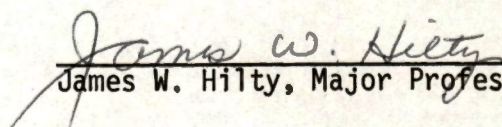
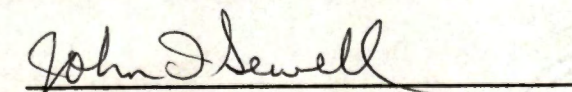
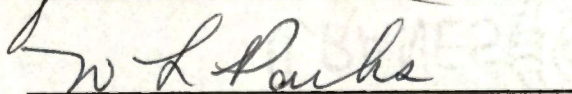


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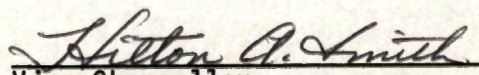
I am submitting herewith a thesis written by Marion Dake Ellis entitled "A Study of Histological Changes in Corn Leaves Under Stress with an Investigation of Their Photographic Density Using Infrared Film." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agricultural Biology.

  
James W. Hilty, Major Professor

We have read this thesis  
and recommend its acceptance:

Accepted for the Council:

  
Vice Chancellor  
Graduate Studies and Research

A STUDY OF HISTOLOGICAL CHANGES IN CORN LEAVES UNDER STRESS  
WITH AN INVESTIGATION OF THEIR PHOTOGRAPHIC DENSITY  
USING INFRARED FILM

A Thesis  
Presented for the  
Master of Science  
Degree  
The University of Tennessee

Marion Dake Ellis

December 1974

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## ABSTRACT

Hickory King corn was germinated in sand and then supplied nutrients using Hoagland's solutions. When the corn plants reached the three-leaf stage, four kinds of stress were induced in separate pots in order to study their photographic density using infrared film as it related to the internal structure of the leaves. The four stresses used in this study were: disease caused by maize dwarf mosaic virus, manganese toxicity, nitrogen deficiency, and water deficiency. Leaves from the four stress conditions and from control plants were examined microscopically to determine what anatomical anomalies could be found to explain their photographic densities as imaged on infrared film.

Photographs were made with a Pentax single lens reflex 35 mm camera using Kodak E-4 Ektachrome infrared film, Batch 36128-UQ, and a Tiffen 12 filter over the lens. Photographs were evaluated using a Macbeth Quantalog Transmission Densitometer, Model TD 404.

Sections were cut from leaves immediately after photographing and fixed in FAA. These sections were then dehydrated in TBA and sectioned in paraffin. Sections were stained in Safranin-O and Fast Green and evaluated for anatomical changes.

Results of this study showed that photographic density increased with increasing leaf thickness when a red densitometer filter was used. Gross morphology of the corn leaves was evaluated qualitatively with respect to the several theories of light reflectance from leaves.

Parameters considered in morphological evaluations included: number and size of vacuoles in parenchymal tissue; presence of air spaces in the mesophyll; size, number, and shape of mesophyll cells; size of air pockets under stomata; number of chloroplasts; size and shape changes in bulliform cells; and the amount of protoplasm in mesophyll cells.

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## CHAPTER I

### INTRODUCTION

A knowledge of how solar radiation interacts with vegetation is essential for the correct interpretation of remote sensing data from agricultural sources. The actinic infrared region, 700-900 nanometers, has proven to be very useful in surveying agricultural resources (Applied Infrared Photography, 1972). The reason for this phenomena can be seen by looking at the reflectance spectrum for a typical plant leaf (Figure 1).

Plant leaves typically have a low reflectance in the visible region of the spectrum (400-700 nanometers) due to the strong absorption by chlorophylls; likewise, in the infrared beyond 1300 nanometers plant leaves have a low reflection due to the strong absorption by water (Gates, et al., 1965). The relatively high reflection peak occurs in the near infrared where all of the incident radiation is scattered within the leaf, with no absorption. A large percent of this scattered radiation is then reflected, giving the high peak shown in Figure 1. Beyond the ends of the range shown in Figure 1, in other words, in the ultraviolet for the shorter wavelengths and in the far infrared for the longer wavelengths, leaf reflectance is at a low and relatively uniform level, generally less than 5 percent (Gates and Tantraporn, 1952).

Since as early as 1918, plant physiologists have offered explanations for the high infrared reflectivity of leaves. These

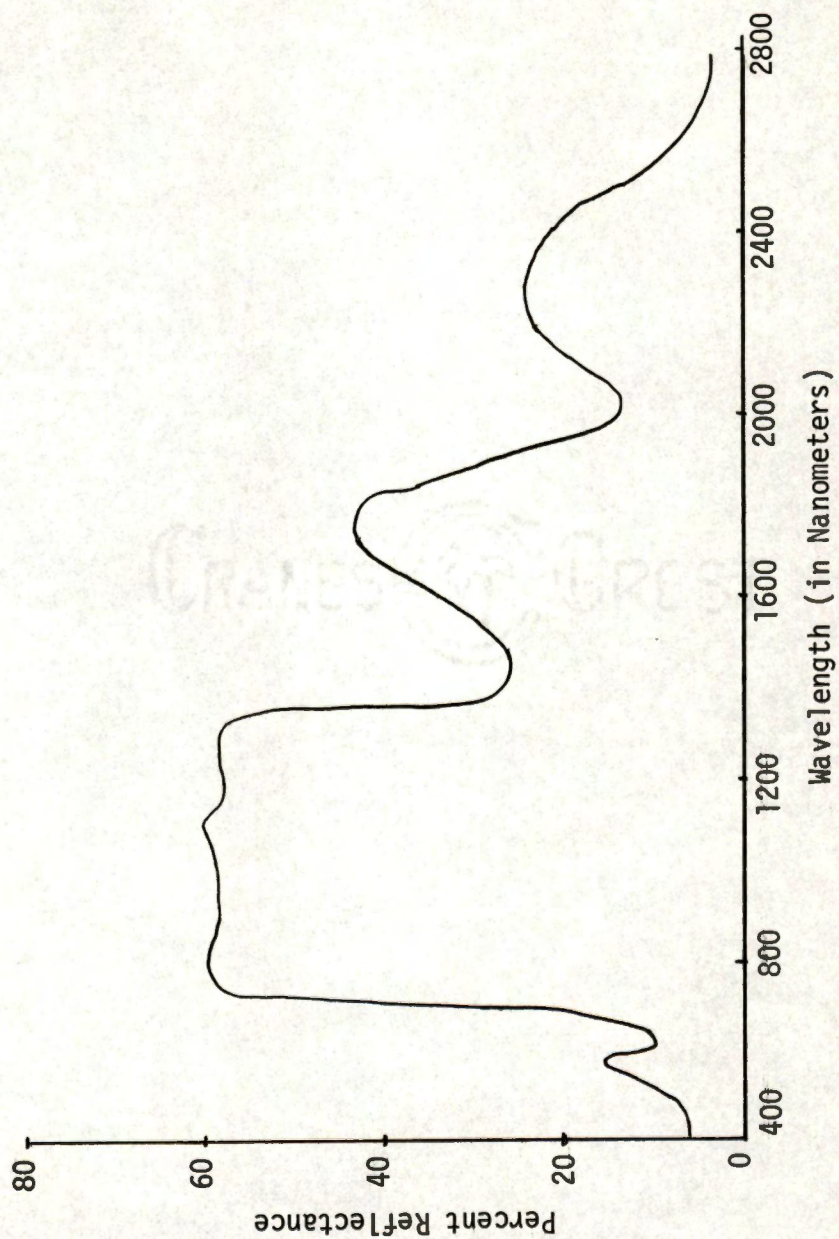


Figure 1. Reflectance spectrum of a tobacco leaf. Taken from Gausman (1971).

theories have not always been agreed on, and since several theories have evolved for structurally different species, it was the purpose of this study to outline parameters meriting further investigations for corn leaves.

## CHAPTER II

### LITERATURE REVIEW

The high infrared reflectivity of leaves appears to be caused by their internal structure. The light entering the leaf has been shown by Willstatter and Stoll (1918) to be critically reflected internally at the cell walls where there is a change in the index of refraction from 1.33 for liquid water to 1.00 for air in the intercellular spaces. This provides an efficient internal reflection at each interface. After several critical reflectances within the leaf, the direction of the original light beam would be reversed and total leaf reflectance could be observed. This theory required the presence of cell walls at oblique angles to incident radiation, and therefore emphasized the spongy mesophyll of leaves as the site of internal leaf reflectance. Mestre (1935) presented a specular leaf model defining diffuse internal reflectance according to the Willstatter and Stoll theory (1918).

More recently, Weber and Olson (1967) and Sinclair (1968) have made photomicrographs of cross sections of leaves suggesting that the important parameter in determining the level of reflectance is the number or total area of the air-wall interfaces and not the volume of air space.

Sinclair (1968) demonstrated the relative nonimportance of surface reflectance from the cuticle. He found reflectances of up to 10 percent for cuticles removed from leaves. Comparison of these results

with the reflectance of the intact leaf showed, however, that the measured values were inflated. A theoretical analysis of the specular properties of the cuticle showed that actually the cuticle of an intact leaf does not reflect more than 5 percent of the incident radiation when the angle of incidence is near perpendicular. Therefore, in the wavelength bands that are highly reflected by leaves, the cuticle probably contributes a relatively small portion of the total leaf reflectance observed.

Gausman, Allen, Cardenus, and Richardson (1969) in studying the maturing process for cotton leaves found reflectance could be predicted from a "void area index." Maturation of a cotton leaf is characterized by development of intercellular air spaces that increase the reflectance and reduce the transmittance of the leaf. The Void Air Index is approximately the average number of air spaces penetrated by a ray passing through the leaf. Their test showed that reflectance of light for the  $0.75\text{--}1.35\ \mu$  wavelength interval is positively correlated with leaf maturation until an age is reached when leaf thickness and numbers of intercellular spaces cease increasing and leaf thickness may actually decrease. They suggested that the reversal with the oldest leaves may be primarily caused by larger cells with increased water contents. The older leaves, as demonstrated by photomicrograph, had larger cells and a much more loosely arranged cellular structure in their mesophylls (lacunose condition) and were associated with comparatively lower transmittance and higher reflectance. Reflectance of older leaves was increased because of an increase in

intercellular spaces of air voids according to their model. In their model, air space is considered to be "compartmentalized" between mesophyll cells. Percentage of intercellular spaces per squared micron of leaf area progressively increased with age. This was associated with increased reflectance and decreased transmittance. In their study, leaf thickness and percentages of intercellular air spaces were determined from photomicrographs. Air spaces were measured on the photomicrographs by planimetry. They fixed their materials in FAA, dehydrated them in a TBA series, and then mounted them in paraffin. Sections were cut at 12  $\mu$  and stained in Safranin-Fast Green.

In a subsequent article by Gausman (1971), he stresses that it is extremely important to consider leaf maturity if reflectance measurements are to be made comparing the reflectance of stressed with nonstressed leaves. Gausman points out that stressed leaves usually have a lower reflectance than nonstressed leaves if they are of the same age; however, if leaf maturity is not considered, he shows how salinity stressed leaves could have a higher reflectance than younger leaves from a control plant. In this same article, Gausman reports on his investigation of the influence of subcellular particles in intact leaves on reflectance of infrared light. He concluded that there was a small contribution, but that it was so small compared to infrared light reflectance caused by the cell wall-air interfaces in the leaf mesophylls that it could be disregarded.

Gates, Keegan, Schleter, and Weidner (1965) suggest that it is probable that the near-infrared reflectance is a function of the cell

shape and size as well as the amount of intercellular space. They emphasize the need for careful experiments to be done relating the reflectance to leaf morphology to establish a morphology-reflectance relationship. Knipling (1970) states that Gates', et al. theory (1965) that non-infrared reflectivity from leaf surfaces is a function of cell shape and size as well as the amount of intercellular space is probably correct. He states that thicker leaves have drastically increased reflectance.

## CHAPTER III

### MATERIALS AND METHODS

#### I. GREENHOUSE PROCEDURE

Hickory King corn plants were grown in the greenhouse with supplementary light provided by grow-lux lamps. Plants were grown in 12-inch styrafoam pots filled with a mixture of three parts sand to one part perlite. After the seeds germinated, they were supplied with Hoagland's nutrient solution (Hoagland and Arnon, 1938) throughout their growth with iron supplied in the form of iron citrate. Three seeds were planted in each pot and two repetitions of each pot were made. It was necessary to change these solutions three times each day to counterbalance the differential absorption rate of ions from the solutions during periods of rapid growth of the plants (F. Bell, personal communication). If too much time elapsed between changes of the solutions, the solutions became too alkaline for optimum nutrient availability. This condition was probably attributable to the rapid uptake of nitrate ions from the solution leaving unbalanced calcium and potassium cations in the solution (F. Bell, personal communication).

When plants were in the three-leaf stage, stress conditions were initiated. Virus symptoms were induced by extracting the juice from infected sorghum (Atlas sorgo) with a mortar and pestle. Small amounts of water and carborandum were added to facilitate the extraction. Healthy plants were mechanically inoculated with this virus extract.

Nitrogen deficiency symptoms were induced by supplying plants with Hoagland's solution deficient in nitrogen (1938). Manganese toxicity symptoms were induced by lowering the pH of a complete Hoagland's solution to 3.5 by the dropwise addition of 0.1 M sulfuric acid. Moisture stress was induced by withholding water and nutrient solutions for four days prior to sampling. Control plants were maintained by supplying complete Hoagland's solution.

## II. SAMPLING PROCEDURE

Samples were taken from corn plants 20 days after the initiation of stress conditions except for water stress. The water stress sample was taken six days after the termination of watering. Samples were taken from the top of the corn plant for all samples but the water stress. It was necessary to take the water stress sample from an older leaf in order to have good symptom development. With the exception of the water stress sample, all samples were taken from leaves of the same age group exhibiting the best symptoms of their respective stress as defined by The Compendium of Corn Diseases (1973).

## III. IMAGERY ACQUISITION

Several photographic systems were tested using both natural and artificial lighting. The most suitable was found to be one employing natural illumination between 11 o'clock and 1 o'clock (Eastern Standard Time) on a clear, cloud free day. This system minimized the possibility

of shadows or uneven lighting. A light blue sky was found to be a suitable background for corn leaves.

Photographs were made with a Pentax 35 mm single lens reflex camera using Kodak E-4 Ektachrome infrared film, Batch 36128-UQ, and a Tiffen 12 filter over the lens. The Tiffen 12 filter is necessary with Ektachrome infrared film to remove blue light before it reaches the film as all three layers of the film are sensitive to blue light. This filter limits the exposure of each layer to only one spectral region. Ektachrome infrared film is a false-color film with the following sensitivities as presented by Fritz (1967):

| Sensitivity        | Dyes in Film Layers    | Resulting Color |
|--------------------|------------------------|-----------------|
| Infrared-sensitive | Cyan positive imate    | Red             |
| Green-sensitive    | Yellow positive image  | Blue            |
| Red-sensitive      | Magenta positive image | Green           |

Photographs were evaluated using a Macbeth Quantalog Transmission Densitometer, Model TD 404. Visual red, green, and blue densitometer filters were used in the evaluation. An aperture of 1.0 mm was used in all cases. Ten density readings were made at random on each leaf and averaged to give a mean density. Density readings were taken in an "X" pattern over the leaf surface on all samples.

#### IV. MICROTECHNIQUE

Sections were cut from corn leaves immediately after photographing and fixed in a 50 percent Formalin-Acetic Acid-Alcohol solution. Sections one millimeter square were taken from the central portion of

the leaves at random. The midvein was avoided in all cases. The fixative was prepared, as suggested by Sass (1951), by combining:

|                                        |       |
|----------------------------------------|-------|
| Ethyl alcohol (95 percent) . . . . .   | 50 cc |
| Glacial acetic acid . . . . .          | 5 cc  |
| Formaldehyde (37-40 percent) . . . . . | 10 cc |
| Water . . . . .                        | 35 cc |

Sections were then put under a vacuum for one hour to remove the air from the tissue and to insure thorough infiltration by the fixative. After the vacuum treatment, fixing solutions had to be changed as unequal evaporation of the constituent chemicals will occur under vacuum.

All sections were left in the fixative for at least 24 hours. After fixing, the sections were dehydrated using tertiary butyl alcohol and then mounted in paraffin. The schedule used in this step was modified from one described by Jensen (1962) as presented in Figure 2. Transverse sections were then made at 10  $\mu$  using a rotary microtome and mounted on slides using an egg albumin-glycerine fixative. Slides were then dried overnight on a warming table at 55° C to allow the adhesive to harden. Slides were then treated with xylene to remove the paraffin and then stained in a Safranin-O and Fast Green according to Sass (1951). A complete schedule is outlined in Figures 2 and 3 for tissue preparation. Slides were mounted in Permount synthetic media and evaluated using a Leitz microscope at 100 X, 250 X, 600 X, and 1000 X.

Leaf thickness was measured using a Filar measuring eyepiece. The measuring eyepiece was calibrated using a stage micrometer which

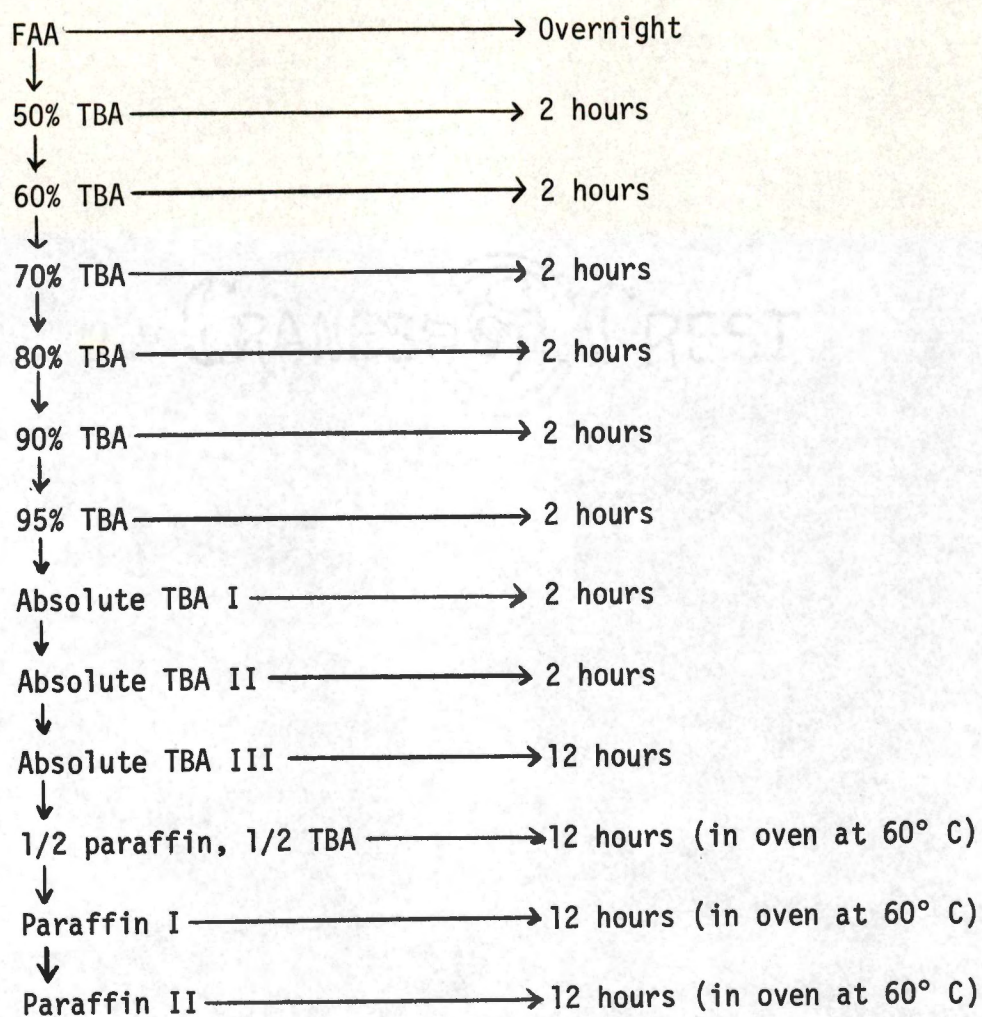


Figure 2. Tissue dehydration and paraffin infiltration schedule.

Sections mounted on slides

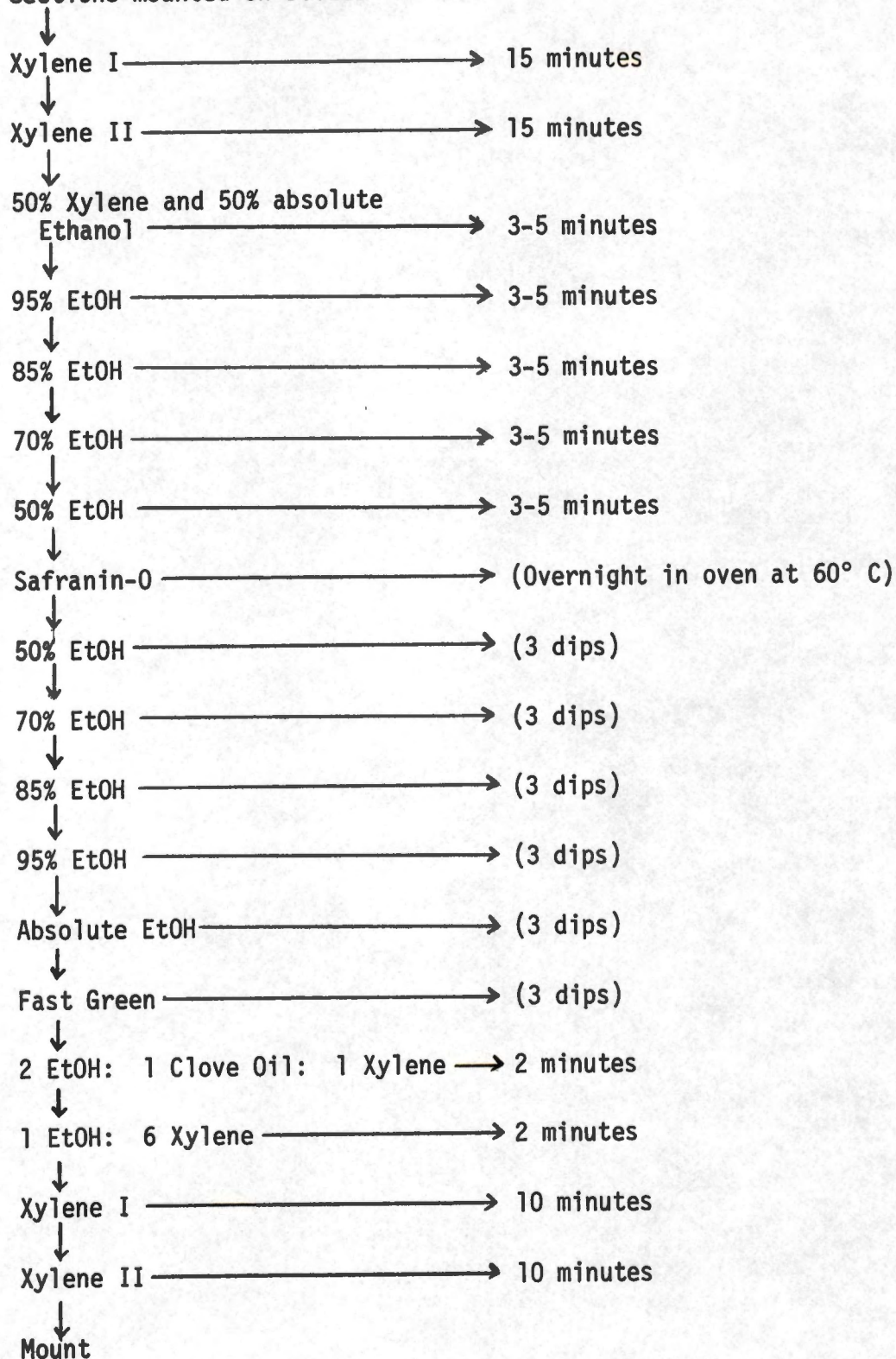


Figure 3. Staining and mounting of sections.

was calibrated into units of 0.01 mm. Measures were accurate to  $\pm 0.005$  mm. All measurements were made between two vascular bundles. All measurements were made from the upper epidermal surface to the lower epidermal surface. Ten points were selected at random from each section for measurement.

Photographs were made using a Leitz 35 mm camera and Kodak Panatonic X black and white film at 100 X, 250 X, 600 X, and 1000 X. Both phase contrast and bright field illumination were used to accentuate points of interest.

## CHAPTER IV

### RESULTS AND DISCUSSION

#### I. PHOTOGRAPHIC DENSITY STUDY

Results of this study demonstrated that the photographic density of infrared film with a Tiffen 12 filter over the lens increased with increasing leaf thickness when a Wratten M-58 red filter was used with a Macbeth Quantalog Transmission Densitometer, Model TD 404 to perform the analysis. The Macbeth densitometer provided density values on a scale of 0.00 to 4.00. Ten density readings were taken from each leaf and the mean of these density readings was plotted against leaf thickness of that same leaf. Leaf thickness was determined by measuring prepared leaf sections at 20 different loci for each leaf. Data from this study are given in Table 1 and Figure 4. The status of the corn leaves assayed is also given in Table 1.

The data from this study agrees with Knipling's finding (1970) that thicker leaves have increased reflectance in the 700-900 nanometer region. Knipling suggests that the shape and size of mesophyll cells along with the amount of air space in the tissue should be further examined for an explanation.

#### II. HISTOLOGY OF CORN LEAF SECTIONS

The evidence for an internal reflection mechanism in leaves is quite strong. One of the most convincing pieces of evidence is given by the

TABLE 1  
LEAF THICKNESS AND PHOTOGRAPHIC DENSITY VALUES USING A WRATTEN M-58  
RED DENSITOMETER FILTER AND A ONE MM APERTURE

| Condition of Leaves          | Mean Leaf<br>Thickness | $\sigma$ | Mean Photographic<br>Density | $\sigma$ |
|------------------------------|------------------------|----------|------------------------------|----------|
| Healthy control              | 540 $\mu$              | 8.4      | 1.62                         | 0.05     |
| Virus stressed               | 380 $\mu$              | 11.0     | 1.49                         | 0.04     |
| Manganese toxicity stressed  | 330 $\mu$              | 10.1     | 1.38                         | 0.09     |
| Nitrogen deficiency stressed | 320 $\mu$              | 7.3      | 1.37                         | 0.06     |
| Water deficiency stressed    | 300 $\mu$              | 6.9      | 1.31                         | 0.04     |

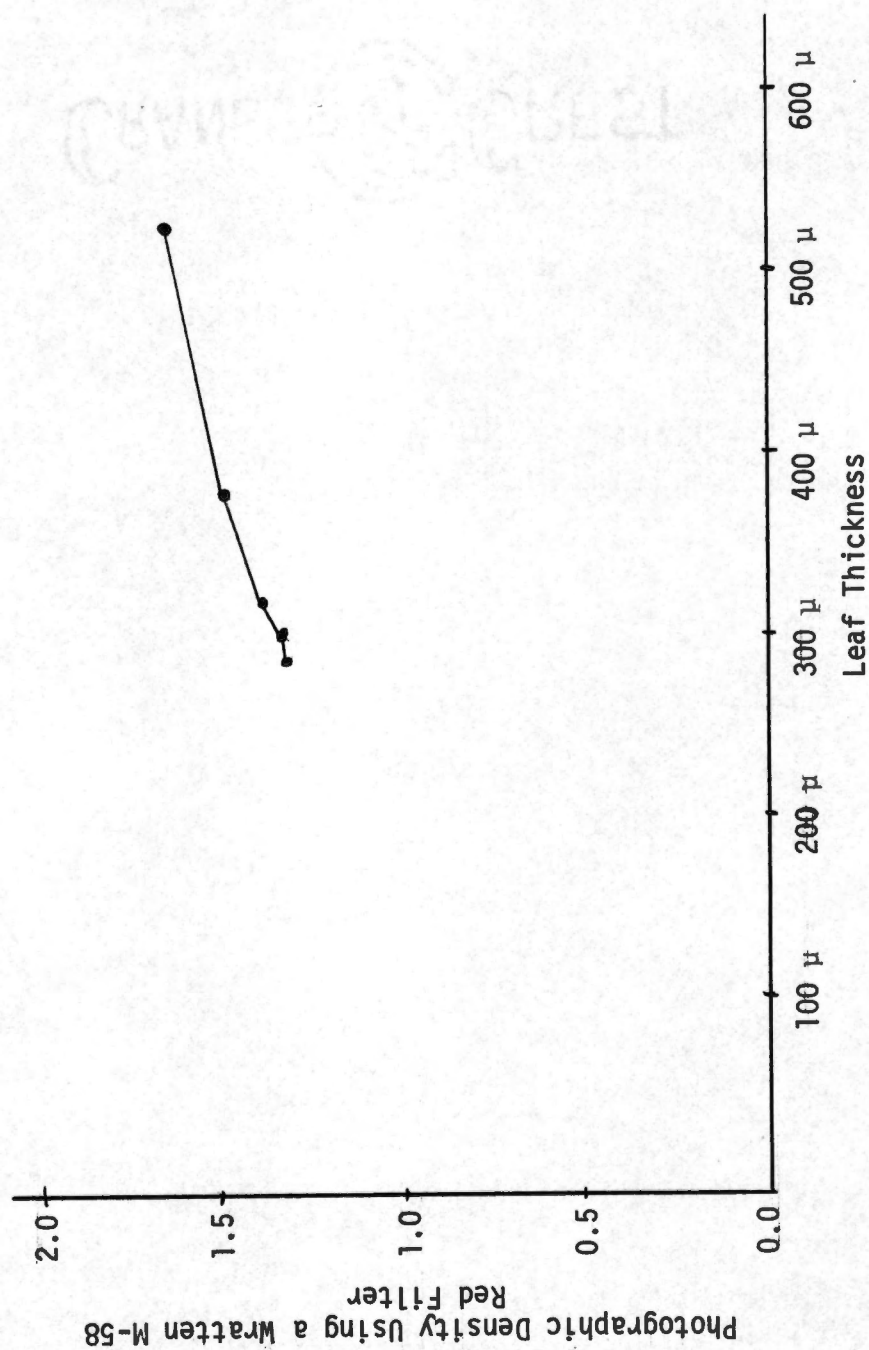


Figure 4. Leaf thickness plotted against photographic density using a Wratten M-58 red densitometer filter and a 1 mm aperture.

drastic reduction in the near-infrared reflectance of a leaf infiltrated with water as described by Knipling (1970). Knipling's explanation was that water had filled the air cavities forming a continuous liquid phase medium throughout the leaf. The elimination of many of the refractive index differences within the leaf was observed to increase transmittance at the expense of scattering.

Corn leaves are characterized as having an undifferentiated mesophyll comprised of cells of irregular shape (Esau, 1953). Corn mesophyll cells are typically compact in arrangement with few inter-cellular air spaces. The mesophyll of corn leaves is not differentiated into a palisade layer as is found in many leaves.

#### Histology of Healthy Corn Leaves

Healthy corn leaves examined were found to have a well developed dark staining cuticle. Sinclair (1968), however, reports that the cuticles he examined did not reflect more than 5 percent of the incident radiation. Nevertheless, if one is to look at the total leaf reflectance as a composite of many factors, as is the opinion of this author, then the presence of a well developed cuticle should be noted as a contributing parameter.

Epidermal cells of healthy corn leaves were found to be made up of 20-30 percent of the total leaf thickness. They can be characterized as a compactly arranged and ovoid to slightly elliptical in shape. Large columnar bulliform cells were found on the adaxial surface of the leaves at regular intervals along the upper epidermis. In healthy corn leaves these bulliform cells were founded and distended and

extended beyond the plane of the epidermal surface. The epidermal cells on both adaxial and abaxial surfaces were rounded and distended. Both surfaces were perforated with numerous stomatae and their accompanying stomatal crypts.

Sinclair's hypothesis is that cell walls act as diffusive surfaces and that one basic pathway for solar radiation in leaves exists. The abundant occurrence of the chlorophyll bearing chloroplasts in the mesophyll and the absence of these light absorbing pigments in epidermal cells indicates that, while some light may be reflected from epidermal cell walls, the greater majority of the light entering the leaf penetrates the epidermis and enters the mesophyll tissue. The substomatal air cavities could be interpreted to have some spectral significance by any of the theories based on void internal spaces in the leaf mesophyll; however, this study did not find enough variation in these air cavities from healthy and stressed corn plants to suggest that they accounted for any difference in reflection between healthy and stressed corn plants.

The mesophyll of healthy corn plants was characterized by an abundance of dark staining chloroplasts within the cells. Mesophyll cells were found to be irregular in shape and number and tightly compacted. Photomicrographs taken at 1000 X revealed that intercellular air spaces were so small that they were not readily distinguishable even at this high magnification. The mesophyll of healthy corn leaves was found to be a continuous tissue in which intercellular air spaces were found to be the exception to the rule.

Any of the theories attributing reflectance to the presence of air cavities in the mesophyll would be questionable in corn leaves. This further agrees with Sinclair's (1968) findings with regard to dorsiventral leaves. Sinclair found that the highest reflectance originated from the ventral side of dorsiventral apple leaves where the palisade tissue is located. The dorsal side having a lacunose spongy mesophyll, in fact, had a lower reflectance rather than higher as was predicted by the Willstatter and Stoll theory. This adds further evidence to the theory that cell walls must be diffuse reflections of incident radiation.

The vascular system of healthy corn leaves is difficult to describe in terms of spectral response since it is not continuous across the leaf (Figures 5, 6, 7, 8, and 9). In all previous works reviewed by this author, it was simply treated as an integral part of the mesophyll. Since no variation was observed in stressed and control corn leaves the author chose to follow this convention also.

#### Histology of Corn Leaves Subjected to Stress

Water-stressed corn leaves. As described earlier in this study, a collapse in overall leaf thickness is characteristic of corn leaves subjected to the stresses assayed in this study. Water-stressed leaves were the most outstanding in this respect. The bulliform cells were observed to be no longer turgid and distended as seen in healthy corn leaves. In water-stressed leaves, bulliform cells were observed as shrunken and greatly reduced in size. A general degeneration of

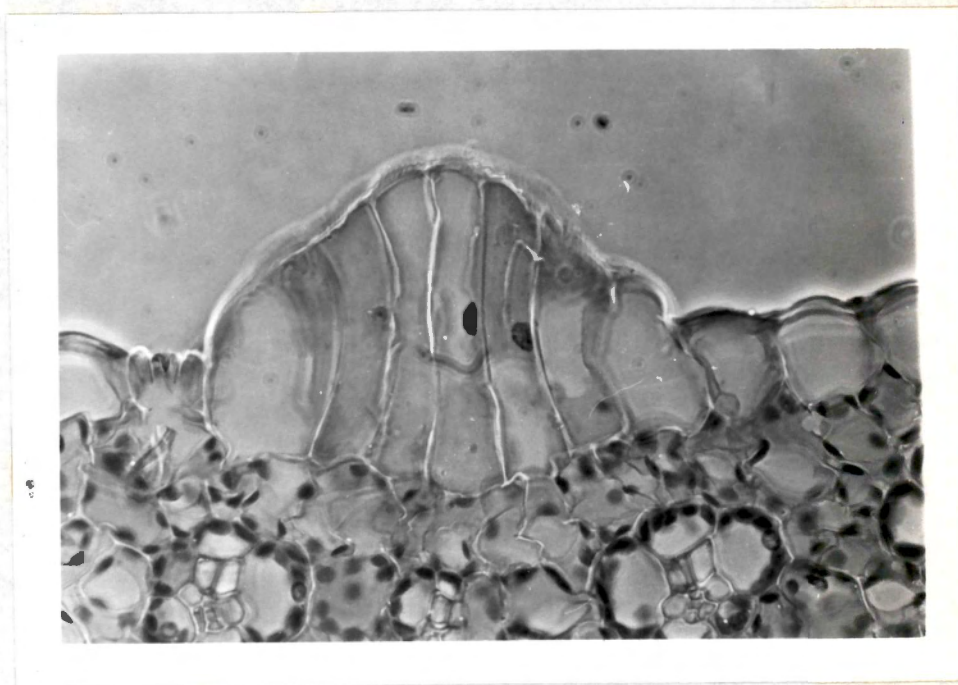


Figure 5. Healthy corn leaf showing bulliform cells in their turgid distended shape (400 X).

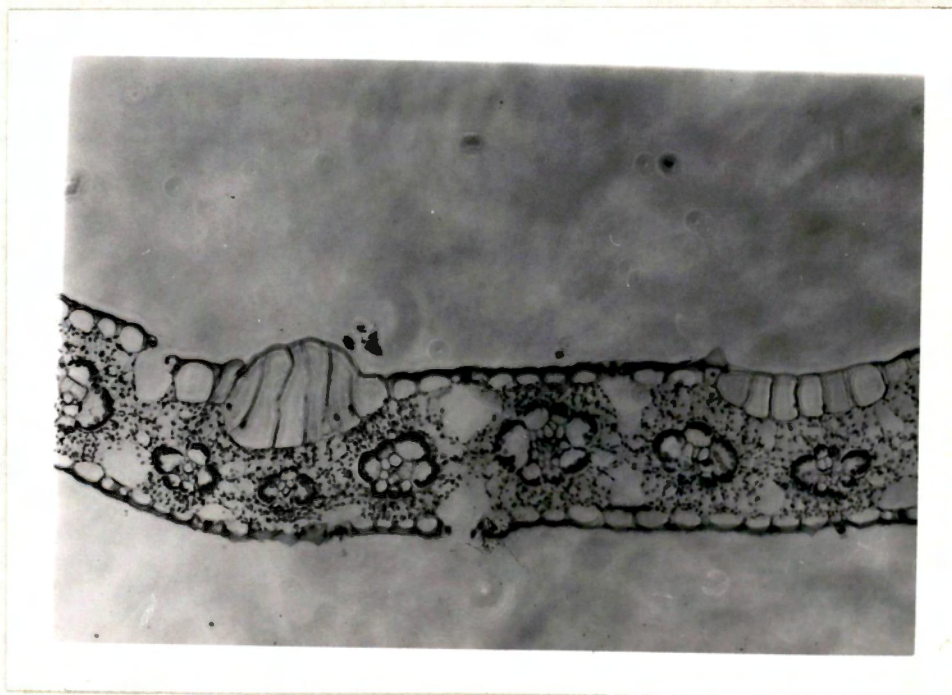


Figure 6. Healthy corn leaf showing dense distribution of chloroplasts in the mesophyll. The substomatal air cavities are also particularly prominent in this photomicrograph (100 X).

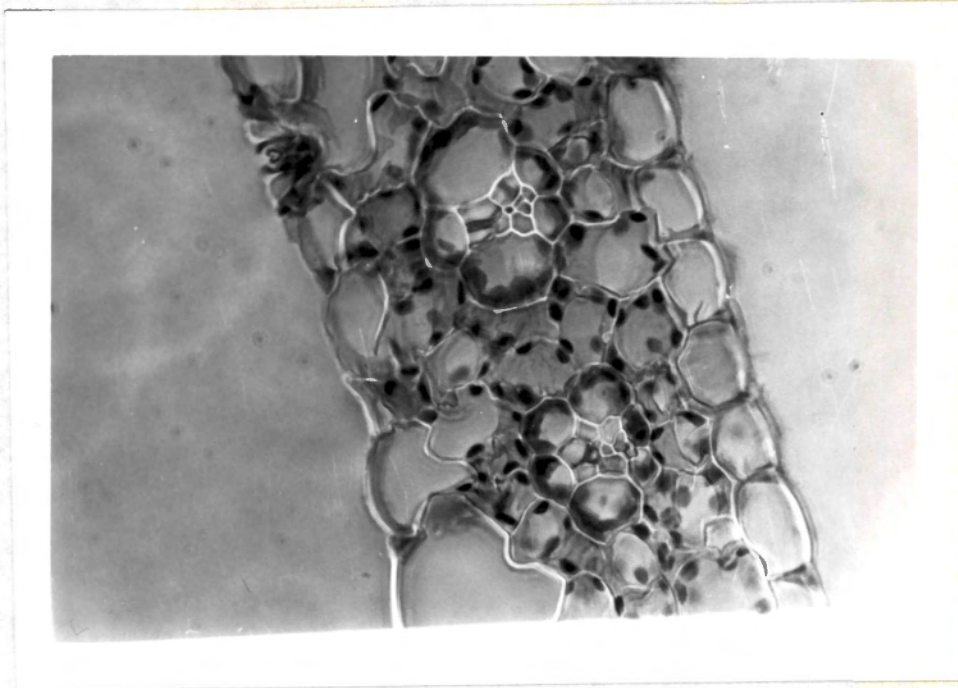


Figure 7. Healthy corn leaves showing compactness of mesophyll cells. The irregular shape of mesophyll cells and the distribution of chloroplasts are also evident here (250 X).

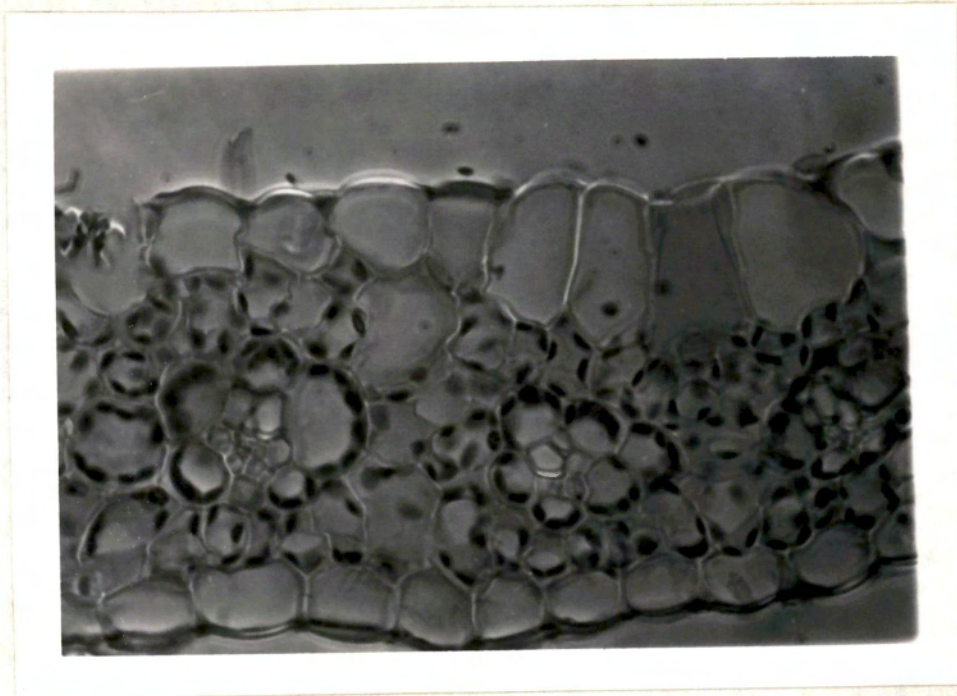


Figure 8. Healthy corn leaf at 250 X showing irregular cell shape and chloroplasts distribution.

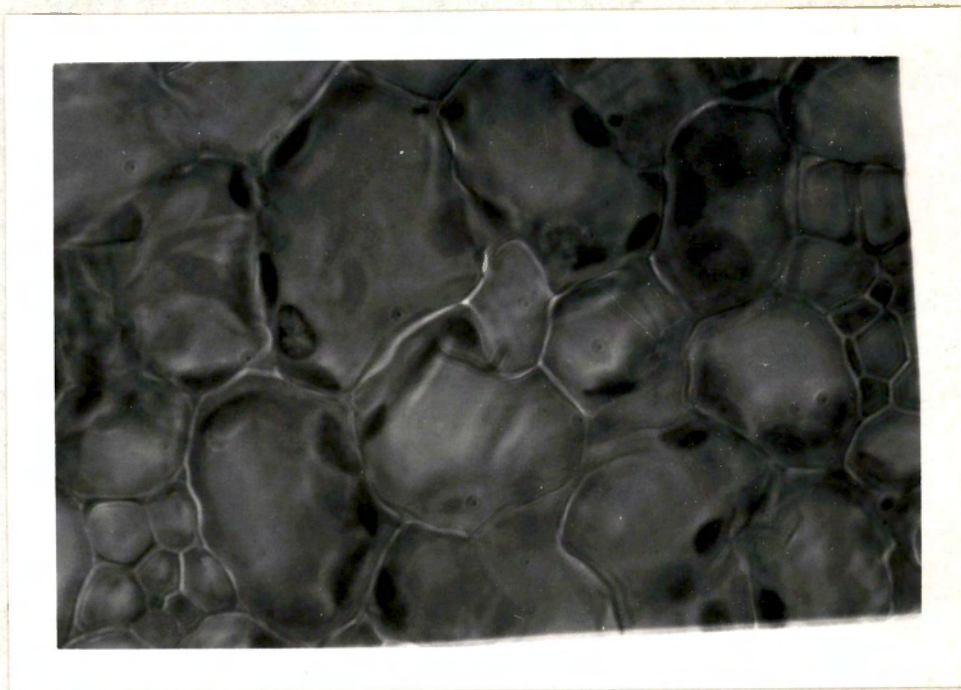


Figure 9. Healthy corn leaf mesophyll at 1000 X showing the compactness of mesophyll cells.

the mesophyll tissue was observed in water-stressed corn leaves (Figure 10). This was evidenced by the small amount of Fast Green stain taken by the mesophyll in prepared slides. Few chloroplasts and light staining cell walls were typical of water-stressed leaves. No changes in the overall shape and size of mesophyll cells were observed.

Corn leaves stressed by virus disease. Corn leaves stressed by virus disease, like water-stressed corn leaves, showed a loss in turgidity and size of bulliform cells. A general loss of chloroplasts, especially between the vascular bundles, was also characteristic. Perhaps the most interesting characteristic of virus-stressed corn leaves was the presence of large vacuoles in many of the mesophyll cells (Figures 11, 12, 13, and 14). Given any of the theories based on void air space in the mesophyll tissue, the presence of vacuoles should contribute to an increased total leaf reflectance. This, however, does not appear to be the case in corn leaves, or at least if it is a factor, the importance is overshadowed by the more important factor of leaf thickness. This finding casts even further doubt on the "void space" theories proposed by Willstatter and Stoll, Knipling, Gausman, et al., Gates, et al., and others. No changes in the overall shape and size of mesophyll cells were observed.

Corn leaves stressed by manganese toxicity. Manganese toxicity-stressed corn leaves were characterized by alternating areas with chloroplast well stained and areas with almost no chloroplast visible. This accounts for the yellow striped appearance of manganese toxicity-

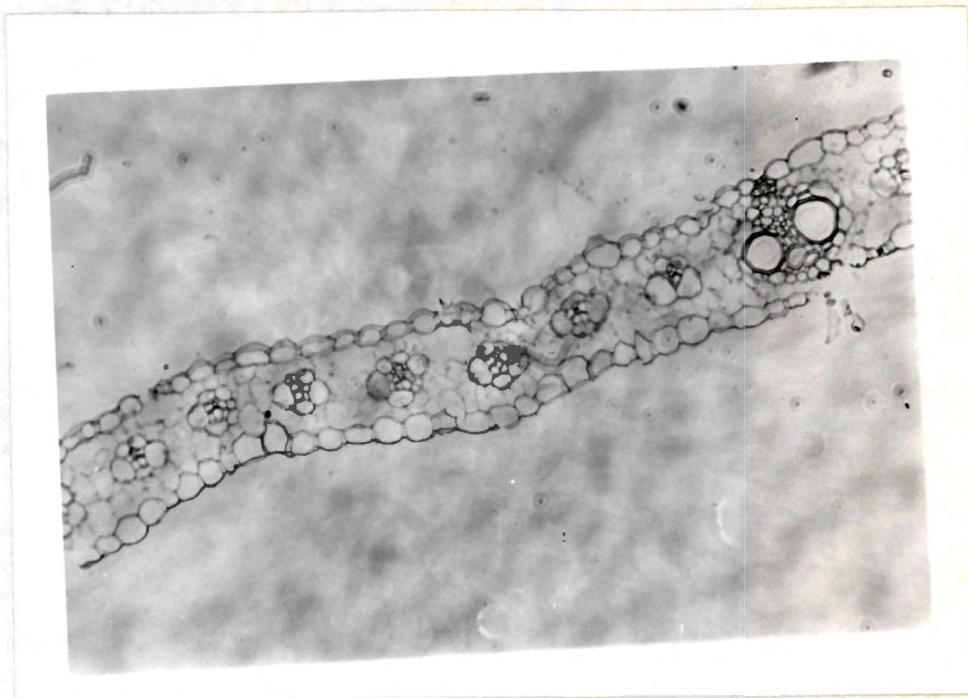


Figure 10. Corn leaf stressed by water stress showing poor chloroplast development and degeneration of mesophyll cells. The collapsed state of the epidermis can also be seen here (100 X).

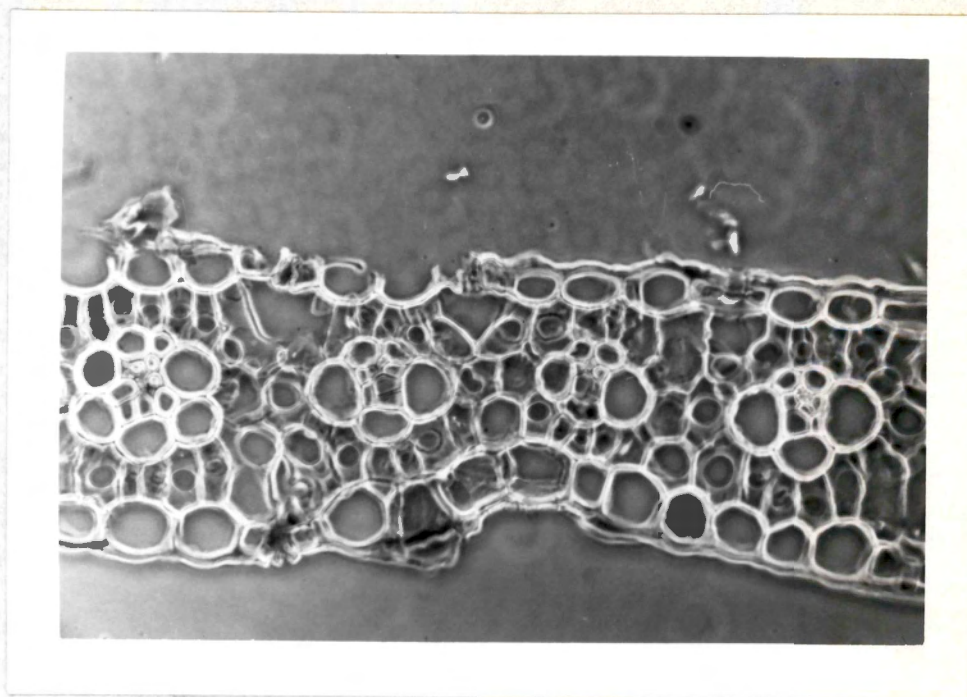


Figure 11. Virus diseased corn leaf at 250 X showing vacuoles in the mesophyll cells.

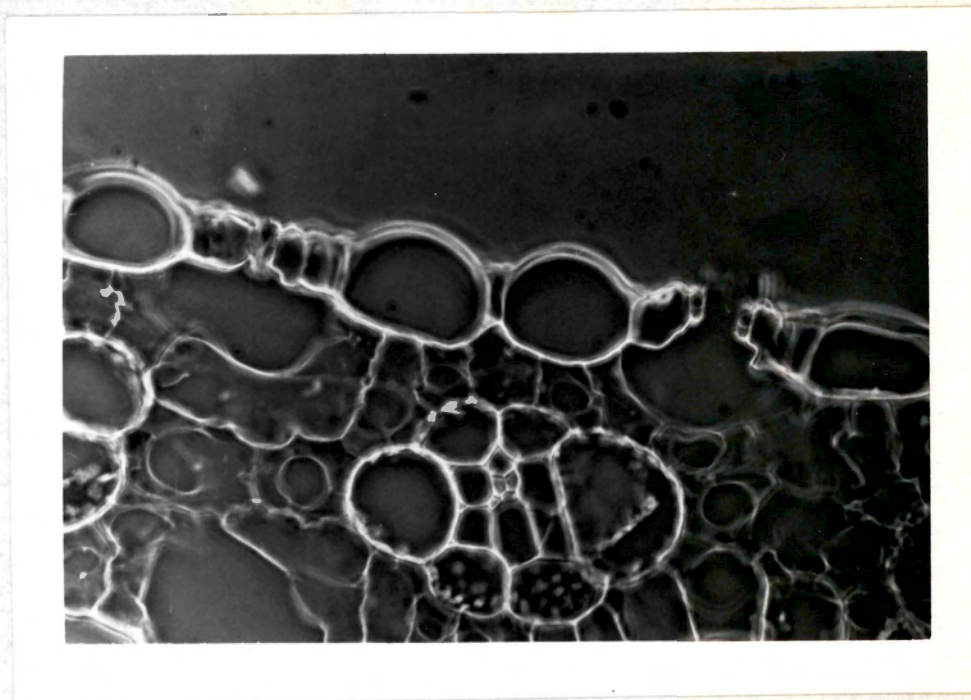


Figure 12. Virus diseased corn leaf showing vacuoles in the mesophyll cells (400 X), view 1.

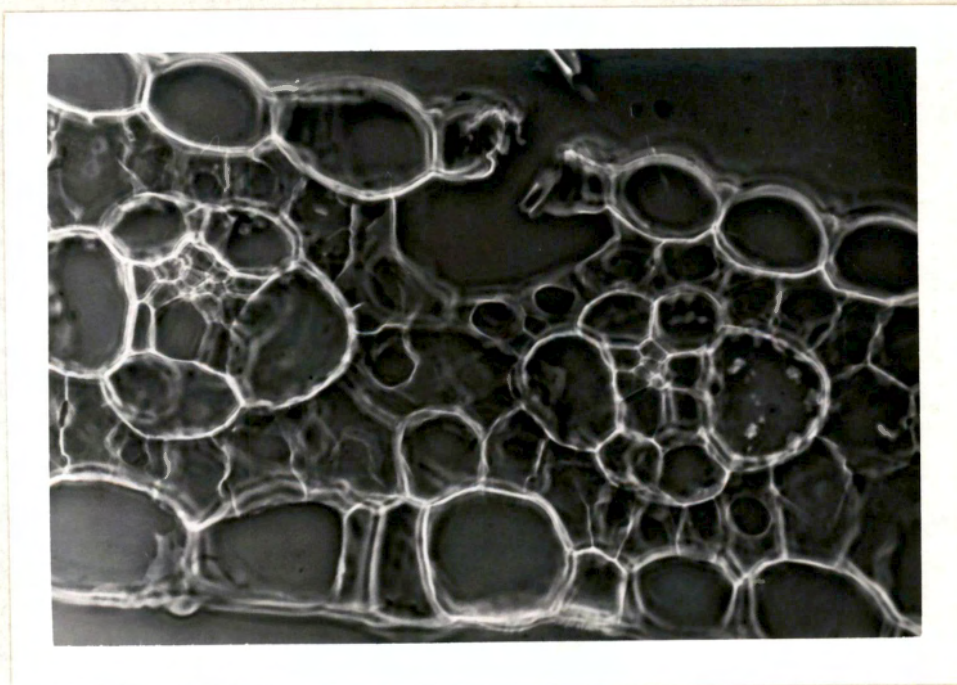


Figure 13. Virus diseased corn leaf showing vacuoles in the mesophyll cells (400 X), view 2.

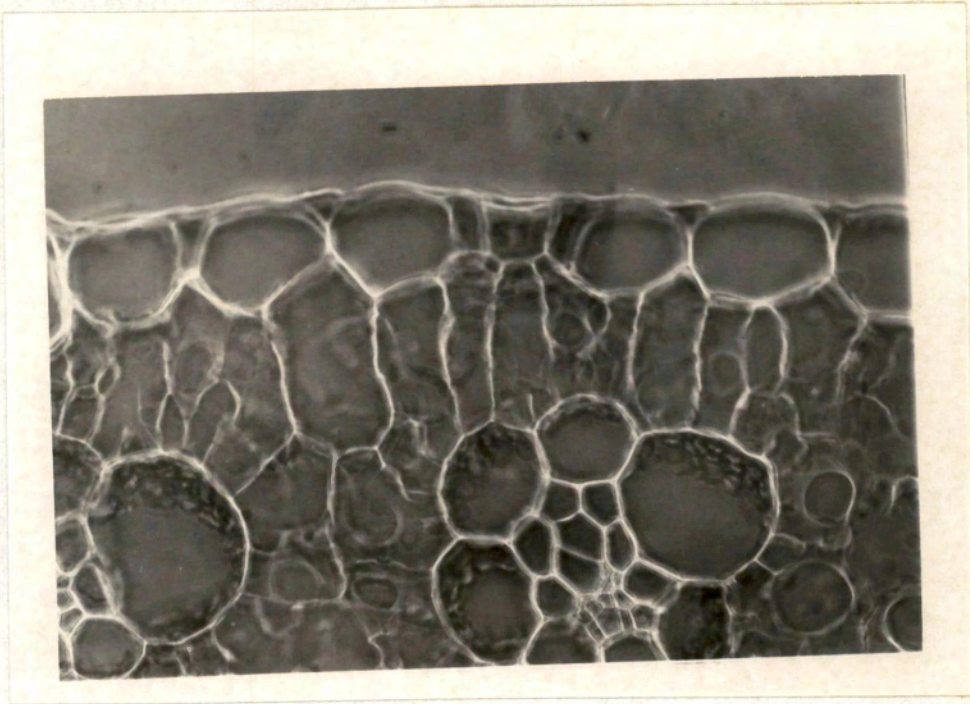


Figure 14. Virus diseased corn leaf showing vacuoles in the mesophyll cells (400 X), view 3.

stressed leaves. Manganese toxicity-stressed leaves, like those of water- and virus-stressed leaves, showed a loss in turgidity of bulliform cells and a light staining of the mesophyll cell walls indicating that a degeneration of the mesophyll was occurring (Figure 15). Except for the presence of vacuoles in the virus-stressed corn leaves, it was difficult to distinguish virus-stressed corn leaf sections from leaf sections of corn stressed by manganese toxicity. No changes in the general shape and size of mesophyll cells were observed.

Corn leaves stressed by nitrogen deficiency. Nitrogen deficiency-stressed corn leaves, as all the other stressed corn leaves observed in this study, were typified by a loss in turgidity of bulliform cells. Chloroplasts were found to be present in much smaller numbers than was observed in healthy corn leaf sections (Figure 16). Mesophyll cells stained lighter than those of healthy control plants, indicating that some degeneration in that tissue had occurred. No changes in the general shape and size of mesophyll cells were observed.

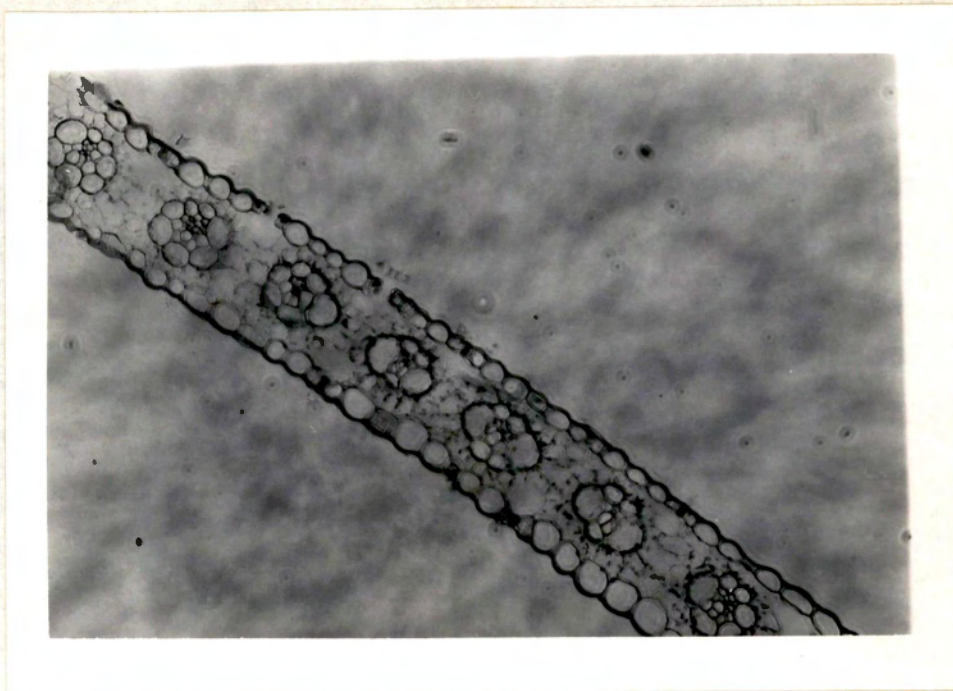


Figure 15. Manganese toxicity-stressed corn leaf showing poor chloroplasts development and general degeneration of mesophyll cells (100 X).

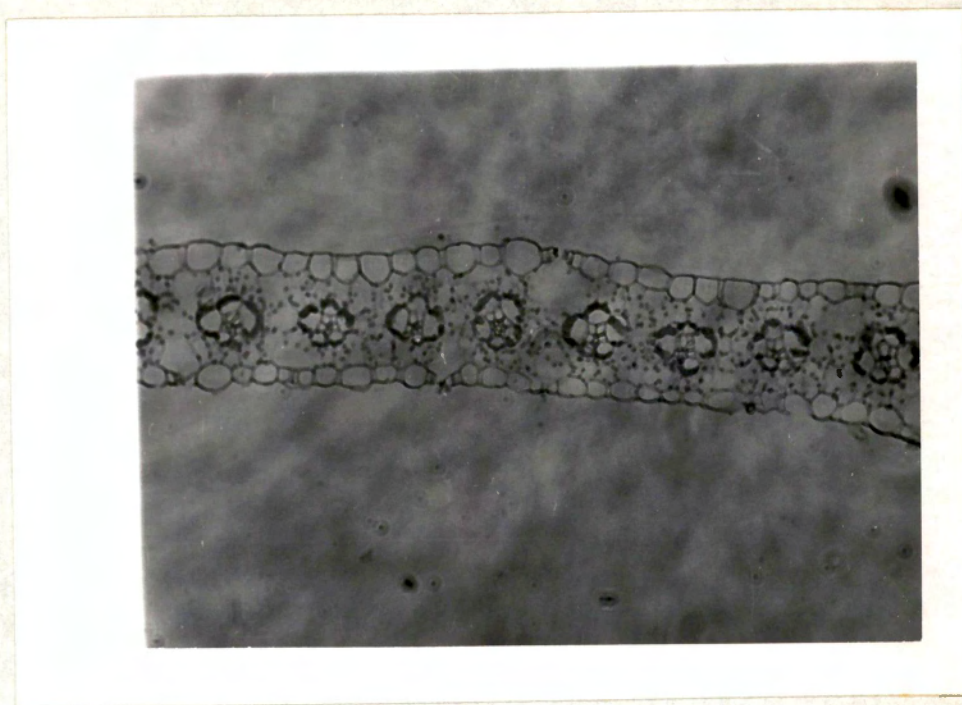


Figure 16. Nitrogen deficiency-stressed corn leaf showing poor chloroplasts development (100 X).

## CHAPTER V

### SUMMARY AND CONCLUSIONS

This research examined the relationship between the photographic density of corn leaves subjected to various stresses as it related to corn leaf morphology. Film-filter combinations were selected so as to measure only the photographic density due to light in the 700-900 nanometer region. This region of the spectrum was chosen for the investigation because leaf reflectance is higher in this region than in any other region imageable on photographic film. The purpose of this research was to examine the various theories of reflectance from leaves in order to determine which theories would apply to corn leaves under a variety of conditions.

The spectral response of leaves can be broken down into reflection, transmission, and absorption. It has long been recognized that a great amount of absorption of visible radiation occurred in leaves. The early investigators recognized that the light which was not absorbed was either transmitted or reflected by the leaf.

Mestre (1935) proposed a leaf model based on diffuse internal reflectance. He attributed diffuse internal reflectance in this model to light scattering which occurred within leaves according to the Willstatter and Stoll (1918) theory. The Willstatter and Stoll theory claimed that internal reflections occurred at cell-air interfaces. They hypothesized that after several critical reflectances within the

leaf the direction of the original light beam would be reversed and total leaf reflectance could be observed.

Sinclair (1968) examined the significance of the surface reflectance from the cuticle and found it did not reflect more than 5 percent of the incident radiation. He also examined the process of internal leaf reflection. Sinclair (1968) concluded that one basic pathway of solar radiation in leaves exists and that once radiation had penetrated the cuticle its pathway was altered by cell walls which acted like diffusive surfaces.

Gausman, et al. (1969) proposed that internal reflectance could be predicted from a "void area index." Gates, et al. (1965) and Knipling (1970) furthered this theory to say that internal reflectance was not only related to the amount of intercellular air space, but that it was also related to the size and shape of mesophyll cells.

The results of this research were found to favor Sinclair's hypothesis that cell walls act like diffusive surfaces. Results further indicated a relationship between photographic density of corn leaves in the 700-900 nanometer region and the thickness of those leaves. Corn leaves subjected to various stress conditions were examined histologically to determine if any morphological changes could be detected which would help explain the differences in photographic density between control and stressed leaves. In this study it was observed that intercellular air spaces were virtually nonexistent in both healthy and stressed corn leaves with the exception of vacuoles in the mesophyll cells of corn leaves stressed by virus

disease. Size and shape of mesophyll cells were observed to change very little in stressed leaves examined in this study. This would at first seem to contradict the reduced leaf thickness found in stressed leaves; however, the irregular structure of corn leaf mesophyll tissue makes changes of this nature difficult to observe. The reduced ability of the mesophyll cells of stressed corn leaves observed in this study to take on Fast Green stain was evidence that a general degeneration of mesophyll cells was occurring.

The conclusion of this investigation is that the leaf reflectance of light in the 700-900 nanometer region by corn leaves is the composite result of several parameters. It was found that of all the theories reviewed that Sinclair's "diffusive cell wall" hypothesis best fitted the observations made of corn leaves under stress conditions.



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