plants. But this method has not been widely used for research due to inconsistent sampling techniques and lack of calibration.

The only developed physical method of tissue analysis now in use is spectrographic. This method imparts energy to the elements in the sample by either heat and high voltage or by X-ray after which each element in the tissue then emits either characteristic light wavelengths or secondary X-rays according to the technique used. The amount of emission indicates the amount of an element present (33).

Either of these methods are useful for certain analyses. Chemical methods are most often used for determination of amount of one or two nutrients whereas, the spectrographic method makes fast work of analysis for several elements simultaneously. Plant analysis is based on the idea that there will be a certain amount of an essential element present in a certain plant part at a given time if the plant is thrifty (33). Plant analysis is not an absolutely positive method of always being able to determine exactly what is needed by growing plants for optimum growth. There are limitations to an analysis' usefulness and often a critical concentration standard has not been established for comparison. A concentration standard for optimum yield is established for a given variety when it is grown under a stated set of environmental factors and

the concentration of the required nutrient in plant parts is recorded.

#### Light

The ultimate source of all useful light for the earth is the sun. By it, three of life's vital processes, photosynthesis, vision, and photoperiodism are controlled. In spite of its universality and its seemingly timelessness, light has never been fully explained.

No complete review of the literature on light and its nature will be attempted, but merely a brief summary of the theories by which research has progressed will be noted. Feinberg (14), in reviewing the theories of light, presented the following information. Sir Issac Newton, in his Optics of 1704, suggested that light was corpuscular in nature, in that it came from a source in discreet bundles of energy (corpuscles) in straight rays. This theory fitted very well into the explanation of reflection but did not lend itself to explaining diffraction, refraction, or interference. Christiaan Huygens, who lived during Newton's time, offered an alternate explanation that light was of wave form--much like the surface waves on water radiating out from a pebble dropped into a pool. This fitted so nicely in explaining all of the known actions of light that Newton's theory was relegated to disfavor for many years. The wave theory was further strengthened by James Clerk Maxwell who proposed that light was merely a portion of a whole family of radiations which included invisible rays such as X-rays and radio waves. All of these radiations, Maxwell explained are composed of electric and magnetic waves oriented normal to each other and traveling at 2.998 x  $10^{10}$  centimeters per second through space. Only during this century have the wave-particle theories been re-examined and reconciliation achieved. Light is associated with wave phenomena of polarization and interference, while it interacts with matter in a manner that suggests that it is made of individual bodies called photons. Therefore, it would appear that radiation has a dual character and its interaction with matter cannot be fully described by using only the corpuscular or the wave theory separately (14) (15).

### Reflection of Light

When light arrives at the interface between mediums with different refraction indices (the speed of light in one medium is different from the light speed in the other) some of the energy is turned back and is termed reflection. By reflection, materials appear either as white, black, colored, transparent, translucent, or opaque according to the response of electrons which are driven by light to vibrate at the atomic and molecular structural level (40).

The vast majority of the objects perceived by the eye are by reflection, with lamps and fire as exceptions, as they are sources and furnish light for reflection by other objects. Light causes the surface electrons of a material to vibrate and emit light. Therefore, reflection is not as descriptive of the process whereby light is turned back and perceived as the word "re-emission." A simplified concept of the process is that electrons act as small oscillators and are driven to oscillate by electric waves of a specified length. The vibrations, though extremely small in magnitude, account for all the light and color we see.

Reflection takes place at an interface which is described as being either optically flat or rough. Light reflected and refracted at an

optically flat or smooth interface obeys Snell's law (39) as shown in Figure 1 (7).

Light strikes a "rough" surface under two possible circumstances. In the first instance the interaction of a plane electromagnetic wave with particles equal or larger than its length can be accounted for by either reflection, refraction, or diffraction. A collection of these particles into a surface (such as a crystal powder) provide randomly oriented reflecting facets which reflect light in all possible angles into the hemisphere from whence the impinging ray arrives. This isotropic angular distribution of specular reflection is termed "diffuse."

In the second case the particles are equal to or smaller than the wavelengths of the impinging light. Reflection, refraction, and diffraction are not distinguishable components of intensity in this case though the reflected light appears diffuse, the term "scattered light" is used to describe this phenomenon.

In both cases the surfaces may be termed as dull or "mat" and the reflection from both deviates very little from Lambert's Cosine Law (27) which is shown in Figure 2 (7).

Clouds and snow appear white because they reflect sunlight without altering the spectral composition. Objects with color selectively absorb certain wavelengths and reflect the rest.

Most natural surfaces such as leaves are "mat". Light refracted at a mat surface may sometimes impinge on a second rough surface and after being reflected and refracted several times with some absorption, re-emerge from the surface on which the light source originally impinged. The intensity of the diffuse light is a combination of the surface reflection and light from the interior, and is a mixture of specular and

1<sub>0</sub>= INCIDENT RAY R = REFLECTED RAY T = TRANSMITTED RAY

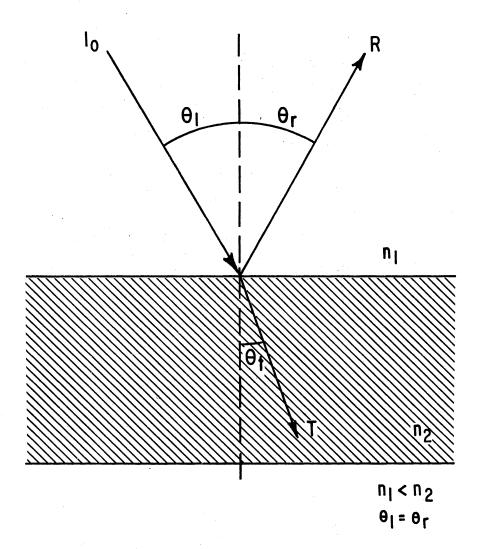


Figure 1. Snell's Law. After Birth, G. S. (7)

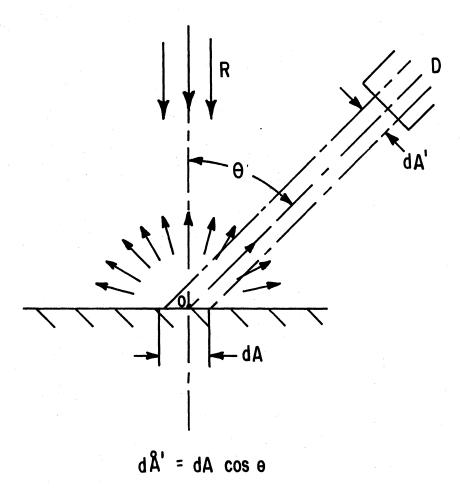


Figure 2. Lambert's Cosine Law. After Birth, G. S. (7)

scattered light. A gloss or sheen from a mat surface is an indication of specular reflectance. The situation described is shown in Figure 3 (7).

To restate, light is reflected whenever it impinges on an interface between two different materials. Electrons are caused to vibrate which normally send forward a strong refracted wave. There is a reflected wave, however, caused by the thin layers of oscillators (about as deep as half a wavelength) whose back radiation is not completely cancelled by interference (40).

#### Structure of Cotton Leaves

Leaves are generally the most obvious plant part. Loosely, they are those flattened green structures which protrude from stems and are the main centers of photosynthesis. They are highly diverse and differences commonly occur in the: presence of a petiole, arrangement on the stem, pattern of blade attachment to the petiole, venation, shape of leaf bases and tips, type of leaf margin, size, and internal structure.

Cotton leaves from <u>Gossypium hirsutum</u> are described as large, cordate, relatively thin, palmately veined, and papery (8). See Figure 4 for a picture of a typical leaf from the Westburn 70 variety. The surface of cotton leaves range from being very hairy to glabrous or almost so, whereas this variety has few hairs and has a relatively smooth surface.

Sketches representing transverse sections of cotton leaves at different stages of leaf maturity are presented in Figures 5 and 6 (21). The sandwich structure of the leaf contains three distinct layers with

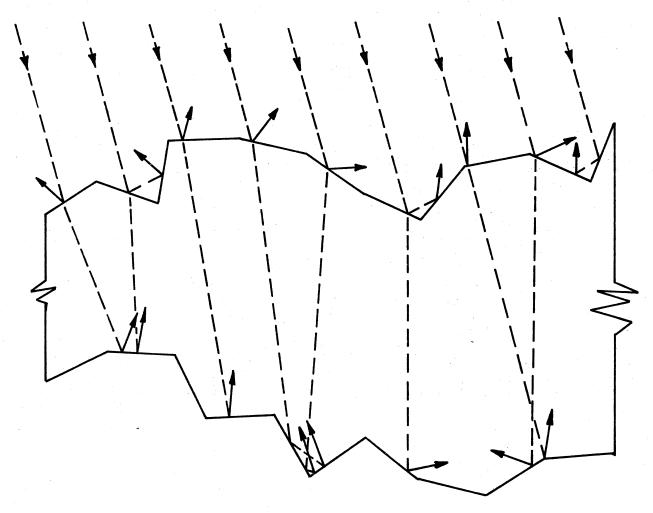
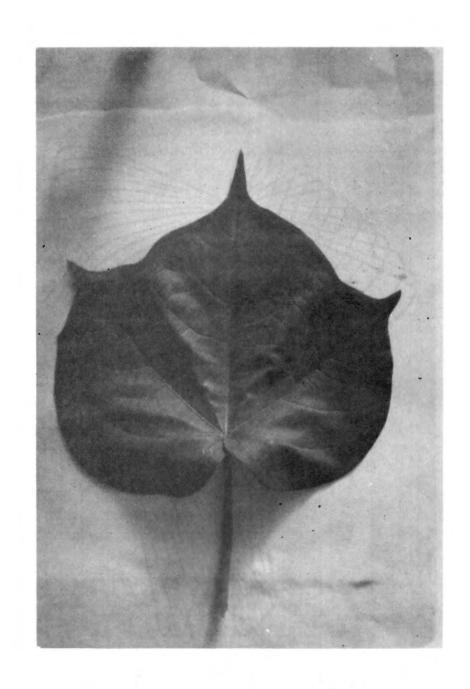


Figure 3. Two Lambertian Surfaces Approximating Transverse Leaf Sections. After Birth, G. S. (7)



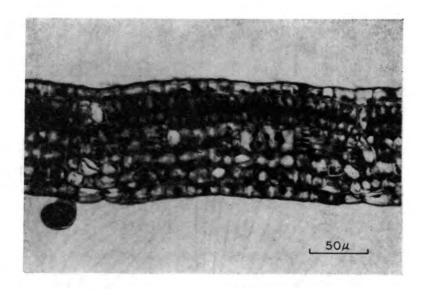


Figure 5. A Sketch Representing a Typical Transverse Section of a Cotton Leaf 3.5 Days of Age Since it Became Macroscopically Visible. After Gausman, H. W., et al. (21)

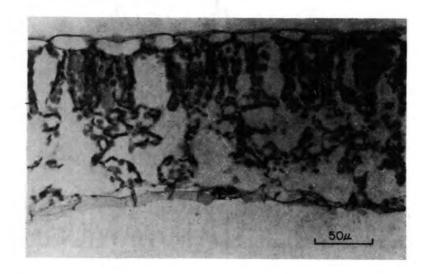


Figure 6. A Sketch Representing a Typical Transverse Section of a Cotton Leaf 10.8
Days of Age Since it Became Macroscopically Visible. After Gausman,
H. W., et al. (21)

the mesophyll comprising the majority of the leaf. The mesophyll is between the upper and lower epidermises. The thin colorless typically transparent cuticle acts as a cover on both upper and lower leaf surfaces and is made primarily of cutin and helps prevent direct evaporation from the underlying cells. The epidermal layers formed of cells one layer thick may thicken with age or with shortages of moisture. These cells typically do not contain chlorophyll and their function appears to be to protect the mesophyll underneath. This covering is punctured by openings (stomata) that permit leaf-air interchange of gases. The number of stomata vary considerably per unit of leaf area with the lower surface of a leaf usually having more than the upper surface.

The mesophyll of leaves is comprised of the palisade and spongy zones. The palisade tissue contains one to four layers of compact cells whose major axes are generally oriented perpendicular to the upper leaf surface, while the spongy mesophyll is a lacunose structure of cells which fills the lower portion of the leaf. The cells of the mesophyll contain the rounded bodies of chloroplasts which give the characteristic green appearance to leaves.

According to Gausman, Allen, and Cardenas (20) a transection of a cotton leaf reveals that it consists of an upper epidermis of brick-like structure; palisade tissue of a single layer of long cells perpendicular to the leaf's upper surface; spongy parenchyma of four or five layers of cells; and the lower irregular surfaced epidermis. See Figure 6.

The palisade cells of a leaf are about  $15000 \times 15000 \times 60000$  nanometers, whereas, the spongy parenchyma and cuticle cells are about

18000 x 15000 x 20000 nanometers. The 50 or more chloroplasts of a cell range from 5000 to 8000 nanometers in diameter with a thickness of about 1000 nanometers. The granum within the chloroplasts may be 500 nanometers in length and as small as 50 nanometers in thickness (18) (38).

Joham (24) determined that a typical leaf blade of cotton contains about 0.17 to 0.19 percent phosphorus before flowering started, after which there was a sharp decrease in phosphorus concentration. He also detected that the amount of each element in the stems, petioles, or leaves varied directly with the amount in the substrate level. Amounts of petiole phosphorus was also inversely related to the amount of nitrogen and calcium in the substrate.

# Light-Leaf Interaction

The interaction of light and leaves is one of nature's most common occurrences when light either directly or indirectly comes from the sun and impinges on leaf surfaces. More specifically, light of nearly all wavelengths strikes the mat surfaces of leaves to be reflected, transmitted or absorbed.

All light incident on a leaf is either transmitted, absorbed, or reflected. Rabideau (30) reviews and concurs with the expression 1 = A + R + T, where A, R, and T account for the fractions of light absorbed, reflected, and transmitted, respectively.

## Spectral Properties of Leaves

Figure 7 depicts the amount of radiant energy reflected, absorbed, and transmitted by a cotton leaf between 500 and 2500 nanometers (26).

Outside the spectral range shown, in the ultraviolet, and in the far

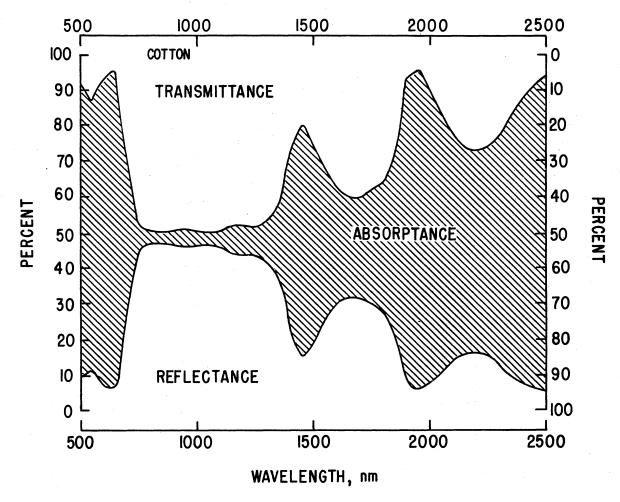


Figure 7. Typical Optical Curves for Leaves.
After Knipling, E. B. (26)

infrared and radio waves of the long wave lengths, the optical properties remain about constant, with a reflectance of approximately five percent. As has been stated before, optical properties of leaves result largely from relatively high absorptance in the visual and far infrared, due to pigments and liquid water plus the reflecting characteristics of the leaf surface. Notice that transmittance and reflectance curves are the same shape and have approximately the same values, whereas absorption is the opposite of the other two. In the visible range and above 1300 nanometers, reflectance and transmission are low but they account for nearly all the energy between 700 and 1300 nanometers. They absorb well in all wavelengths of the visual range but have highest absorption in the blues and reds. Highest reflectance of approximately 10 percent occurs at approximately 500 nanometers which explains the green color of leaves. The specific absorption of the extracts of chlorophyll A and B are shown in Figure 8 (43). The absorption spectra beyond 1300 nanometers appears to be due to the presence of water in the leaves, since the absorption curve for water and for leaves beyond that point are quite similar, as shown in Figure 9 (16).

Gates (18) describes the leaf as being wonderfully adapted to its environment. It is an efficient absorber of the visual light wavelengths from whence energy is used for photosynthesis, but it is a poor absorber in the near infrared where the majority of the sunlight energy exists. Absorption of these wavelengths would lead to high leaf temperatures and protein denaturization. The leaf is once again a good absorber (and emitter) in the infrared region above 1300 nanometers.

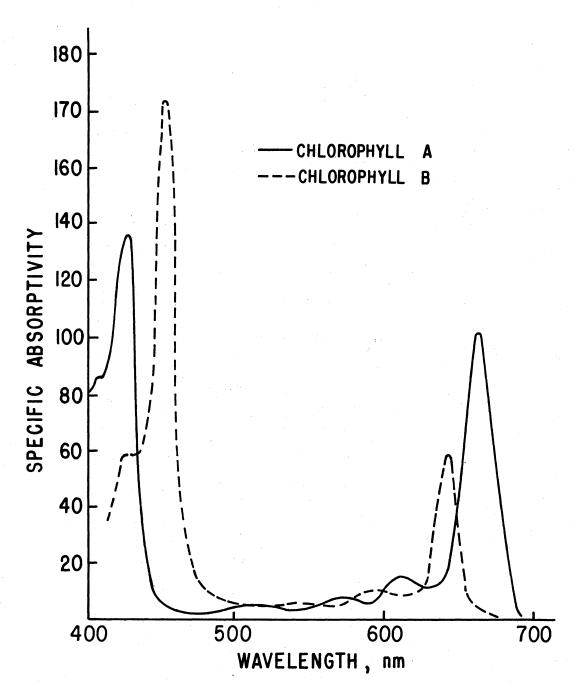


Figure 8. Absorption Spectra of Chlorophyll A and B Extracted in Ether. After Zscheile, F. P. and C. L. Comar (43)

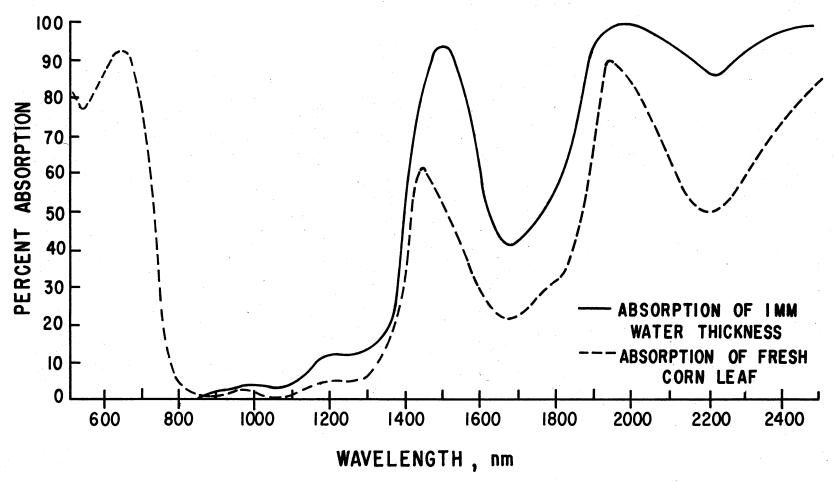


Figure 9. Absorption Spectra of a 1 mm Thickness of Water and a Fresh Corn Leaf. After Forsythe, W. E. and F. L. Christison (16)

#### Reflectance of Leaves

In the visual range there are several leaf characteristics that influence reflectance. Knowing the reflectance and transmission which can be directly measured, gives an indirect method for calculating the absorptance. Absorbed light is that portion of light that is utilized by the plant in photosynthesis, whereas reflected light facilitates vision and hopefully can indicate the internal conditions of a plant. For example, plant diseases, which will not be detailed in this study, do affect the reflectance and thereby dictate the amount of useful absorptance by a given plant. It is believed that healthy leaves of the same age and of a given plant variety which are grown under optimum conditions will have a characteristic reflectance (signature) curve which can be used as a standard for comparison.

# Leaf Structure

Generally thick leaves reflect light less than thin leaves.

Rabideau (30) found that pineapple leaves reflect less than Morning

Glory leaves and less than the leaves of cabbage and lettuce. Moss and

Loomis (28) determined that a fig leaf reflected less than bean,

spinach, swiss chard, or tobacco leaves as shown in Figure 10. For

thick leaves the transmitted component is diminished as leaf thickness

increases. Investigations (29) on reflectance of stacked leaves con
cluded that the energy of the visual light waves was reflected by the

surface of the top leaf.

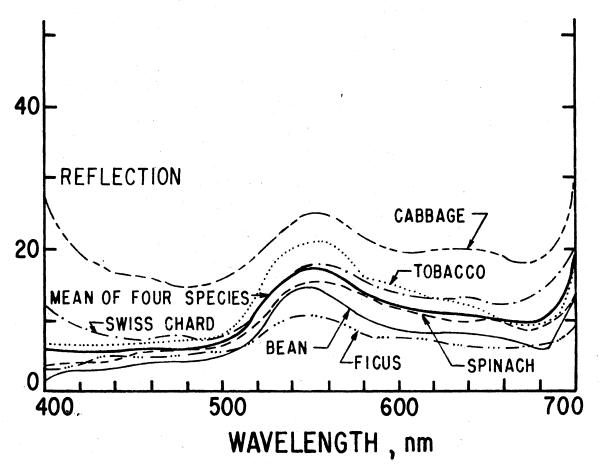


Figure 10. Reflectance of Light by Leaves of Six Species.
After Moss, R. A., and W. C. Loomis (28)

### Pubescence

Shull (31) and Coblentz (9) found that hairs on mullein did not markedly increase reflectance over glabrous leaves nor was reflectance particularly enhanced by the shiny cuticle of the red mulberry. It was found, however, that the hairs on the leaves of white popular and cucumber tree did increase reflectance. Later, Billings and Morris (5) noted that white sage, a desert plant with dense stellate hairs had higher reflectance than other desert plants in the visual wavelengths. Gausman and Cardenas (19) continued along this line of investigation by measuring the reflectance of a velvet plant before and after the removal of leaf hairs. They found little differences in the amount of diffuse reflectance in the visual range but did note a small increase in reflectance for the shaved leaf in the infrared beyond 1300 nanometers as shown in Figure 11.

# Turgidity

Studies in Russia relate reflectance from small leafed linden, English Oak, and flax leaves to the moisture content of the soil in which the plants were growing (12). Highest reflectance was recorded for plants on soil at 20 percent of total capacity, whereas the lowest occurred with soil moisture at 60 and 80 percent of capacity. Thomas, Myers, Heilman, and Wiegand (36) found that reflectance at 540 nanometers wavelength increased as water content of the leaf decreased. This increase in reflectance may be due to solute concentration within the cell as the moisture escapes. The results are given in Figure 12.

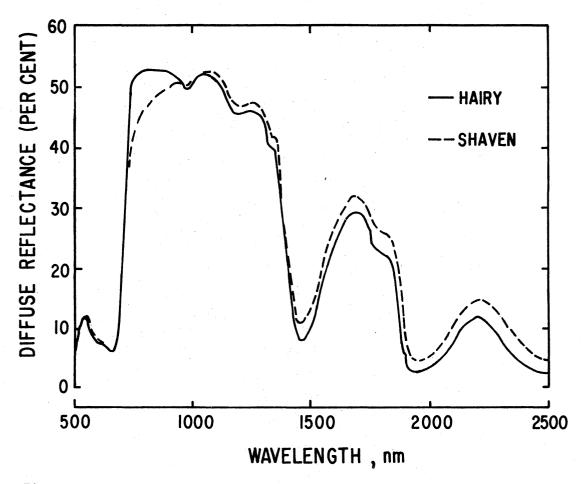
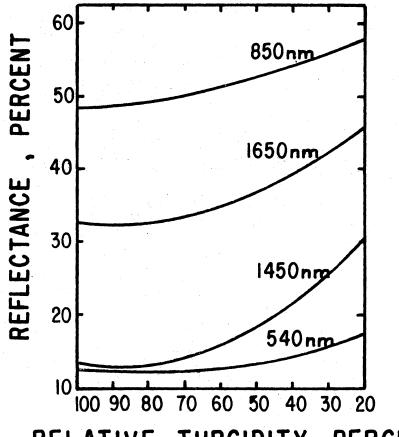


Figure 11. Influence of Hair Removal From Leaves of Velvet Plant on Diffuse Reflectance of Light. After Gausman, H. W., and R. Cardenas (19)



# RELATIVE TURGIDITY, PERCENT

Figure 12. Effect of Relative Turgidity of a Cotton
Leaf on the Level of Spectral Reflectance at Selected Wavelengths. After
Thomas, J. R., et al. (36)

#### Age

Young leaves evolve from the plant's apex with the result that leaves are progressively more mature downward along the stalk. The majority of investigations conclude that reflectance decreases with leaf age. Shull (31) associated the decreasing reflectance of leaves with age with the increase of chlorophyll concentration in the cells which increases very rapidly at first and then more gradually for about two months or until the final mature deep green has been reached. Tageeva, Brandt, and Derevyanko (34) demonstrated that as the chlorophyll content increased in the leaves of birches and lindens in the Spring, the light absorptance coefficient also increased until the optimal chlorophyll content of 2 mg/100cm<sup>2</sup> of leaf area was reached. Absorptance remained rather constant from that time until in the Fall. when the chlorophyll content fell below the optimum amount. A record of the reflectance of white oak leaves through the growing season by Gates, Keegan, Schleter, and Weidner (18) show the variance of optical properties during this period. The young leaf displays very little chlorophyll absorption; but as chlorophyll developed in the leaf, reflection decreased and remained almost constant until late in the growing season when senescence started. A more detailed study conducted later on the reflectance of leaves from the time they became macroscopically visible up to 12 days of age revealed that reflectance in the 500 to 700 nanometer range increased moderately with leaf age up to about 10 days of age, after which the reflectance curve was slightly lower (21). Gausman, Allen, Cardenas and Richardson (22) confirmed

previous results that young leaves (second node from apex) reflect slightly less in the visual range than do more mature leaves from nodes further down the stem. Thomas, Myers, Heilman, and Wiegand (36) found that the reflectance for the same leaf over a 30 day period at 550 nanometers plotted against leaf area decreased from about 15 to 9 percent as the leaf grew from 20 to  $100 \text{ cm}^2$  in area.

Very young leaves which apparently absorb more and reflect less than older leaves are characterized by compact mesophyll. One theory attributes reflectance to cell wall-air interface of the spongy mesophyll which is most developed in mature leaves (41). This theory is somewhat contradicted by recent findings of Sinclair (32) who hypothesized after a thorough investigation that leaf reflectance is not so much a result of cell wall-air interface as it is the result of light striking plant cell walls which have diffuse characteristics. A shift to this hypothesis would give more importance to the compact palisade parenchyma with its greater barrier of cell walls than the lacunose spongy parenchyma layer and explain the lower reflectance of young leaves. Figures 13 and 14 depict theories of light-leaf cell interaction as discussed.

## Geographic Location

The variance of reflectance with geographic location is pronounced. Billings and Morris (5) made a study of plants from five different environments of the western Great Basin. Reflectance was highest for desert species and lowest for shaded species in the visible radiation. Dadykin, Stanko, Gorbunova, and Igumnova (11) conducted studies on "optical adaptability" by comparing optical properties of plants from

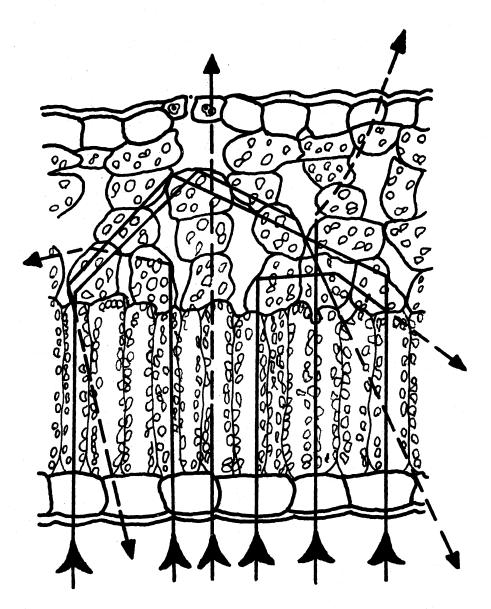


Figure 13. Schematic Drawing Depicting One Theory on the Pathway of Light Through Leaves.

After Willstatter, R., and A. Stoll (41)

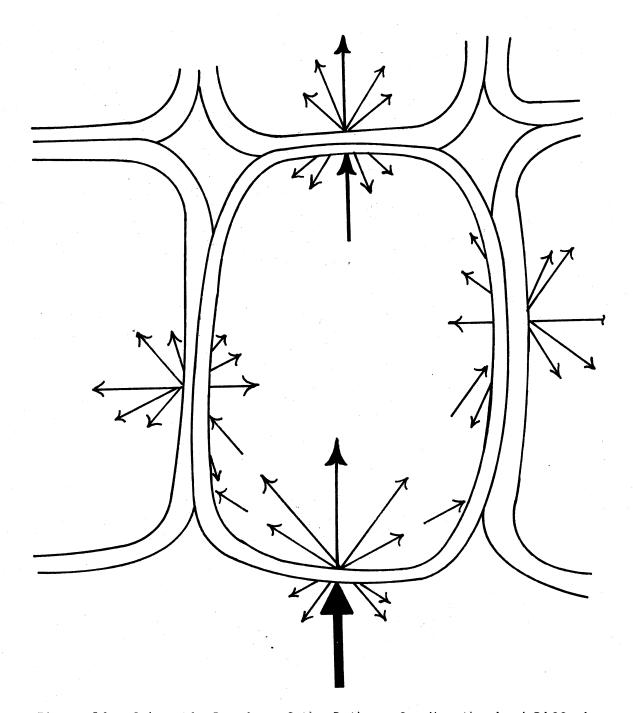


Figure 14. Schematic Drawing of the Pathway for Hypothesized Diffusive Reflected and Transmitted Radiation from Cell Walls.
After Sinclair, T. R. (32)

Yakutsk (62<sup>o</sup>N) and Tikso (71.6<sup>o</sup>N). Plants of the same species and age on comparison showed that the plants from the more severe climatic conditions were more efficient absorbers and reflected less radiant energy. Dadykin and Bedenko (12) on further investigation of optical adaptation selected two longitudes (37<sup>o</sup>E and 128<sup>o</sup>E approximately) with three different latitudes stretching from approximately 50<sup>o</sup>N to 70<sup>o</sup>N. In every case the plants of a species grown in the northernmost latitude reflected least, those grown at the intermediate latitude reflected next least, and those of the southernmost position reflected the most in the visual range.

## Surface Characteristics

Coulson (10) reviews and summarizes the research into the polarizing characteristics of natural surfaces. Reflected light from leaves in the visual range is highly polarized. Much of this investigative work has been done for better interpretation of data gathered in remote sensing programs. Though the light is polarized, it continues to be diffuse, and, therefore, has no influence on this investigation.

#### Nutrition

A leaf's reflectance curve is influenced by anything environmental or genetical that causes a morphological change in leaf structure.

Nutritional factors are of importance and a few have been studied.

Recently, Thomas and Oerther (35) were able through reflectance studies to determine the nitrogen content of field grown sweet peppers. The pepper leaves continuously reflected more light as the nitrogen content of the leaf dropped. Earlier work by Benedict and Swidler (4), in trying

to find ways to estimate chlorophyll content by nondestructive methods, found there was a close correlation between reflectance and chlorophyll content of soybean and orange leaves. The reflectance reading for a given chlorophyll content changed with species and varieties of plants under study. The concentration of chlorophyll (to a lesser degree the other pigments) and the amount of water in leaves has an indirect influence on light disposition by the leaf.

Characteristic reflectance and transmittance curves for a cotton leaf without apparent deficiencies in nutrition or moisture are given in Figure 7.

#### CHAPTER III

#### METHODS AND MATERIALS

### Cotton Plant Propagation

Seeds of Westburn 70 ( $\underline{Gossypium hirsutum L.}$ ) were germinated in vermiculite and transplanted to modified Hoagland solution in a controlled environment chamber. The recipe for the solution is given in Table I. The basic recipe was from Zyngas (44) with modification as suggested by Dr. Glenn Todd (37). Distilled water was used to make the solution and the pH was maintained at 6.0. The environmental chamber was scheduled for 12 hours of darkness at  $26.6^{\circ}$ C. ( $80^{\circ}$ F.) and 12 hours of light at  $29.4^{\circ}$ C ( $85^{\circ}$ F.).

Two cotton plants were grown in each of 26 alphabetically designated containers. Treatments 1, 2, 3, and 4 were randomly applied to the plants. The treatments represented 10, 5, 2.5 and 1 milliliters respectively, of sodium phosphate stock solution added to the 10 liters of solution in the containers. A sketch of container placement within the chamber and the treatment for each is presented in Figure 15. A picture of the arrangement is presented in Figure 16.

The plants were supported with their roots in solution by inserting the stem through a 1.3 cm hole in a small piece of wood attached to the lid of the container. A small steel bolt was vertically inserted in the wood to provide additional plant support, as shown in Figure 17.

TABLE I

MODIFIED HOAGLAND'S SOLUTION USED FOR GROWING COTTON PLANTS

Chemica1	Stock Solution Concentration (mg/liter)	Quantity of Stock Solution/10 liter H <sub>2</sub> 0		
CA(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	472.00	10 m1		
MgS0 <sub>4</sub> ·7H <sub>2</sub> 0	246.00	10 ml		
KNO <sub>3</sub>	250.00	25 ml		
NaH <sub>2</sub> PO <sub>4</sub> ·7H <sub>2</sub> O	123.00	10,5,2.5, 1 ml		
H <sub>3</sub> BO <sub>3</sub> —	2.40	•		
MnS0 <sub>4</sub> ·H <sub>2</sub> 0	1.20			
ZnS0 <sub>4</sub> ·7H <sub>2</sub> 0	0.36	10 . 1		
l l	race nerals 0.03	10 m1		
CoC1 <sub>2</sub> ·6H <sub>2</sub> 0	0.06			
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.20			
Fe-HEEDTA	(2 ppm Fe) —	10 m1		

All chemicals except the Fe-HEEDTA were reagent grade.

## Leaf Sampling

According to Gausman, Allen, Cardenas, and Richardson (22) there is a definite change in the reflectance of cotton leaves with age due to decrease in compactness of the internal cellular structure. Furthermore, a pronounced lacunose condition develops in the mesophyll of an aging leaf. Therefore, leaves were sampled from the fourth node down in

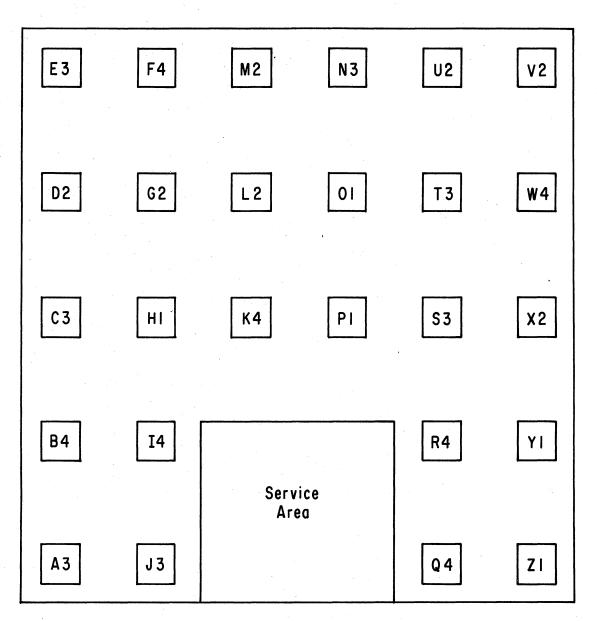


Figure 15. Layout of Containers in Growth Chamber, Alphabetically Designated, with Treatments 1, 2, 3, or 4 Given.



Figure 16. Cotton Plants Being Grown Hydroponically.



Figure 17. The Method of Supporting Tall Cotton Plants.

attempting to sample leaves of the same age. The leaf at the growth point was counted as leaf number one if it was approximately two centimeters or more in width. A leaf was plucked from each plant, the petiole clipped under water in order to eliminate the possibility of bubbles that might inhibit the uptake of water when the petiole was inserted into a test tube of water. The test tubes were supported upright in a bed of vermiculite contained in a common styrofoam container. This technique was used to prevent the loss of turgidity that occurs when leaves are removed from their source of moisture—the stem. The styrofoam container also permitted the easy transport and handling of samples with the petioles constantly immersed in water and the leaves protected from ambient temperatures. Moisture in the vermiculite bed supplied moisture to the air around the leaves, stabilized the material so it would support the test tubes, and maintained a lower than ambient temperature.

### Measurement of Leaf Thickness

Leaf thickness was measured immediately after collection and while leaves were near full turgidity. Thickness was measured with a linear variable displacement transducer (23) with the signal recorded by a Sandborn Recorder Model 321. The recorder's indicator was displaced one centimeter on the chart for each one-tenth millimeter of leaf thickness. For thickness measurements a leaf was positioned so that the venation on the abaxial side was up and the probe could be placed so as not to receive an inflated thickness value due to the heavy veins. The round probe was designed so that its diameter would be small enough to allow it to fit between the prominent rib-like veins. The probe had

a diameter of 0.558 cm and a cross-sectional area of 0.244  $\text{cm}^2$ . Figures 18 and 19 show location of typical thickness samples and measurement method.

## Sampling for Chemical Analysis

The check for this series of tests was to be the chemical analysis. In order to run a chemical analysis for phosphorus, a sample, 2.550 cm in diameter was cut from each leaf. The sample was taken between the characteristic main rib-like veins as shown in Figure 18. The samples were dried 24 hours at 100°C before being transferred to the Soil and Water Service Laboratory, Agronomy Department, Oklahoma State University, for analysis. The results were reported in micrograms per sample which were divided by sample dry weight to result in micrograms of phosphorus per gram of dried leaf sample. The data included sample dry weights, micrograms of phosphorus per sample (laboratory report), and phosphorus concentration in micrograms per gram (ppm) in the dried samples.

The average fresh leaf thickness for each sample was multiplied by the sample area and divided into the oven dried weight of the sample to give grams of dry material per cubic centimeter of green leaf tissue.

#### Leaf Reflectance Measurements

After each leaf was measured for thickness and a portion removed for chemical analysis, reflectance was measured on the remainder. The circular sample for chemical analysis was cut from one side of the leaf's mid-rib while the reflectance was measured on the upper surface of the opposite side of the leaf. The leaves were kept in the insulated

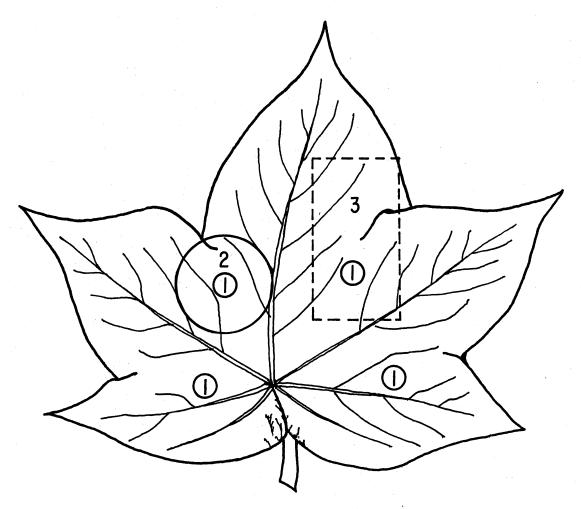


Figure 18. Sample Locations for: Four Leaf Thickness Measurements (1); Chemical Analysis (2); and Reflectance (3).

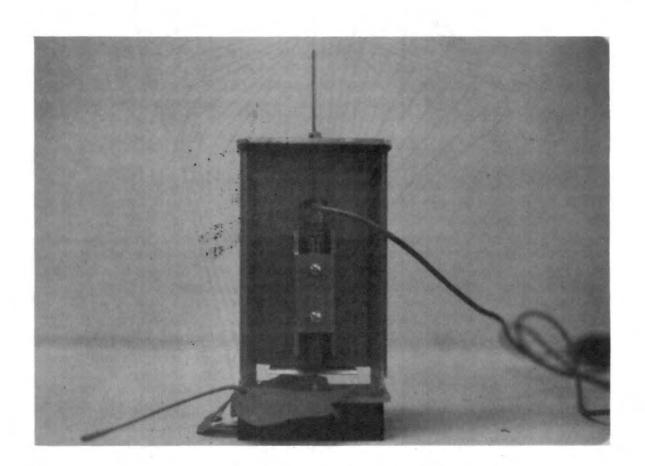


Figure 19. Measurement of Leaf Thickness.

chests with their petioles immersed in water until their reflectances were measured.

A Beckman DU-2 Spectrophotometer with a reflectance chamber attachment was used to measure the relative amounts of diffuse reflectance of each leaf between 400 and 700 nanometers. The instrument is designed to permit measurement of relative diffuse reflectance of each wavelength either by automatic or manual scanning as selected by the monochromator. A diagram of the optics of the monochromator is given in Figure 20. Path of its light beam through the reflectance chamber is shown in Figure 21, with a picture of the instrument and the recorder shown in Figure 22. Technical data, instrument descriptions and specifications are given in Beckman Preliminary Instructions 1291 (3). The light source used was the tungsten lamp, since all testing was to be in the visible range. It is used as one of the standards in spectrophotometry and is designated as CIE Source A which operates at a color temperature of 28540K.

The beam of radiant energy from the standard source A is reflected from either the sample or reference. Those diffuse rays reflected between 35° and 55° from the vertical are focused by an ellipsoidal mirror ring segment to strike a frosted quartz diffusing screen before reaching a phototube which measures the amount of energy that has at the screen (Figure 21). All reflectance measurements were relative, since the amount of reflected light was being compared to the amount reflected by a magnesium carbonate reference standard. Reflectance values from this instrument are different than those taken from the integrating-sphere method, in that the indicated values are relative and not absolute.

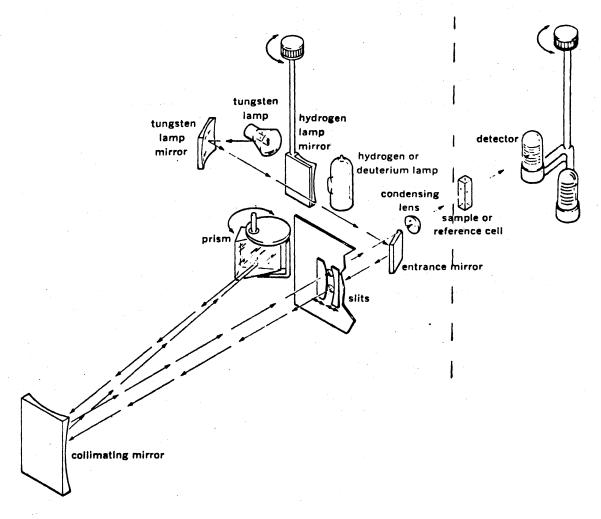


Figure 20. Optical Diagram for Beckman DU-2 Spectrophotometer (Absorptance) (3).

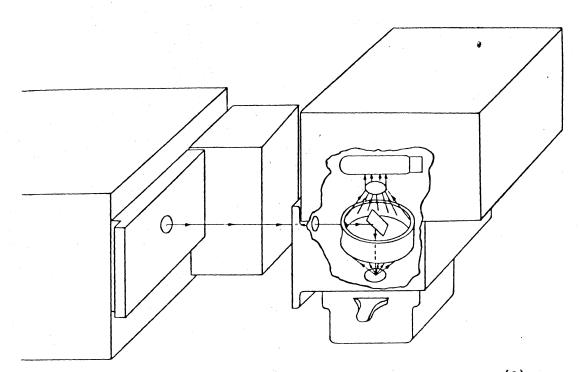


Figure 21. Optical Diagram for Reflectance Attachment (3).

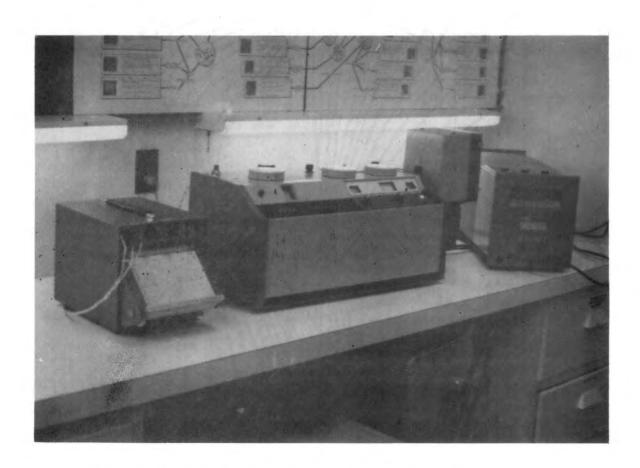


Figure 22. Beckman DU-2 Spectrophotometer and Recorder.

In order to get a reflectance curve from a sample, it was placed in the sample drawer along with the magnesium carbonate reference (Figure 23). The instrument was adjusted to read 100% reflectance for the reference standard at 550 nanometers, as this is the wavelength at which maximum reflectance occurs for cotton leaves.

The recorded response from the spectrophotometer was a curve giving the amount of reflectance of visual light from a cotton leaf, wavelength by wavelength as compared to the light reflected (100%) from a magnesium carbonate block at 550 nanometers.

After the reflectance curve was determined for each sample the relative percent reflectance was determined for the sample at selected wavelengths by manual operation of the spectrophotometer. At each selected wavelength and with the reference standard in position, the null meter was balanced at 100% reflectance. The sample was then moved into the beam and the null meter was again balanced to give a direct reading of relative percent reflectance on the instrument. The wavelengths selected were: 400, 495, 505, 525, 536, 550, 562, 610, 625, 675, and 700 nm. These wavelengths were selected after studying the reflectance curves of several cotton leaves and noting the positions of rapidly changing slope.

This method of determining reflectance for these specific wavelengths was more sensitive than the first described method whereby a continuous reflectance curve was constructed.

## The CIE Colorimetric System

The presence or absence of a nutrient, specifically phosphorus, may affect a leaf's color (13). An effort was made to relate lightness,



Figure 23. Sample Drawer of the Reflectance Attachment.

purity, and the dominant wavelength of a cotton leaf's color to the concentration of phosphorus in the leaf tissue.

One system of orderly specification and description of color is the CIE<sup>1</sup> system (2) (25). It is similar to other systems in that color determination is based on reflected light detected by a standard observer. The reflected light is from a standard source lamp. Products of the energy of the lamp, the percent reflectance, and the color matching functions (42), for wavelengths at a stated interval throughout the visual range are summed together to yield tristimulus values. These values constitute an intermediate step toward color specification.

Color matching functions are measures of the amount of three primary colors required to match a given spectral color. For convenience, the color matching functions,  $\bar{x}$ ,  $\bar{y}$ , and  $\bar{z}$  have been multiplied by the spectral energy distribution ( $E_A$ ) of CIE source A and listed by wavelength in three columns in Table XXI of Appendix D (42). To compute the tristimulus values X, Y, and Z, reflectance values at given wavelengths must be multiplied by the values of the respective columns of Table XXI. The equations for X, Y, and Z are as follows:

$$X = \sum_{\lambda} E_{A} \bar{x} R \tag{1}$$

$$Y = \sum_{\lambda} E_{A} \bar{y} R \qquad (2)$$

$$Z = \sum_{\lambda} E_{A} \bar{z} R \qquad (3)$$

Originally recommended in 1931 by the International Commission on Illuminations and designated in English-speaking countries generally as the ICI system, the system is now designated by the official abbreviation CIE adopted in 1951 from the French name, Commission Internationale de l'Eclairage (2, p.934).

X, Y, Z-----Tristimulus values

E<sub>A</sub>------ Spectral energy distribution, or relative energy at each wavelength for CIE Source A (color temperature of 2854<sup>O</sup>K)

 $\bar{x}$ ,  $\bar{y}$ ,  $\bar{z}$ ----- Color matching functions

R----- Percent reflectance

 $\lambda$  ----- Over all wavelengths in the visual range at a specified interval

The tristimulus values are an intermediate step in deriving useful values to specify color. Only Y which is a measure of brightness (luminance) has immediate use. The three values are used to calculate chromaticity coordinates x, y, and z for the CIE chromaticity diagram of Figure 24.

$$x = \frac{X}{X + Y + Z} \tag{4}$$

$$y = \frac{Y}{X + Y + Z} \tag{5}$$

$$z = \frac{Z}{X+Y+7} \tag{6}$$

Since x+y+z=1, only two of the coordinates, usually x and y, are used to locate a sample color on the chromaticity diagram. The locus of the diagram is a line connecting the points representing the coordinates of spectrum colors and is characteristically horseshoe in shape. The coordinates for the CIE standard source A are x=0.448 and y=0.408 (42). When the reflectance values of a surface are known for several wavelengths across the visual spectrum, the calculated chromaticity coordinates locate the surface's color on the diagram. A line is drawn from source A, through the point to locus intersection. At the intersection the dominant wavelength can be read and color purity calculated as demonstrated in Figure 24.

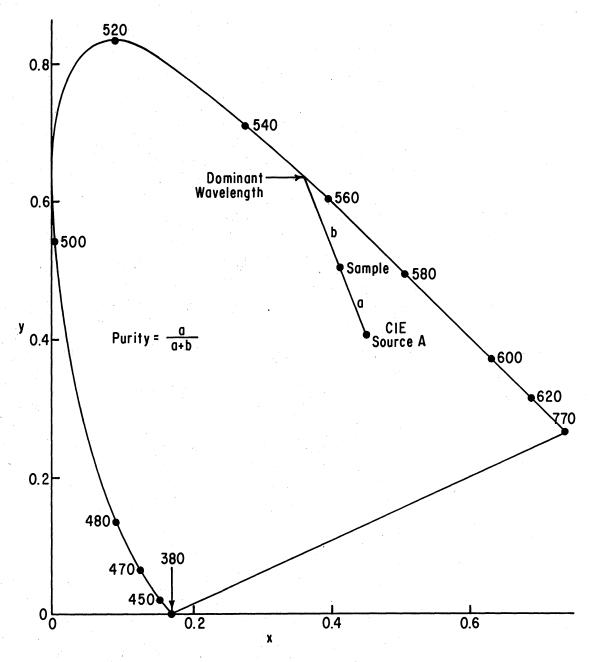


Figure 24. CIE Chromaticity Diagram with Dominant Wavelength and Purity Demonstrated.

#### CHAPTER IV

#### ANALYSIS AND PRESENTATION OF DATA

Raw data consisted of measurements of leaf thickness, quantitative analyses of phosphorus in leaf samples, weights of dried leaf samples before being subjected to chemical analysis, and relative light reflectances for selected wavelengths. Two sets of data were collected for the latter; one being compiled from readings directly from the spectrophotometer, and the second taken from curves of the instrument's readout. Data from leaves of plants growing in the same container were averaged for mean values.

Statistical analyses were done using the Statistical Analysis System, SAS, on the IBM 360-65 digital computer.

### Chemical Analysis

The Soil and Water Service Laboratory reported that the phosphorus content of the cotton leaf samples varied from 860 to 27,555 ppm. The array of analyses revealed that the top four values (27,555, 19,034, 15,669 and 12,555 ppm) were separated from the rest of the data, with 12,555 being 4,898 ppm above the next lower reading. These four data were discussed with the Head of the Analysis Laboratory<sup>1</sup>, who advised

Personal communication with Dr. John Baker, Soil Specialist, Cooperative Extension Service, Oklahoma State University, Stillwater, Oklahoma.

that these readings be dropped as being aberrant. These four analyses were not only isolated from the main body of data but were well outside the range of phosphorus content values as reported in other research (24). The phosphorus content value for sample F2 was not available due to miscalculation of sample dried weight. The remaining data ranged in phosphorus content from 0.086% to 0.76%, or 860 to 7657 ppm respectively. These data, arranged by treatment, are graphically displayed in Figure 25.

During plant growth, it was hypothesized that a decrease of phosphorus concentration in the substrate would be accompanied by a decrease of phosphorus concentration in the leaf tissue. An analysis of variance of the sample values is given in Table II.

TABLE II

ANALYSIS OF VARIANCE OF PHOSPHORUS CONCENTRATION

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	
Treatment	3	31624740	10541580	
Error	21	48890214	2328105	
F = 10541	580/2328105 = 4.53	Probability	> F = 1.33%	

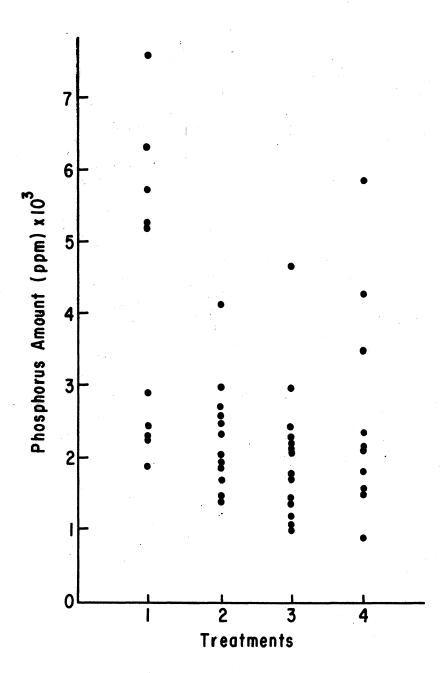


Figure 25. Phosphorus Concentration in Cotton Leaf Tissue.

The variance between the treatments is 4.53 times larger than the variance within the mean. Only 1.3% of similar comparisons would result in F values equal to or greater than this one; therefore, the null hypothesis of identical populations is rejected.

Although the means did not continuously decrease with reduced amounts of  $NaH_2PO_4$  added to the substrate as shown in Table III, it appears that the availability of phosphate did have an overall influence on phosphorus concentration, as there was an apparent shift of quantity values to lower amounts with decrease in availability, although treatment 4 does not fit smoothly into the trend.

TABLE III

PHOSPHORUS CONCENTRATION, TREATMENT
AND OVERALL MEANS

Treatment	Mean (micrograms/gm)	Difference (micrograms/gm)
1	4187	
4	2575	1612
2	2277	298
3	1980	297
Overal1	2667	2207

Seventy-three percent of the difference between treatments was between treatments 1 and 4. The remainder was evenly apportioned to the differences between treatments 4 and 2 and treatments 2 and 3. Plants grown in the solution of least available phosphorus had the second highest concentration of phosphorus present in their leaf tissue. These results are not without precedence, as similar results were reported by Johan (24). In general, the decreasing phosphorus concentration in leaf tissue was associated with lower amounts of available phosphorus, with the exception noted. The relatively high phosphorus content for leaves produced under treatment 4 caused further investigation. An analysis of variance of plant height based on phosphorus availability resulted in an F value of 9.75. This statistic indicates that phosphorus availability did control plant height, but due to the small growth under treatment 4, the small leaves were richer in phosphorus than those of treatments 2 and 3. Plant heights of treatments 2 and 3 favorably compared with plants grown under treatment 1.

Means of the treatments are listed in Table IV. These values indicate that little relative difference in height was experienced for treatments 1, 2 and 3 but plants of treatment 4, with the second highest phosphorus content, were on an average 18.6 cm shorter than plants from treatment 3. The plants' growth was inhibited by the low phosphorus application.

Statistical analyses of variance of other selected dependent variables whose regression on phosphorus content were inspected, are summarized in Table V. Those variables considered were reflectance values at 550 nm and the highest reflectance encountered under automatic spectrophotometer operation, luminance (Y), dominant wavelength,

and color purity. The latter three variables resulted from the processing of curve reflectance data to arrive at CIE colorimetric system values.

TABLE IV
PLANT HEIGHT MEANS (cm)

Treatment 1	Treatment 2	Treatment 3	Treatment 4
52.0	56.1	49.3	30.7

TABLE V

REGRESSION OF DEPENDENT VARIABLES ON CHEMICAL ANALYSIS (CHEM)

Dep. Var.	F. Value	Prob. > F	Corr. Coeff.	Mean	S. D.
R550	0.002	95.3%	0.000	11.1	1.18
HREF	0.002	96.7%	0.000	11.1	1.17
Υ	0.092	76.3%	0.002	6.2	0.76
DWL	2.847	9.9%	0.061	557.92	5.75
PUR	1.896	17.6%	0.041	0.573	0.05

All of the F values and correlation coefficients are low and indicate that none of these variables regress on the phosphorus content of the leaves. Each of these relationships will be discussed further under appropriate later sections.

#### Reflectance

All raw reflectance data are given in relative percentages as the reflectance of the leaves are compared to a standard as described under "Methods and Materials." Two sets of data were recorded for each leaf sample. One was read from reflectance curves which were automatically drawn by the recorder attached to the spectrophotometer, while the second set of data was created by taking individual reflectances of selected wavelengths impinging on the samples.

#### Reflectance Values at Selected Wavelengths

The reflectance data as recorded from individual observations for eleven selected wavelengths are listed in Table XX of Appendix C. From these data, Table VI gives the means for treatments for the selected wavelengths and gives the overall mean for each of them. Three curves are drawn to display the data, with a curve for treatment 1, a curve for treatment 4, and a curve for treatments 2 and 3, and the overall mean. These curves are shown in Figure 26. The curve representing the two treatments and the overall mean is drawn without discernible distortion as the maximum reflectance difference between the three sets of means is 0.15%. The reflectance curves of treatments 1 and 4 represent the maximum and minimum reflectances encountered respectively.

TABLE VI

MEANS OF MONOCHROMATIC REFLECTANCE CURVES FOR COTTON
LEAVES GROWN WITH DIFFERENT LEVELS OF PHOSPHORUS AVAILABLE IN THE SUBSTRATE
(% REFLECTANCE)

Wavelength (nm)	Trt. 1	Trt. 2	Trt. 3	Trt. 4	Overall Mean
400	5.01	4.83	4.86	4.58	4.81
495	4.74	4.55	4.51	4.58	4.60
505	5.40	5.07	5.00	5.00	5.12
525	9.27	8.41	8.46	8.04	8.54
536	11.25	10.17	10.19	9.55	10.29
550	12.06	10.96	10.96	10.14	11.03
562	11.29	10.18	10.21	9.54	10.31
610	6.39	5.71	5.71	5.62	5.86
625	5.85	5.24	5.22	5.18	5.37
675	4.53	4.27	4.24	4.28	4.33
700	8.01	7.55	7.41	7.27	7.56

The leaves from the substrate with the highest application of phosphorus (treatment 1) also had the highest reflectance, whereas, the substrate with the lowest phosphorus application produced plants whose leaves reflected the lowest amounts of all wavelengths.

The relationships between reflectances and treatment means are shown more distinctly in Figure 27. In every case, the reflectance for

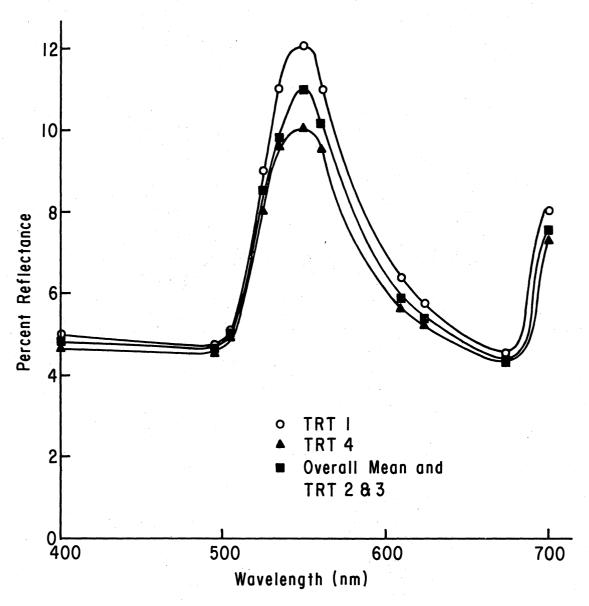


Figure 26. Light Reflectance Curves for Cotton Leaves, Spectrophotometer Under Manual Operation.

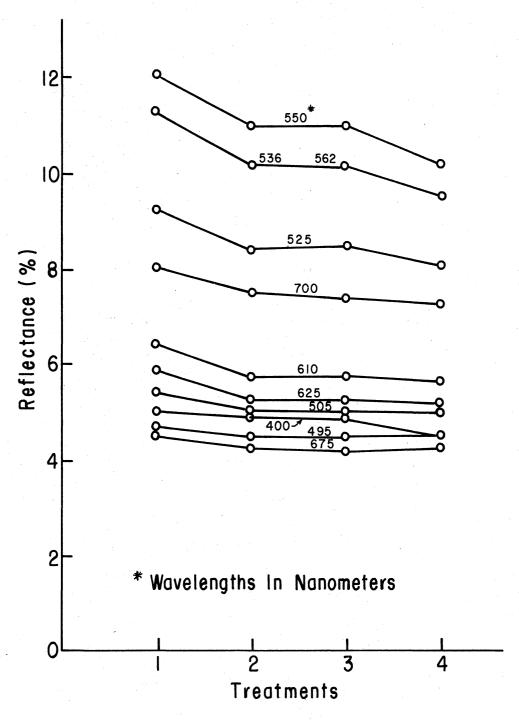


Figure 27. Reflectance Values at Selected Wavelengths for Four Levels of Phosphorus Availability.

treatment 1 was highest of all treatments. All wavelength reflectance means for treatment 2 and 3, except that for 700 nm, were within 0.07% of each other. They were of equal value twice, while treatment 2 exceeded treatment 3 values five times and the reverse occurred four times. Treatment 4 had the lowest values in every case, except for wavelengths 495, 505, and 675 nm, in which case they exceeded values of treatment 2 twice by a maximum of 0.03% and exceeded treatment 3, three times with a maximum of 0.07%.

It is not known under which treatment the cotton plants would have been the most productive, but according to data of this study, only plants of treatment 4 showed height deficiency. High, intermediate and low phosphorus applications can be detected by inspecting plant heights and comparing reflectance levels. As evidenced by treatments 2 and 3, there is a range of phosphorus availability that does not correspondingly cause a change in the magnitude of reflectance values. If later tests should show that treatments 1 and 4 are excessive and deficient in phosphorus availability, respectively, the data displayed here indicates that these levels could be reflectively detected. Likewise, reflectance that falls within the values spanned by treatments 2 and 3 would indicate that a sufficient level of phosphorus is available for satisfactory production.

An analysis of variance was carried out on the selected wavelength reflectance data to compare the treatment means with the pot means.

The pot means were comprised of data from each of the two plants growing in the pot. The results are listed in Table VII.

A study of the F ratios reveals there is no evidence that the treatments positively affected the reflectance at 495 and 675 nm since

TABLE VII

ANALYSIS OF VARIANCE FOR SELECTED
WAVELENGTH DATA

Wavelength (nm)	Source	s.s.	D.F.	M.S.	F Ratio	Prob. > F.
400	Between Trt. Within Trt.	1.15 2.82	3 22	0.38 0.12	2.99	5.2%
495	Between Trt. Within Trt.	0.32 3.42	3 22	0.11 0.16	0.69	57.0%
505	Between Trt. Within Trt.	1.14 4.09	3 22	0.38 0.18	2.04	13.6%
<b>5</b> 25	Between Trt. Within Trt.	8.98 17.10	3 22	2.99 0.77	3.85	2.3%
536	Between Trt. Within Trt.	16.94 22.93	3 22	5.65 1.04	5.41	0.6%
550	Between Trt. Within Trt.	21.44 23.85	3 22	7.15 1.08	6.60	0.3%
562	Between Trt. Within Trt.	18.01 24.23	3 22	6.00 1.10	5.45	0.6%
610	B <b>etw</b> een Trt. Within Trt.	4.14 9.72	3 22	1.38 0.44	3.13	4.5%
<b>62</b> 5	Between Trt. Within Trt.	3.29 8.73	3 22	1.10 0.40	2.75	6.5%
675	Between Trt. Within Trt.	0.58 4.13	3 22	0.19 0.19	1.00	39.9%
700	Between Trt. Within Trt.	3.41 10.11	3 22	1.14 0.46	2.47	8.8%

the means of the treatments are not as disperse as would be expected if they were from different populations. The probabilities of computing larger F ratios from means of the same population are 57% and 39.9% respectively for the two wavelengths. On the other hand, for wavelengths 525, 536, 550, 562, and 610 nm the statistical evidence is strong that the means are from different populations, as the probability of a larger F ratio for means of the same populations would be 4.5% or less. Roughly, reflectances in the outer thirds of the visual range do not appear to be as affected by phosphorus availability as do reflectances in the middle third or in the zone of green wavelengths.

The selected arbitrary levels of phosphorus produced detectable reflectance differences in the wavelengths spread from 525 to 625 nm. Further work on relating reflectance to phosphorus content should be directed to wavelengths in this range, as the response was more evident in this area. There was not a reflectance-phosphorus content relationship that was apparent on an individual plant basis as demonstrated by Figure 28. Predictions as to phosphorus content from reflectance values must be on a treatment and not individual basis. Data given here indicates that the reflectance of light at 550 nm was the most responsive to treatment influence.

# Reflectance Values at 10 nm Intervals from 400 to 700 nm

The set of data as given in Table XIX, Appendix C, were taken from reflectance curves drawn on strip charts. Before the reflectance curve was drawn, the spectrophotometer's null meter was calibrated to read 100% reflectance at 550 nm for the reference magnesium carbonate block. After calibration, a sample was moved into position, into the monochro-

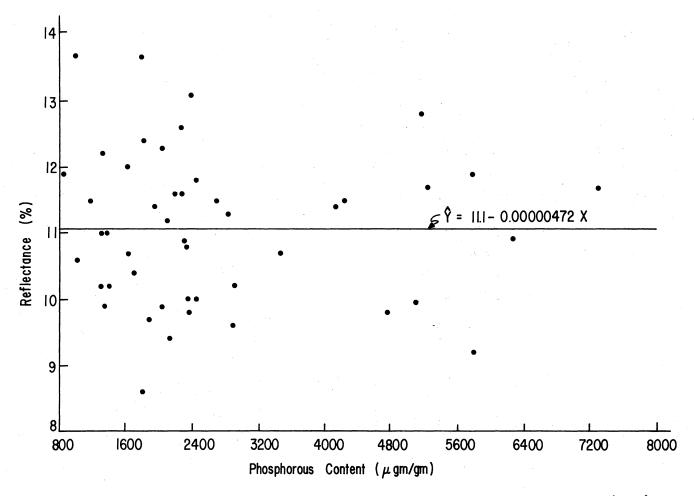


Figure 28. Regression of Relative Reflectance on Phosphorus Concentration in Cotton Leaf Tissue.

matic light beam, and a continuous reflectance curve was drawn for lightwaves between 400 and 700 nm. Curves for the means of the treatments and for the overall mean are shown in Figure 29 and the values from which the curves were plotted are given in Table VIII. The curves depict low reflectance in the blue and red spectrums but relatively high reflectance in the green wavelengths. The maximum reflectance occurred at or near 550 nm. The curves are coincident at both extremes of the visual range but diverge at wavelengths in the 500 to 600 nm range. The peaks of the curves from 530 to 580 nm have been amplified and are presented in Figure 30.

# Tristimulus Values and Luminance

As was discussed under CIE Colormetric System the raw reflectance data was used to generate other data to be used in studying whether the color of leaves as determined by colormetric parameters could be related to the phosphorus concentration within the leaves.

The method of data generation was by the weighted ordinate method as detailed earlier. The resulting parameters were: the tristimulus values X, Y, and Z; the chromaticity coordinates x, y, and z; dominant wavelength; and color purity. These values are tabulated in Table XXII of Appendix D. A summarization is given in Table IX, which lists the maximum, minimum and mean for all parameters for each treatment and for overall values. All data used in statistical analysis were container means.

The tristimulus values represent the amounts of the three primary spectral lamps required to describe a color. Only Y is used as computed as it is a measure of the luminance or brightness of a sample's color.

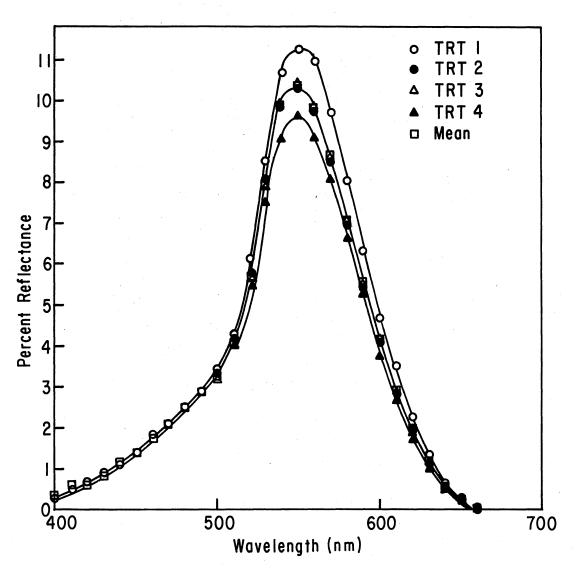


Figure 29. Light Reflectance Curves for Cotton Leaves, Spectrophotometer Under Automatic Operation.

TABLE VIII

MEAN REFLECTANCE VALUES FROM CURVE DATA
(%)

iavelength		Treatment Means				
(nm)	<u> </u>	2	3	4	Mean	
400	0.33	0.34	0.34	0.30	0.33	
410	0.50	0.49	0.50	0.48	0.49	
420	0.77	0.74	0.71	0.69	0.72	
430	0.90	0.89	0.88	0.86	0.88	
440	1.09	1.10	1.13	1.10	1.11	
450	1.45	1.42	1.41	1.40	1.42	
460	1.84	1.82	1.79	1.81	1.82	
470	2.12	2.09	2.09	2.09	2.10	
480	2.49	2.44	2.44	2.48	2.46	
490	2.89	2.87	2.83	2.89	2.87	
500	3.41	3.33	3.25	3.37	3.33	
510	4.29	4.21	4.16	4.16	4.20	
520	6.13	5.89	5.81	5 <b>.6</b> 6	5.85	
530	8.58	8.09	8.11	7.57	8.05	
540	10.66	9.90	9.94	9.19	9.86	
550	11.29	10.31	10.31	9.62	10.31	
5 <b>6</b> 0	10.93	9.80	9.74	9.16	9.83	
570	9.69	8.51	8.55	8.07	8.63	
580	8.00	6.90	6.91	6.61	7.04	
590	<b>6.</b> 35	5.33	5.32	5.22	5.50	
600	4.72	3.89	3.82	3.87	4.03	
610	3.49	2.71	2.62	2.69	2.84	
620	2.30	1.73	1.68	1.82	1:85	
630	1.33	1.40	0.94	1.05	1.16	
640	0.67	0.72	0.47	0.52	0.59	
650	0.25	0.37	0.19	0.22	0.26	
660	0.12	0.15	0.09	0.09	0.11	
670	0.01	0.00	0.02	0.01	0.01	
680	0.00	0.00	0.00	0.00	0.00	
690	0.00	0.00	0.00	0.00	0.00	
700	0.00	0.00	0.00	0.00	0.00	

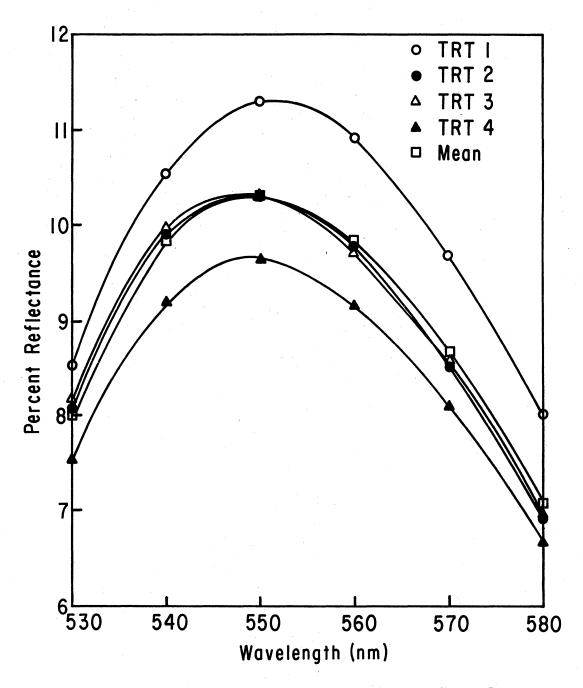


Figure 30. Amplification of the Upper Portion of the Reflectance Curves of Figure 29.

TABLE IX
SUMMARY OF COLORMETRIC PARAMETERS

Para-	•		Trea	tment		Overall
meter		1	2	3	4	Mean
Х	Min.	4.10	3.76	3.31	3.53	3.31
	Max.	5.74	5.67	6.43	5.81	6.43
	Mean	5.22	4.53	4.43	4.32	4.58
Υ	Min.	5.84	5.99	5.04	4.63	4.63
	Max.	7.74	7.25	8.01	7.12	8.01
	Mean	6.84	6.11	6.07	5.80	6.16
Z	Min.	0.654	0.616	0.614	0.560	0.560
	Max.	0.796	0.812	0.836	0.803	0.836
	Mean	0.725	0.714	0.725	0.681	0.711
х	Min.	0.377	0.356	0.358	0.363	0.356
	Max.	0.424	0.415	0.423	0.425	0.425
	Mēan	0.408	0.398	0.393	0.398	0.398
y	Min.	0.523	0.519	0.527	0.521	0.519
	Max.	0.558	0.567	0.561	0.556	0.567
	Mean	0.534	0.538	0.542	0.536	0.538
Z	Min.	0.0515	0.0543	0.0496	0.0542	0.0543
	Max.	0.0650	0.0769	0.0813	0.0810	0.0813
	Mean	0.0572	0.0635	0.0646	0.0660	0.0628
-Pur.	Min.	0.553	0.501	0.469	0.468	0.468
	Max.	0.649	0.627	0.660	0.628	0.660
	Mean	0.609	0.571	0.566	0.553	0.572
DWL	Min.	552.04	544.84	543.55	545.19	543.55
	Max.	565.06	563.06	565.23	565.30	565.30
	Mean	561.01	<b>5</b> 57.91	556.28	557.35	557.92

The Y value for a perfectly reflecting surface is normalized to 100% in the following manner (42):

$$Y = \sum_{\lambda} \bar{y} E_{A} R$$
 (normalizing factor) = 100.00 (7)

normalizing factor = 
$$100/1078.96 = 0.09268$$
 (8)

Equation (7) differs from equation (2) in that a normalizing factor has been used which permits all surfaces to be compared to the reference surface on a percentage basis. All surfaces will have brightnesses between 0 and 100%. The Y axis is perpendicular to the chromaticity plane of the chromaticity diagram at the CIE source as shown in Figure 31. Samples located on the diagram close to the axis will have relative high brightness values, whereas those colors located close to the spectrum locus will have low luminance.

The treatments did affect luminance as there was more variance between means of the treatments than the variance between container means. The analysis of variance is given in Table X.

TABLE X

ANALYSIS OF VARIANCE OF LUMINANCE (Y)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Treatment Error	3 22	6.60 11.76	2.20 0.53
F = 2.20/0.53 = 4.11		Probability > F	= 1.8%

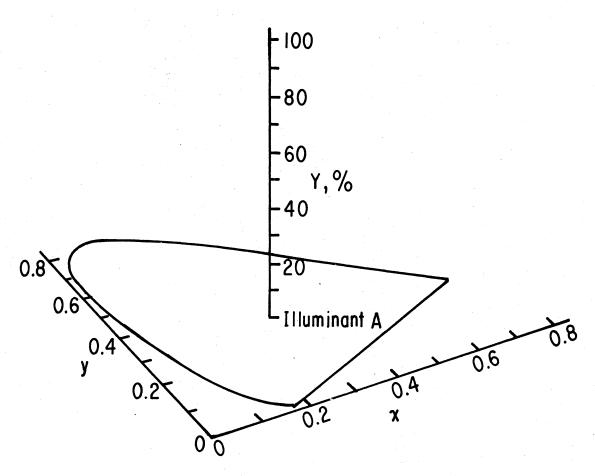


Figure 31. Luminance Axis for CIE Source A Located on the Chromaticity Diagram (6).

It is known that certain nutrients' presence within leaf tissue will affect its color. Apparently the availability of phosphorus in the substrate did influence its concentration in the leaf tissue, which in turn affected the quantity and quality of light reflected.

A relationship between Y values and the chemical analyses was sought, but the resulting F value of 0.09 and correlation coefficient of 0.002 (Table V) did not suggest that such a relationship existed.

The relationship between luminance and the reflectance values at 550 nm was studied. This wavelength was selected for study in relation to Y due to its proximity to the wavelength at which maximum reflectance was experienced for cotton leaves. Inspection of Table IX also reveals that values for Y of the three tristimulus values would be the largest for reflectance from green surfaces.

By the SAS program the regression of Y on reflectance at 550 nm was investigated. The regression equation was:

$$Y = -.018 + 0.563R_{550 \text{ nm}}$$
 (9)

The F ratio given in Table XI indicates that the variance of the regression from population mean was considerably higher (298.6 times) than the variance of the deviations from regression. The Y values were not accurately estimated by a mean since they change with reflectance values. The data points lie close to the calculated regression line and the correlation coefficient of 0.86 also indicates close relationship between luminance and reflectance.

The data did not indicate phosphorus content discrimination. Some leaves with high phosphorus content had relatively low Y values, whereas, leaves with low phosphorus content not uncommonly had average or high values.

TABLE XI

ANALYSIS OF REGRESSION VARIANCE OF Y

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Regression	1	241791.6	241791.6
Deviations from Regression	49	39670.9	809.6
F = 241791.6/8	09.6 = 298.6	Probability > F = (	).01% r = 0.8

# **Chromaticity Coordinates**

Data from Table XIX, Appendix C, were first used to compute the tristimulus values after which these newly computed values were used to compute chromaticity coordinates x, y and z by formulas (4), (5), and (6). As explained under the CIE Colormetric System, only x and y were utilized for further investigation. For each leaf reflectance curve, the data were used to calculate these two values which were the coordinates for leaf colors on the chromaticity diagram. A summary of means by treatment is presented in Table XII.

Analysis of variance on the data yielded F ratios of 0.82 and 0.74 for the variables x and y respectively. These statistical values indicate that the availability of phosphorus to the plant did not significantly affect the mean, as there was more variance within treatments than between them.

TABLE XII
CHROMATICITY COORDINATES, TREATMENT, AND OVERALL MEANS

Treatment	Coordina	ates
	<b>X</b>	У
1	0.408	0.535
2	0.398	0.538
3	0.393	0.542
4	0.398	0.536
verall Mean	0.398	0.538

The coordinates for all samples were plotted and are shown on an enlarged portion of the chromaticity diagram of Figure 32. A regression of y on x was assumed and a curve computed from the data points. The sample regression equation of y on x as computed by the SAS program was:

$$y = 0.77 - 0.59x$$
 (10)

The correlation coefficient was -0.87 which indicates a close relationship between the two variable coefficients.

The overall means for the x and y chromaticity coordinates are used to locate a sample in Figure 33, which depicts the average position of all samples.

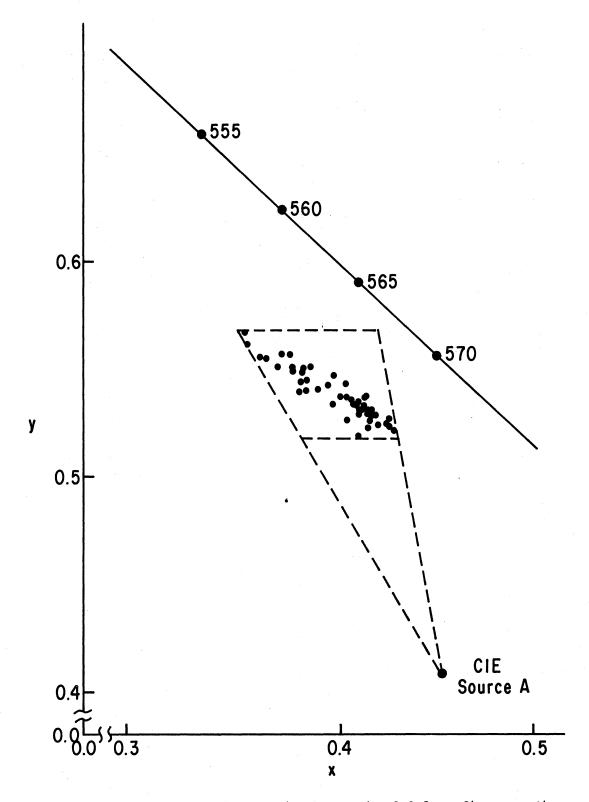


Figure 32. Chromaticities of the Cotton Leaf Colors Shown on the Pertinent Portion of the CIE Chromaticity Diagram.

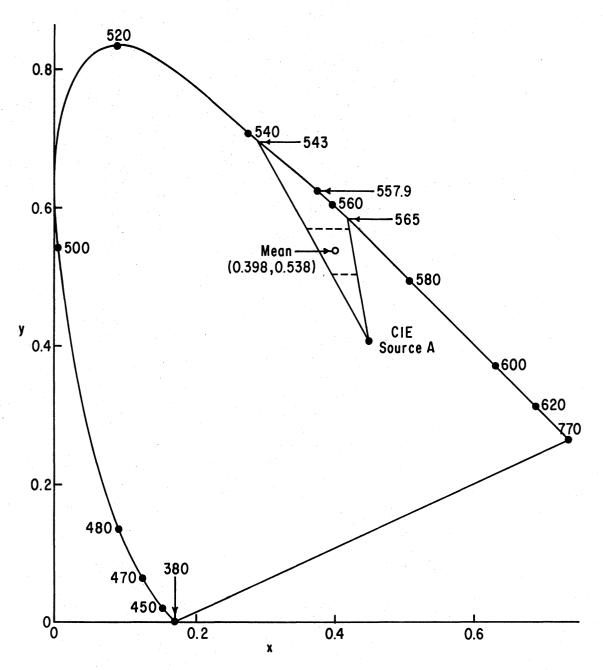


Figure 33. Location of the Overall Chromaticity Mean and Dominant Wavelength for the Cotton Leaf Samples Tested.

# Dominant Wavelength and Purity

Each surface presents a color to the standard observer that is a composite of the reflected wavelengths. The color can be represented by a single wavelength which has a theoretical color purity of 100%, and lies on the spectrum locus—a spectrum color.

The mean dominant wavelength of 557.9 nm for all observations is shown in Figure 33. The minimum, maximum and mean dominant wavelengths for each treatment are listed in Table IX. As would be expected, the sample dominant wavelengths lie within the green portion of the chromaticity diagram. They are spread from 543.6 to 565.3 nm, spanning 21.7 wavelengths on the locus. The analysis of variance of dominant wavelengths given in Table XIII showed that the treatments did not have an affect on the means, as the F ratio was below unity. There was as much variance within the treatment means as there was between them.

Regression of dominant wavelength and purity of colors of the samples on phosphorus concentration did not give F and r values (Table V) that indicated significant relationships. The phosphorus content in the leaf was not the controlling element in relation to leaf color and purity.

## Leaf Thickness

Four thickness readings were taken for each leaf and averaged for a mean value. The strip chart of the recorder was graduated to permit estimation of leaf thickness to the nearest five thousandths of a millimeter. The average measured thickness for all leaves of all treatments

TABLE XIII

ANALYSIS OF VARIANCE OF DOMINANT WAVELENGTHS

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Between Trt.	3	137.5	45.8
Within Trt.	22	1157.2	52.6
F = 45.8/5	2.6 = 0.87	Probability > F	= 52.6%

TABLE XIV

ANALYSIS OF VARIANCE OF LEAF THICKNESS

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Treatment	3	0.0055	0.0018
Error	22	0.0545	0.0025
F = 0.00	018/0.0025 = 0.744	Probability >	F = 54%

was 0.207 mm which compares favorably with an average thickness of 0.212 mm for 200 mature cotton leaves as determined in other work (1). The array of individual thickness measurements followed by container and treatment means are listed in Table XVI, Appendix A.

Results of the analysis of variance (Table XIV) indicate that the availability of phosphorus had no measurable effect on leaf thickness. The null hypothesis that the population means are the same for all treatments is supported by the small value of the F statistic. Fifty-four percent of the time a larger F value would be realized due to either an increase in the effect of the treatment or a decrease in sampling variance.

The curve for the regression of reflectance at 550 nm on leaf thickness is given in Figure 34. Equation for the curve is:

$$Y = 9.52 + 6.94X. (11)$$

The 95% confidence interval includes only 14 of the 51 data points. The correlation coefficient for the data is 0.04, which is evidence for a null relationship. The predicted reflectance values for the regression curve spanned upward from 10.42% to 11.46%, a spread of 1.04%, whereas the data ranged from 8.6% to 13.7%, a difference of 5.1%. The variation in thickness did not affect reflectance and collaborates the results of Myers (29), who concluded that reflectance was the same for one leaf or any number of stacked leaves within the visual wavelength range.

## Specific Weight

Oven dry weights, average leaf thicknesses, and specific weights, in graps per cubic centimeter for the samples are listed in Table XVII,

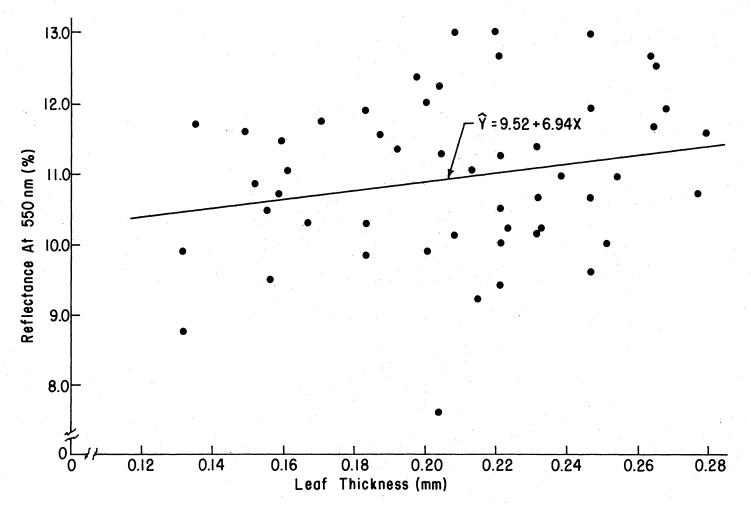


Figure 34. Regression of Relative Reflectance on Cotton Leaf Thickness.

Appendix A. Means of specific weights are listed in Table XV.

TABLE XV

SPECIFIC WEIGHTS, TREATMENT AND OVERALL MEANS (gm/cm<sup>3</sup>)

Treatment				Overall
1	2	3	4	<del>-</del>
0.157	0.193	0.182	0.142	0.171

According to the mean specific weight values listed above, it appears that the mean value for treatment 1 does not follow the trend suggested by the other three. While phosphorus availability did not consistently dictate the leaves' specific weights, an F value of 3.03 from an analysis of variance does give evidence of a weak influence. There was a 5.1% probability of experiencing a larger ratio for this population. This analysis, which indicated a weak effect of phosphorus availability on specific weight, was in agreement with the results of the regression of specific weight on phosphorus concentration as shown in Figure 35. The scatter of data and a correlation coefficient of -0.27 also indicates a loose relationship between the two factors.

Regression of the reflectance--specific weight relationship is

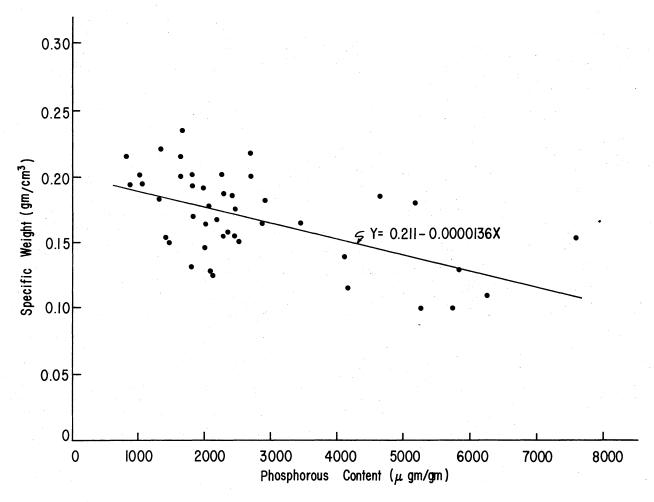


Figure 35. Regression of the Specific Weight on Phosphorus Concentration in Cotton Leaves.

shown in Figure 36. An r value of 0.09 gave very strong indication there was no correlation between the two characteristics.

#### Discussion

The array of treatment reflectance means in Table VI and the reflectance curves for different levels of phosphorus availability given in Figures 26 and 29 reveal that leaves of plants grown under treatment 4 had the lowest reflectance levels. The differences in reflectance for the treatments were most pronounced between 525 and 562 nm. The highest reflection values and widest spread of the reflectance curves for all treatments occurred at the 550 nm wavelength.

In view of the above findings, it is demonstrated that phosphorus availability levels could be indicated by reflectance percentages.

Reflectance was not closely related to phosphorus content of the leaves, as shown in Figure 28, but did consistently indicate by lower reflectance means those plants grown under low phosphorus availability levels.

The monochromatic reflectance data collected during this work suggests that an instrument capable of supplying light and measuring its reflectance in the wavelengths about 550 nm could be used to detect levels of phosphorus availability. This idea is reinforced by noting the reflectance patterns in Figure 27 of monochromatic light at the 525, 536, 550, and 562 nm wavelengths.

Although it is interesting to note that the mean overall dominant wavelength of 557.92 lies in the yellowish green area of the chromaticity diagram (6) and that the overall purity was 57% (Table IX), these chromaticity values are so closely related to reflectance values that

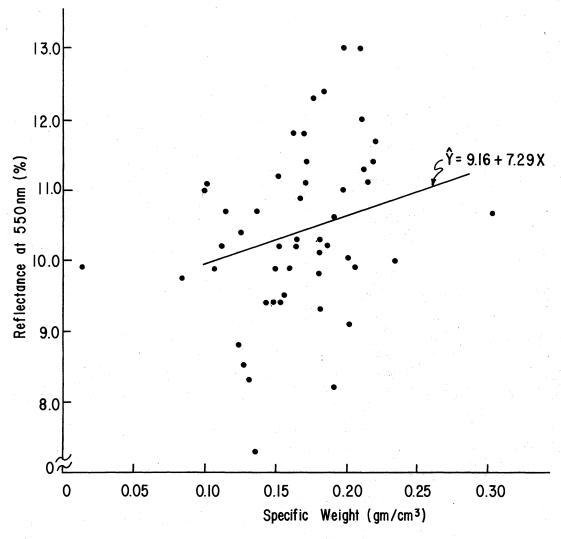


Figure 36. Regression of Relative Reflectance on the Specific Weight of Cotton Leaves.

they do not suggest a solution of phosphorus availability discernment distinct within themselves.

#### CHAPTER V

#### SUMMARY AND CONCLUSIONS

The objectives of this research were to: (1) Determine if the quantity and quality of light reflected from a cotton leaf can be used to give an indication of the amount of phosphorus concentration in the leaf; (2) Find the relationship between reflectance and the phosphorus concentration if it exists; (3) Establish a signature reflectance curve in the visual range for the cotton variety used in the study; (4) Determine if the colormetric parameters can be useful in relating to a cotton leaf's phosphorus concentration; and (5) Investigate the possibility of relationships between light reflectance, specific weight, and leaf thickness.

Cotton plants were grown hydroponically in a modified Hoagland solution with  $NaH_2PO_4$ , one constituent of the solution being applied in four different concentrations. A leaf of the same morphological age was plucked from each of the plants.

The leaves were measured for thickness, specific weight, phosphorus concentration, reflectance quantity and quality in the visual spectrum from 400 to 700 nm.

The reflectance measurements from one set of data were converted to colormetric parameters in order that a study of relationships between phosphorus presence and the parameters could be examined.

#### Conclusions

Conclusions drawn from analysis of the data are:

- 1. The different levels of NaH<sub>2</sub>PO<sub>4</sub> availability did affect the phosphorus content but not in a linear manner. The amount of phosphorus in an aqueous solution does not necessarily indicate the amount that will be found in the cotton leaf's tissue. Plants grown under the treatments did have means that were significantly different from each other.
- 2. The lowest phosphorus availability treatment resulted in poor plant growth with rather small leaves which were often higher in phosphorus content than leaves from the higher availability treatments.
- 3. The reflectance curves gave an indication of phosphorus availability, not phosphorus concentration in the leaves. The highest availability treatment resulted in highest reflectance values and likewise the lowest availability yielded data which gave the lowest reflectance curve.
- 4. Reflectance differences of leaves grown under different treatments were most discernible at 550 nm.
- 5. The physical properties of leaf thickness and specific weight did not indicate phosphorus content or reflectance character-istics.
- A characteristic reflectance curve for a cotton population can be determined, but curves for different populations may coincide.

- 7. Colorimetric parameters are easily computed from reflectance data and can be used to determine the purity, dominant wavelength, and lightness characteristics. Although these values define a color in three dimensions there does not appear to be a pragmatic relationship between a leaf sample's color and phosphorus deficiency.
- 8. All colors of the samples were located on the chromaticity diagram so that a curve describing the regression of y on x had the equation:

$$y = 0.77 - 0.59 x \tag{12}$$

#### Recommendations for Further Study

The results of this study have demonstrated that monochromatic light is reflected in different amounts by leaves of plants grown under different levels of phosphorus availability.

Additional studies should be carried out to determine if reflectance of the 550 nm wavelength or some close by wavelength gives maximum sensitivity to levels of phosphorus availability. The number of treatments need to be increased with small phosphorus availability differences to test the sensitivity of the reflection response.

Since there was detection of availability levels at the 550 nm wavelength, further investigation needs to be initiated to study the feasibility of constructing an instrument for the rapid detection of these phosphorus levels.

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# APPENDIX A

LEAF THICKNESS AND SPECIFIC
WEIGHT DATA

TABLE XVI

LEAF THICKNESS MEASUREMENTS (mm)

Leaf			ding		Leaf	Pot
Sample	1	2	3	4	Avg.	Avg.
A13 A23	0.210 0.255	0.210 0.240	0.190 0.210	0.280 0.210	0.222 0.229	0.226
B14 B24	0.320	0.210 0.220	0.235 0.240	0.225 0.245	0.248 0.231	0.240
C13 C23	0.220 0.150	0.195 0.175	0.190 0.175	0.195 0.165	0.200 0.166	0.183
D12 D22	0.240 0.180	0.200 0.180	0.200 0.195	0.180 0.195	0.205 0.188	0.196
E13 E23	0.120 0.250	0.155 0.200	0.125 0.245	0.160 0.190	0.130 0.221	0.176
F14 F24	0.190 0.230	0.200 0.230	0.210 0.255	0.180 0.265	0.195 0.244	0.220
G12 G22	0.150 0.200	0.145 0.230	0.160 0.260	0.170 0.230	0.156 0.230	0.193
H11 H21	0.200 0.250	0.210 0.205	0.190 0.170	0.225 0.185	0.206 0.202	0.204
I14 I24	0.210 0.245	0.235 0.235	0.190 0.220	0.250 0.215	0.221 0.229	0.225
J13 J23	0.235	0.285 0.250	0.250 0.290	0.275 0.305	0.261 0.280	0.270
K14 K24 L12	0.325 0.305	0.250 0.235	0.220 0.240	0.305 0.195	0.275 0.244	0.260
L22 M12	0.250 0.260 0.215	0.255 0.230	0.205 0.295	0.265 0.260 0.210	0.244 0.261	0.252
M22 N13	0.155 0.170	0.200 0.160 0.175	0.185 0.185 0.230	0.145 0.160	0.202 0.161 0.184	0.102
N23 011	0.170 0.135 0.245	0.145 0.265	0.230 0.145 0.265	0.195 0.230	0.155 0.251	0.170
021 P11	0.245 0.225 0.265	0.270 0.250	0.260 0.255	0.230 0.220 0.270	0.244	0.240
P21 Q14	0.210 0.210 0.210	0.230 0.230 0.220	0.195 0.210	0.240 0.240	0.219 0.220	0.210
Q24 R14	0.245 0.210	0.160 0.215	0.195 0.245	0.205 0.185	0.201 0.214	0.240
R24 S13	0.260 0.220	0.270 0.190	0.285	0.250 0.185	0.266	0.210
S23 T13	0.215 0.240	0.230 0.210	0.240 0.220	0.200 0.205	0.221 0.219	0.216
T23 U12	0.215	0.195 0.140	0.230 0.140	0.210 0.150	0.212 0.152	0.156
U22 V12	0.160 0.215	0.190 0.175	0.145 0.165	0.140 0.175	0.159 0.182	0.182

TABLE XVI (CONTINUED)

		<del></del>				
Leaf Sample	1	Rea 2	ding 3	4	Leaf Avg.	Pot Avg.
V22 W14 W24 X12 X22 Y11 Y21 Z11 Z21	0.110 0.140 0.130 0.195 0.260 0.210 0.175 0.160	0.125 0.155 0.170 0.180 0.280 0.150 0.170 0.130	0.140 0.155 0.160 0.170 0.215 0.175 0.185 0.125	0.145 0.135 0.170 0.185 0.195 0.145 0.180 0.125	0.130 0.146 0.158 0.182 0.238 0.170 0.182 0.135	0.138 0.170 0.204 0.158
Mean (TRT)	0.211	0.190	0.207	0.218		
Overal	l Mean					0.207

TABLE XVII

COTTON LEAF SPECIFIC WEIGHTS

Leaf Sample	Oven Dry Wt. (gm)	Avg. Leaf Thickness (cm)	Specific Weights (gm/cm <sup>3</sup> )
A13	0.0206	0.0222	0.182
A23 B14	0.0243 0.0193	0.0229 0.0248	0.208 0.152
B24	0.0178	0.0231	0.150
C13	0.0161	0.0200	0.158
C23	0.0154	0.0166	0.182
D12	0.0161	0.0205	0.154
D22	0.0210	0.0188	0.219
E13 E23	0.0122 0.0163	0.0130 0.0221	0.184 0.144
F14	0.0163	0.0195	0.164
F24		0.0244	
G12	0.0161	0.0156	0.202
G22	0.0225	0.0230	0.192
H11 H21	0.0195 0.0170	0.0206	0.185
114	0.0142	0.0202 0.0221	0.165 0.126
124	0.0177	0.0229	0.151
J13	0.0238	0.0261	0.178
J23	0.0282	0.0280	0.197
K14	0.0155	0.0272	0.110
K24 L12	0.0207 0.0219	0.0244	0.166
L12	0.0219	0.0244 0.0261	0.176 0.170
M12	0.0229	0.0202	0.170
M22	0.0268	0.0161	0.326
N13	0.0159	0.0184	0.169
N23	0.0186	0.0155	0.235
011 021	0.0197	0.0251	0.154
P11	0.0262 0.0283	0.0244 0.0260	0.210
P21	0.0200	0.0219	0.213 0.179
Q14	0.0176	0.0220	0.156
Q24	0.0139	0.0201	0.135
R14	0.0141	0.0214	0.129
R24 S13	0.0292 0.0221	0.0266	0.215
S23	0.0221	0.0200 0.0221	0.216 0.128
T13	0.0221	0.0219	0.128
T23	0.0198	0.0212	0.183
U12	0.0145	0.0152	0.187

TABLE XVII (CONTINUED)

Leaf Sample	Oven Dry Wt. (gm)	Avg. Leaf Thickness (cm)	Specific Weights (gm/cm <sup>3</sup> )
U22	0.0113	0.0159	0.139
V12	0.0123	0.0182	0.132
V22			
W14	0.0129	0.0130	0.194
W24	0.0087	0.0146	0.117
X12	0.0163	0.0158	0.202
X22	0.0079	0.0182	0.085
Y11	0.0139	0.0238	0.114
Y21	0.0089	0.0170	0.102
Z11	0.0093	0.0182	0.100
Z21	0.0105	0.0135	0.152
*			

Diameter of sample = 2.550 cm. Area = 5.107 cm<sup>2</sup>.

Specific weight =  $\frac{\text{Oven dried sample weight, gm}}{(5.107 \text{ cm}^2) \text{ (Avg. thickness, cm)}}$ 

APPENDIX B

CHEMICAL ANALYSIS DATA

TABLE XVIII

DETERMINATION OF PHOSPHORUS CONTENT

	*		
Leaf Sample	Dry Weight (gm)	Laboratory Report Amt. of P (µgm)	Phosphorus Concentration (µgm/gm or PPM)
A13	0.0206	26.8	1301
A23	0.0243	25.1	1033
B14	0.0193	28.5	1477
B24	0.0178	26.8	1506
C13 C23	0.0161 0.0154	38.5 45.2	2391 2935
D12	0.0154	40.2	2497
D22	0.0210	57.0	2714
E13	0.0122	57.0	4672
E23	0.0163	33.5	2055
F14	0.0163	33.5	2055
F24		33.5	
G12	0.0161	46.9	2913
G22	0.0225	45.2	2009
H11	0.0195	46.9	2405
H21 I14	0.0170 0.0142	48.6 30.2	2859 2127
114 124	0.0142	41.9	2367
J13	0.0238	50.2	2109
J23	0.0282	33.5	1188
K14	0.0155	427.1	27555
K24	0.0207	72.0	3478
L12	0.0219	55.0	2511
L22	0.0226	41.9	1854
M12	0.0229	31.8	1389
M22 N13	0.0268 0.0159	38.5 35.2	1436 2214
N23	0.0139	31.8	1710
011	0.0197	45.2	2294
021	0.0262	48.6	1855
P11	0.0283	65.3	2307
P21	0.0200	103.9	5195
Q1	0.0176	335.0	19034
02	0.0139	217.8	15669
R14	0.0141	82.1	5823 860
R24 S13	0.0292 0.0221	25.1 36.9	1670
S23	0.0221	30.2	2097
T13	0.0221	21.8	986
T23	0.0198	26.8	1354
U12	0.0145	33.5	2310

TABLE XVIII (CONTINUED)

Leaf Sample	Dry Weight (gm)	Laboratory Report Amt. of P (μαm)	Phosphorus Concentration (µgm/gm or PPM)
U22	0.0113	46.9	4150
V12	0.0123	23.4	1902
V22			
W14	0.0129	23.4	1814
W24	0.0087	36.9	4241
X12	0.0163	26.8	1644
X22	0.0079	100.5	12722
Y11	0.0139	87.1	6266
YŽĪ	0.0089	46.9	5269
ŽII	0.0093	53.6	5763
Z21	0.0105	80.4	7657

APPENDIX C

REFLECTANCE DATA

TABLE XIX

REFLECTANCE VALUES OF COTTON LEAF SAMPLES FROM SPECTROPHOTOMETRIC CURVES

																				1											
	400	410	420	430	440	. 450	460	470	480	490	500	510	( λ = 520	400 530	.700,2 540	λ= 10 550	<u>)</u> 560	570	580	590	600	610	620	630	640	650	660	670	680 6	90	700
A1 A2 B1 B2 C1 C1 D1 D2 F1 E2 F1 F2 G1 H1 L1 L1 L2 M1	0.3 0.5 0.4 0.4 0.4 0.4 0.4 0.4 0.4 0.5 0.4 0.3 0.3 0.3 0.3 0.3 0.3 0.3 0.3 0.3 0.3	0.5 0.6 0.6 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5	0.7 0.8 0.8 0.7 0.6 0.6 0.6 0.6 0.7 0.8 0.8 0.7 0.7 0.7 0.7 0.7 0.7 0.7 0.7 0.7 0.7	0.9 1.0 0.8 0.8 0.8 1.0 0.9 0.8 0.8 1.0 0.9 0.8 0.8 0.9 0.9 0.9 0.9 0.9 0.9	1.2 1.3 1.3 1.2 1.1 1.3 1.4 1.2 1.3 1.4 1.2 1.0 0.9 1.1 1.1 1.0 1.0 1.1 1.1 1.1 1.1 1.1 1.1	1.4 1.6 1.6 1.4 1.3 1.5 1.6 1.6 1.6 1.6 1.5 1.3 1.3 1.3 1.3 1.5 1.6 1.6 1.6 1.5 1.4 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5	1.8 1.9 2.1 2.0 1.8 1.6 1.9 1.6 1.6 1.6 1.6 1.6 1.6 1.8 1.8 1.8 1.8 1.8 1.8 1.8 1.8 1.8 1.8	2.2 2.3 2.1 2.2 2.3 2.2 2.2 2.3 1.9 1.9 2.2 2.0 2.0 2.0 2.0 2.0 2.0 2.0 2.0 2.0	2.4 2.6 2.7 2.4 2.5 2.5 2.5 2.5 2.7 2.7 2.7 2.5 2.5 2.5 2.5 2.7 2.5 2.5 2.5 2.5 2.5 2.5 2.5 2.5 2.5 2.5	2.9 3.1 3.2 2.8 3.0 2.9 3.3 3.1 2.6 3.0 2.9 3.8 3.0 2.9 2.9 3.8 2.9 2.9 3.8 2.9 3.0 2.9 3.0 2.9 3.0 2.9 3.0 2.9 3.0 2.0 3.0 3.0 3.0 3.0 3.0 3.0 3.0 3.0 3.0 3	3.3 3.6 3.8 2.3 3.6 3.1 4.1 3.7 4.1 3.1 4.3 3.3 3.5 3.3 3.2 3.3 3.2 3.3 3.3 3.3 3.3 3.3 3.3	4.0 4.6 4.5 4.2 4.5 5.0 4.3 3.5 4.6 4.0 3.3 5.0 4.6 4.0 3.3 7.5 4.2 4.0 3.3 7.5 4.0 4.0 3.3 4.0 4.0 4.0 4.0 4.0 4.0 4.0 4.0 4.0 4.0	5.0 5.3 5.8 4.3	7.1 9.0 8.9 8.1 8.0 10.5 8.0 10.5 8.6 8.0 10.5 8.8 7.8 9.0 10.5 8.8 7.3 7.6 9.0 7.5 7.6 9.0 7.5 9.0 10.5 10.5 10.5 10.5 10.5 10.5 10.5 10	9.2 9.9 9.1 9.9 9.4 11.4 9.7 9.7 10.5 10.5 10.7 10.0 9.3 11.9 10.7 10.0 10.5 11.6 9.3 11.4 11.6 9.3 11.4 11.6 9.3 11.4 11.6 11.6 11.6 11.6 11.6 11.6 11.6	11.4 11.8 11.6 10.5 10.9 10.0 12.1 12.3 10.0 7.2 8.5 11.3 11.1 10.4 12.8 10.3 10.2	9.9 8.7 8.6 8.5 8.0 8.5 8.2 9.9 9.0 8.1 10.3 10.3 10.3 10.0 11.4 10.7 8.1 10.0 11.8 12.0 9.1 10.8 10.0 11.8 10.0 11.8 10.0 11.8 10.0 10.0	9.0 7.1 7.2 6.6 7.2 7.9 7.0 6.9 9.1 8.9 9.1 8.9 9.0 8.9 9.0 10.0 8.8 9.9 9.0 10.0 8.9 9.0 9.0 9.0 9.0 9.0 9.0 9.0 9.0 9.0 9	7.0.66.5.41.6.5.9.6.5.9.6.5.9.6.5.9.6.5.9.6.5.9.7.7.7.5.6.0.7.7.0.9.4.8.1.1.3.0.9.6.5.5.6.5.0.0.7.7.0.9.4.8.5.5.6.5.0.0.0.7.7.0.9.4.8.5.5.6.5.0.0.0.0.7.7.0.9.4.8.5.5.6.5.0.0.0.0.0.0.0.0.0.0.0.0.0.0.0.0	5.7 3.6 4.0 4.0 4.5 5.0 4.3 4.4 4.0 5.0 4.3 5.0 6.0 6.0 7.5 6.0 7.5 7.5 7.5 7.5 7.5 7.5 7.5 7.5	4.4 2.3 3.2.8 2.7 2.1 2.4 2.1 3.5 3.5 2.3 3.5 2.4 4.1 4.3 3.6 3.6 3.6 3.6 3.6 3.6 3.6 3.6 3.6 3	1.4 2.2	2.1 0.8 1.4 0.7 1.1 1.0 1.7 1.2 1.2 1.2 1.4 1.4 1.9 2.4 2.0 0.8 1.7 1.1 1.2 1.2 1.2 1.2 1.3 1.1 1.0 1.1 1.1 1.0 1.1 1.1 1.0 1.1 1.0 1.0	1.2 0.4 0.8 0.5 0.3 0.6 0.6 0.6 0.7 0.8 1.0 0.8 1.0 0.8 1.0 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0	0.6 0.2 0.2 0.2 0.3 0.3 0.3 0.4 0.4 0.3 0.3 0.4 0.4 0.3 0.3 0.4 0.4 0.2 0.2 0.3 0.3 0.3 0.3 0.3 0.3 0.3 0.3 0.3 0.3	0.2 0.1 0.2 0.1 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2	0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1	0.1	(No Ref Record		nce
W1 W2 X1 X2 Y1 Y2 Z1 Z2	0.2 0.3 0.2 0.3 0.4 0.3	0.4 0.5 0.5 0.4 0.5 0.5 0.5	0.7 0.8 0.6 0.7 0.8 0.8	0.8 0.9 0.9 0.8 0.8 1.0	1.0 1.1 1.0 1.0 1.0 1.2 1.1	1.3 1.4 1.4 1.2 1.3 1.6 1.6	1.7 1.9 1.7 1.6 1.7 1.9 2.0	2.0 2.1 2.0 1.8 2.0 2.2 2.2	2.3 2.6 2.3 2.0 2.2 2.6 2.6 2.5	2.7 3.0 2.8 2.4 2.6 3.0 3.0 2.9	3.0 3.5 3.0 2.9 3.0 3.3 3.4 3.2	3.8 4.3 3.9 3.6 3.6 4.0 4.2 4.0	4.9 6.1 5.0 4.9 5.8 5.9 5.2	6.5 8.4 7.3 7.0 7.0 8.0 8.0 7.8	8.0 10.1 9.2 9.0 9.1 10.0	10.1 9.7 10.1 11.0	8.0 10.0 10.0 9.2 10.1 11.0 10.8	7.0 9.0 9.0 8.1 9.2 10.0 9.7 9.9	4.4 5.8 5.9 5.1 6.3 6.9	3.3 4.2 4.5 4.0 5.0 5.0 5.0	2.3 3.0 3.2 3.0 3.8 4.0 3.8	1.6 2.0 2.1 2.0 2.6 2.8 2.5 2.9	1.0 1.3 1.0 1.7 1.7 1.4	0.4 0.6 0.7 0.5 0.8 0.8	0.2 0.3 0.2 0.3 0.3 0.3	0.1 0.1 0.1 0.1 0.1 0.2 0.1	0.1				

TABLE XX

REFLECTANCE VALUES OF COTTON LEAF SAMPLES
AT SELECTED WAVELENGTHS (%)

	400	495	505	Ligh 525	t Wave 536	length 550	(nm) 562	610	625	675	700
A13 A23 B14 B24 C13 C13 C12 E13 E23 F14 F24 G12 H11 H21 H21 H21 H21 H21 H21 H21 H21 H	5.4.8.7.8.6.9.9.3.1.4.9.5.5.5.4.4.4.4.5.5.5.5.4.4.4.4.5.5.4.4.4.5.5.5.5.4.4.4.4.5.5.5.5.4.4.4.4.5.5.5.5.4.4.4.4.5.5.5.5.4.4.4.4.5.5.5.5.4.4.4.4.5.5.5.5.4.4.4.4.5.5.5.5.4.4.4.4.5.5.5.5.4.4.4.4.5.5.5.5.4.4.4.4.5.5.5.5.4.4.4.4.5.5.5.5.4.4.4.4.5.5.5.5.4.4.4.4.5.5.5.5.4.4.4.4.5.5.5.5.4.4.4.4.5.5.5.5.4.4.3.4.4.5.5.4.4.5.5.5.5	4.6697328604.30791231489777765418906667287502 4.6697328604.30791231489777765418906667287502	55555445555555555555555555555555555555	8.1.90689647.12681.525183425458067.50391337.52 10.1.2.6.8.1.52.5.1.8.3.4.2.5.4.5.8.0.6.7.5.0.3.9.1.3.3.7.5.2	9.4 9.7 9.5 9.5 9.5 9.7 9.7 10.5 10.1 10.7 10.7 10.7 10.7 10.7 10.7 10.7	10.2 10.6 9.2 10.6 9.2 10.5 10.5 10.5 10.5 11.3 10.6 11.6 10.7 11.6 11.6 11.6 11.6 11.6 11.6 11.6 11	9.4 9.3 9.5 9.6 9.5 9.6 9.6 9.6 9.6 9.6 9.6 9.6 9.6	55.6.4.4.1.3.8.1.1.8.0.4.0.1.9.2.7.6.1.0.8.3.4.4.6.8.3.2.5.7.7.8.2.8.7.1.0.1.6.1.6.1.6.1.6.1.6.1.6.1.6.1.6.1.6	9312991378271454873674877237871049616641 65555654465555555555556666534655655655	14222952020945022896936664298608543904389	77.6.6.6.0.2.7.7.1.3.8.2.2.9.6.9.9.6.7.7.7.7.7.7.8.8.8.7.6.6.8.7.7.7.7.7.7

TABLE XX (CONTINUED)

	400	495	505	Ligh 525	t Wave 536	length 550	(nm) 562	610	625	675	700
U12 U22 V14 V24 W14 W24 X12 X22 Y11 Y21 Z11 Z21	4.5 5.0 4.9  4.8 4.6 5.0 4.3 4.8 5.3 5.3	4.2 4.5 4.6  4.3 4.7 4.4 4.0 4.3 4.8 5.0 4.7	4.7 5.3 4.9  4.5 5.4 4.8 4.5 5.6 5.3	8.0 8.8 7.3  6.8 9.6 8.1 7.6 8.2 9.0 9.2 8.9	10.2 10.7 8.5  8.2 10.7 10.0 9.6 10.1 11.1 11.0 10.9	10.8 11.4 9.7  8.6 11.5 10.7 10.0 10.9 11.7 11.9 11.7	9.9 10.6 8.6  7.9 10.9 10.0 9.2 10.1 11.0 11.0	5.4 6.0 4.7  4.4 5.9 5.6 5.3 5.6 6.1 6.0	5.0 5.5 4.4  4.3 5.4 5.1 4.8 5.2 5.6 5.7 5.5	3.9 4.4 3.7  3.7 4.1 4.3 3.8 4.1 4.4 4.4	7.3 8.1 7.1  6.4 7.2 7.3 7.7 7.5 7.7 8.1 8.0

APPENDIX D

COLORIMETRIC TABLES

PRODUCTS OF THE RELATIVE SPECTRAL ENERGY VALUES OF CIE STANDARD SOURCE A AND THE COLOR MATCHING FUNCTIONS OF THE 1931 STANDARD OBSERVER (42)

Wavelength (nm)	ΧE	$\sqrt{YE}$ ( $\lambda = 400700n$	
(nm)  400 410 420 430 440 450 460 470 480 490 500 510 520 530 540 550 560 570 580 590 600 610 620 630 640	0.21 0.77 2.82 7.00 10.00 11.12 11.00 8.38 4.61 1.73 0.29 0.61 4.59 13.10 24.96 40.27 59.45 81.68 104.86 124.93 137.07 136.69 122.71 96.89 70.76	0.01 0.02 0.08 0.29 0.66 1.26 2.27 3.90 6.71 11.21 19.33 33.23 51.48 68.21 82.00 92.45 99.50 102.04 99.56 92.15 81.42 68.58 54.72 39.97 27.65	m;Δλ = 10nm)  1.00 3.67 13.56 34.18 50.14 58.64 63.13 55.20 39.23 25.08 16.28 10.45 5.67 3.34 1.74 0.81 0.39 0.23 0.19 0.13 0.10 0.04 0.03 0.00 0.00
650 660 670 680 690 700	46.79 28.36 15.62 8.68 4.36 2.26	17.66 10.49 5.72 3.15 1.57 0.81	0.00 0.00 0.00 0.00 0.00

x = 0.448, y = 0.408 = Chromaticity coordinates for CIE Source A

TABLE XXII

COLORIMETRIC PARAMETERS

Pot Plant Trt.	X	Y	Z	x	У	Z	Pur.	DWL
A13 A23 B14 B24 C13 C23 D12 E13 E23 F14 F24 G12 G22 H11 H21 J13 J23 K14 K24 L12 M12 M12 M12 M13 N23 O21 P11 P21 Q14 R24 R24 R24	4.70 3.86 3.56 3.584 3.584 4.17 4.610 1.77 4.610 4.71 4.71 4.71 4.71 4.71 4.71 4.71 4.71	6.10 6.347 5.47 5.47 5.47 5.47 5.49 5.50 6.884 6.15 6.89 6.163 6.16	0.712 0.777 0.803 0.795 0.692 0.660 0.739 0.812 0.664 0.774 0.769 0.774 0.654 0.643 0.606 0.779 0.694 0.756	0.408 0.358 0.363 0.363 0.366 0.374 0.382 0.371 0.380 0.385 0.410 0.387 0.387 0.387 0.387 0.392 0.413 0.385 0.414 0.409 0.383 0.415 0.407 0.407 0.407 0.403 0.411 0.405 0.405 0.425	0.530 0.561 0.556 0.558 0.558 0.558 0.559 0.551 0.5550 0.5531 0.5531 0.531 0.531 0.5337 0.5337 0.5337 0.5331 0.5331 0.5331 0.5331 0.5331	0.0619 0.0816 0.0792 0.0810 0.0765 0.0689 0.0736 0.0769 0.0769 0.0774 0.0706 0.0753 0.0720 0.0701 0.0650 0.0650 0.0659 0.0648 0.0559 0.0648 0.0559 0.0557 0.0557 0.0557 0.0533 0.0533 0.0543 0.0563 0.0563 0.05594 0.0653 0.05594	0.578 0.469 0.468 0.470 0.541 0.503 0.545 0.545 0.523 0.524 0.553 0.553 0.5541 0.553 0.561 0.591 0.592 0.627 0.630 0.649 0.558 0.649 0.558 0.649 0.558	560.95 543.55 550.43 545.19 547.39 551.58 552.01 544.84 553.98 552.02 552.04 552.04 555.85 562.55 554.13 563.63 561.64 562.55 563.63 561.64 562.55 559.80 559.80 559.80 559.80 559.80 559.80

TABLE XXII (CONTINUED)

Pot Plant Trt.	X	Υ	Z	X	у	Z	Pur.	DWL
V12	4.15	5.24	0.652	0.413	0.522	0.0649	0.557	561.80
W14	3.73	5.01	0.655	0.397	0.534	0.0697	0.527	556.80
W24	4.75	6.38	0.735	0.400	0.538	0.0620	0.582	559.01
X12	4.81	<b>6.</b> 18	0.677	0.412	0.530	0.0580	0.605	562.28
X22	4.38	5.74	0.616	0.408	0.535	0.0573	0.610	561.37
Y11	5.17	6.38	0.655	0.424	0.523	0.0536	0.632	565.06
Y12	5.52	6.92	0.752	0.418	0.524	0.0570	0.608	563.64
ŽII	5.31	6.78	0.758	0.413	0.528	0.0589	0.598	562.32
Z21	5.47	6.82	0.711	0.421	0.524	0.0547	0.625	564.39

VITA V

## Yack Clayton Moseley Candidate for the Degree of Doctor of Philosophy

Thesis: DETECTION OF THE PHOSPHORUS LEVEL IN COTTON LEAVES BY

SPECTROPHOTOMETRY

Major Field: Agricultural Engineering

Biographical:

Personal Data: Born near Izoro, Coryell County, Texas, June 20, 1927, the son of Randall L. and Mildred Moseley; married to Genevela Getchell Moseley, and father of Jeffrey Alan, John Randall, Brenda Gail and Scott Andrew.

Education: Graduated from Pearl High School in Pearl, Texas in 1944; commissioned into the U. S. Air Force in June, 1948, after completing the Air Force Officer Candidate School; received wings as a military pilot in May of 1950 after completing the U. S. Air Force Flying School in Enid, Oklahoma; graduated from Texas A & M University with Bachelor of Science in Agricultural Engineering in May, 1956; received the Master of Science degree in Agricultural Engineering from Oklahoma State University in August, 1958; completed the requirements for the Doctor of Philosophy degree from Oklahoma State University in May, 1975.

Professional Experience: Graduate teaching assistant from September, 1956 to January, 1958 in Agricultural Engineering Department at Oklahoma State University; Extension Agricultural Engineer for New Mexico State University, Las Cruces, New Mexico from March, 1958 to June, 1962; Assistant Professor of Agricultural Engineering, College of Agriculture, Haile Sellassie I University, Dire Dawa, Ethiopia, June, 1962 to August, 1968; Graduate Research Assistant in the Agricultural Engineering Department, Oklahoma State University, September, 1968 to June, 1973; Assistant Professor, Department of Agriculture, Sam Houston State University, Huntsville, Texas, until the present.