

# REMOTE SENSING APPLICATIONS IN FORESTRY

REMOTE SENSING OF CHANGES IN MORPHOLOGY AND  
PHYSIOLOGY OF TREES UNDER STRESS

N 7<sup>by</sup> - 28037

Charles E. Olson, Jr.  
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Jennifer M. Ward

School of Natural Resources  
University of Michigan

Annual Progress Report

30 September 1971

*A report of research performed under the auspices of the*

Forestry Remote Sensing Laboratory,  
School of Forestry and Conservation  
University of California  
Berkeley, California

*A Coordination Task Carried Out in Cooperation with*  
The Forest Service, U. S. Department of Agriculture

*For*

EARTH RESOURCES SURVEY PROGRAM

OFFICE OF SPACE SCIENCES AND APPLICATIONS

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

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## ABSTRACT

This is the fifth annual progress report describing results of continuing studies of forest trees subjected to varying types of stress. Both greenhouse and field studies are included and six studies were active during the year.

Measurements on foliage samples collected from several drought and salt treated plants revealed that leaf thickness decreased with increasing severity of the drought treatment, increased with increasing severity of treatment with NaCl, but remained essentially unaffected by treatment with CaCl<sub>2</sub>. No evidence of "physiologic drought" due to treatment with either NaCl or CaCl<sub>2</sub> was discernible.

Statistical analyses of foliar reflectance and foliar moisture content data led to a species independent regression equation for predicting the oven-dry-weight foliar moisture content from near infrared reflectance data. This regression equation gave a multiple correlation coefficient of 0.945 and a standard error of estimate of 0.014 percent.

Work with sugar maple (Acer saccharum Marsh.), yellow poplar (Liriodendron tulipifera L.), white ash (Fraxinus americana L.) and red oak (Quercus rubra L.) confirmed earlier indications that changes in reflectance characteristics of tree foliage vary with the structure of the xylem tissues. Results also indicate that emittance properties of ring-porous and diffuse-porous species differ, with increase in emittance of trees under moisture stress greatest in ring-porous species, and greater in white ash than in red oak.

Airborne data collected in 1969 and 1970 with the University of

Michigan multispectral scanner (MSS) were used in preparing simulated color, color IR and a series of "false color" images of areas with known infestations of Fomes annosus. Results indicate that "false color" images utilizing the 1.5 to 1.8 and 2.0 to 2.6 micrometer bands provide selective enhancement of the diseased areas. Comparison of simulated and actual aerial color and color IR photography revealed that the color renditions of the MSS simulations agreed closely with those of the actual photography.

Detailed analyses of the effect of interpreter capabilities on detection of Fomes annosus in pine plantations from color photography and MSS data were initiated. Personnel demands imposed by the 1971 "Corn Blight Watch" prevented completion of this study.

Work with reflectance changes in red oak seedlings infected with the oak wilt fungus (Ceratocystis fagacearum [Bretz] Hunt.) indicates that the development of visible symptoms is so rapid, once infection occurs, that pre-visual remote detection of this disease would be of limited practicality.

## ACKNOWLEDGEMENTS

The research described in this report was conducted as part of the Earth Resources Survey Program in Agriculture/Forestry sponsored by, and with financial assistance from, the National Aeronautics and Space Administration (Contract Number R-09-038-002). The work was a cooperative undertaking of the Forest Service, U. S. Department of Agriculture, and the University of Michigan, School of Natural Resources, administered as Supplement 27 to Cooperative Agreement Number 12-11-099-16744.

The spectrophotometer used in obtaining the reflectance data was furnished by the Office of Naval Research, U. S. Navy.

The generous support of Dr. Warren H. Wagner, Jr., former director of the University of Michigan Matthaei Botanical Gardens, and the entire staff of the Botanical Gardens, is gratefully acknowledged.

The study of reflectance changes during oak wilt pathogenesis (Study VI) could not have been conducted without the voluntary contributions of Joseph A. Bruno, who performed all laboratory work and data analysis without direct financial support from this project.

The assistance of the University of Michigan Willow Run Laboratories in processing the multispectral data was invaluable.

Special thanks are also extended to:

Professors Robert Zahner and Gary W. Fowler for their assistance in interpreting the data and guiding the statistical analyses, and to Professor Harrison L. Morton for his assistance in guiding the oak wilt pathogenesis phase of this work (Study VI).

Robert D. Dillman, Charles V. Nazare and Drew Urbassik for their

willing assistance in completing the measurements of leaf thickness for Study I, and for their efforts in setting up and initiating Study V.

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INTRODUCTION

Early detection of stress in vegetation is one of the keys to correcting the condition causing that stress. Modern remote sensors offer considerable promise for early detection of plant stress when the energy relationships associated with such stress are understood.

Controlled studies of reflectance and emittance characteristics of foliage on trees subjected to varying kinds and severity of stress have been conducted at the University of Michigan for several years. A list of reports describing work supported entirely, or in part, under National Aeronautics and Space Administration Contract Number R-09-038-002 will be found at the end of this report. This report summarizes work completed under this same contract during the period from 1 October 1970 through 30 September 1971.

STUDY I: EFFECTS OF SALINITY ON REFLECTANCE AND EMITTANCE PROPERTIES OF TREE SEEDLINGS (Study Leader - J. M. Ward)

During 1968 and 1969, several groups of sugar maple (Acer saccharum Marsh.) seedlings were grown in the greenhouse with different concentrations of  $\text{NaCl}_2$  in the soil. Methods used were described in our 1968



Annual Report (Olson and Ward, 1968) and observed changes in foliar reflectance were summarized in the 1969 Annual Report (Olson, Ward and Rohde, 1969). Work during 1970 and 1971 was confined to analyses of anatomical changes in the foliage of the treated plants. Results of these analyses are described below and this report represents the final report on this study.

### Salinity and Physiological Drought

In 1898, Schimper used the term "physiological drought" to describe the osmotic inhibition of water uptake by plants growing in soils with high salinity. Even when soil moisture was high, salinity was thought to produce conditions equivalent to drought. More recently, Bonner and Galston (1952) and Slayter (1967) have pointed out that this may be true when no saline solutes enter the plant; but that, in most plants, osmotic adjustment takes place by uptake of solute ions, i.e., "the internal osmotic pressure of the plant increases by an amount approximately equal to the osmotic pressure of the applied solutes" (Slayter, 1967). The plant is not, at this point, fully turgid, since its water potential is reduced by an amount equal to the osmotic pressure of the substrate. Slayter concludes that the main effect of salinity is not "physiological drought", but is related to excessive ion accumulation, possibly combined with reduced uptake of essential mineral elements.

Bonner and Galston (1952) defined halophytic (salt-tolerant) plants as those which take up salt, particularly the non-essential  $\text{Na}^+$  ion, from the saline soil and thus achieve an osmotic equilibrium with the soil

solution, while glycophytic (salt-intolerant) plants cannot increase their osmotic pressure by absorbing sodium, and are therefore subject to physiological drought. Other workers have noted that halophytic plants frequently develop succulence under saline conditions (Strogonov, 1962; Chapman, 1968). Strogonov termed this "halosucculence", and claimed that it occurs in response to excess  $\text{Cl}^-$  salinity, while excess soil  $\text{SO}_4^{--}$  results in "haloxerism" (non-succulent leaves).

Slayter (1967) stated that "salt tolerance is associated with relatively low rates of absorption of chloride and sodium ions", and that increased levels of these salts in cells causes succulence by affecting protoplasmic viscosity and volume relationships, protein hydration and increased cytoplasmic and vacuolar osmotic pressure. He noted that excess electrolytes, especially  $\text{Na}^+$  and  $\text{Cl}^-$  will gradually cause dislocation of metabolism and that, as salinity increases, so does the respiration rate of many species, requiring increased energy for uptake of essential minerals from the soil and suppressing growth.

If sugar maple is glycophytic as defined by Bonner and Galston (1952), the sugar maple seedlings used for this experiment should not have absorbed  $\text{Na}^+$ ,  $\text{Ca}^{++}$  or  $\text{Cl}^-$  ions from the soil solution and the physiological effect would be similar to that produced by drought. If, however, sugar maple is able to absorb small amounts of the ions in question and achieve osmotic adjustment to the saline soil solution, physiological drought would not have developed, although the plants might become succulent and ultimately die from the toxic effects of excess ion accumulation.

### Anatomical Measurements

Leaves from plants subjected to each treatment were fixed, imbedded in paraffin and cross-sectioned (vertically, from lower to upper surface of the lamina) on a rotary microtome. The 10 micrometer sections thus obtained were mounted on glass slides, approximately 60 sections per slide, the paraffin removed in xylol/alcohol solutions and the sections stained in a Safranin/Fast Green series and permanently mounted.

Representative cross-sections were chosen from each treatment for anatomical analysis. Enlarged "tracings" of each section were prepared using a projection microscope at 315X magnification. On each tracing the thicknesses of the upper epidermis, palisade layer, spongy mesophyll and lower epidermis were measured (Table 1).

The data in Table 1 make it appear as if succulence did develop in the plants treated with NaCl. The difference in mean leaf thickness between the control and 0.50% NaCl treatments is not significant at the 10% level, however. Succulence did not develop in the CaCl<sub>2</sub> treated plants, but no evidence of physiological drought can be seen either. The plants from the drought treatments had a mean leaf thickness which became progressively smaller as the severity of the treatment increased, while the difference in leaf thickness between the control and the two CaCl<sub>2</sub> treatments is obviously insignificant. These measurements provide some evidence to support our earlier conclusion that the response of sugar maple to NaCl differs from its response to CaCl<sub>2</sub>.

Portion of Leaf	Severity and Type of Treatment (All measurements in centimeters)						
	Control	Moderate Drought	Severe Drought	0.25% NaCl	0.50% NaCl	0.25% CaCl <sub>2</sub>	0.50% CaCl <sub>2</sub>
Upper Epidermis	0.313	0.316	0.264	0.366	0.326	0.351	0.315
Palisade	1.103	0.934	0.968	1.320	1.346	1.026	1.107
Spongy Mesophyll	0.978	0.880	0.768	0.974	1.178	0.961	0.952
Lower Epidermis	0.275	0.198	0.208	0.227	0.262	0.254	0.225
Total Leaf Thickness	2.669	2.328	2.208	2.007	3.112	2.592	2.599
Number of Measurements	195	25	25	55	60	65	65
Standard Deviation (Total)	0.388	2.205	0.367	0.371	0.871	0.334	0.390
All measurements made on pencil tracings prepared at 315X with a projecting microscope.							

Table 1. Average thickness of foliage from control, drought treated and salt treated sugar maple seedlings.

### Future Plans

This study has been completed. A report describing the results of these anatomical investigations is being prepared for publication in the botanical literature.

### STUDY II: THE RELATIONSHIP BETWEEN LEAF REFLECTANCE AND LEAF WATER CONTENT (Study Leader - W. G. Rohde)

Work completed through September 1970 led to a series of regression equations for predicting foliar moisture content (oven-dry weight basis) from laboratory spectral reflectance curves. These were species dependent, and this limited their usefulness for remote sensing applications. Also, the data transforms utilized in the regression analyses are difficult to adapt to existing multispectral data processors, such as the University of Michigan Spectral Analysis and Recognition Computer (SPARC). Work on this study during 1970-71 was directed towards overcoming these two problems.

#### An Equation Independent of Species

Previous work indicates that foliar reflectance at wavelengths between 1.5 and 2.6 micrometers ( $\mu\text{m}$ ) is closely related to moisture content while reflectance at shorter wavelengths is influenced more by cell structure and pigmentation. For this reason, attention was turned almost exclusively to the 1.5 to 2.6  $\mu\text{m}$  band. Plots of linear reflectance values over moisture content (Figure 1) suggested a polynomial relationship of some sort, and were the basis for testing the quadratic and cubic terms included in the final regression equation.

Subsequent results of analyses of transformed data which are compatible

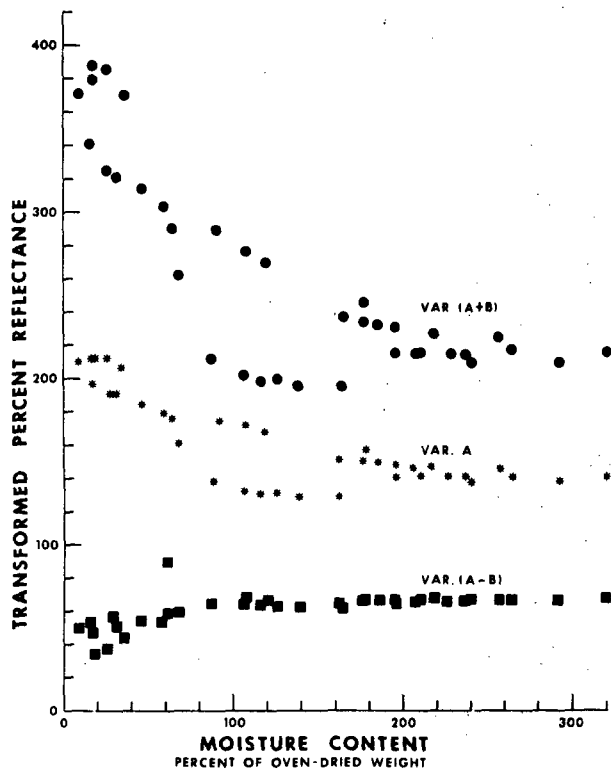


Figure 1. Plots of the linear reflectance terms against mean moisture content indicate a polynomial relationship.

with multispectral data processors yielded an equation, independent of species, which has only slightly lower prediction capabilities than the individual equations for yellow birch (Betula alleghenensis Britton --  $r = 0.98$ ), sugar maple (Acer saccharum Marsh. --  $r = 0.94$ ) and white ash (Fraxinus americana L. --  $r = 0.93$ ). The regression equation derived from the transformed data is:

$$\log \text{M.C.} = 1.015 + 0.915 [(A + B)/(A-B)] - 0.201 A^3 \cdot 10^{-7} + 0.420(A-B)^3 \cdot 10^{-6}$$

where: M.C. = leaf moisture content (oven-dry weight basis)

$$A = R(1.64) + R(1.75) + R(1.78)$$

$$B = R(2.00) + R(2.19) + R(2.30) + R(2.60)$$

$R(\ )$  = percent reflectance at the wavelength (in  $\mu\text{m}$ ) in the ( ).

This equation is based on data for yellow birch, sugar maple and white ash, and the calculated multiple correlation coefficient is 0.945, with a standard error of estimate of 0.014.

### Discussion

A review of these and previously reported results of this study leads to some understanding of the complexity of the moisture content/reflectance relationship.

The first response of leaves deprived of water is a transient opening of stomata, soon followed by gradual closure of them (Kozlowski, 1968). Incident solar radiation, leaf water content and temperature are three important parameters affecting stomatal activity, but when leaf water deficits occur leaf water content appears to be the most important. Decreasing leaf turgor results in stomatal closure and reduced

transpiration. Stomatal response to moisture stress is accompanied by changes in reflectance properties of foliage.

Although an increase in reflectance with decreasing leaf moisture content was observed at all wavelengths longer than 0.80  $\mu\text{m}$ , the largest differences within and between species occurred at wavelengths between 1.30 and 2.60  $\mu\text{m}$ . The graphs presented as Figure 2 indicate a curvilinear relationship between leaf reflectance and leaf moisture content, which may result from changes in transpiration rates, stomatal activity and cell structure.

Variation in reflectance between species, and the several inflection points shown in the graphs of Figure 2, point to the existence of critical thresholds at different moisture contents that may be partially explained by variation in transpiration rates, stomatal activity and leaf water content for the three species studied. The occurrence of inflection points at different leaf moisture contents may be a function of differences in species morphology, or may be due to the measurement of leaf water content on an oven-dry weight basis. Although the inflection points occur at different moisture contents in each of the three species studied, there is a noteworthy similarity in the general pattern of reflectance change with decreasing moisture content. With sugar maple, the inflection point near 90 percent moisture content may mark the beginning of rapid stomatal closure after the disappearance of loosely bound water in the outer-cellular spaces. As such water loss continues, deterioration of cell structure occurs and the inflection point near 20 percent moisture content may mark the onset of this event.



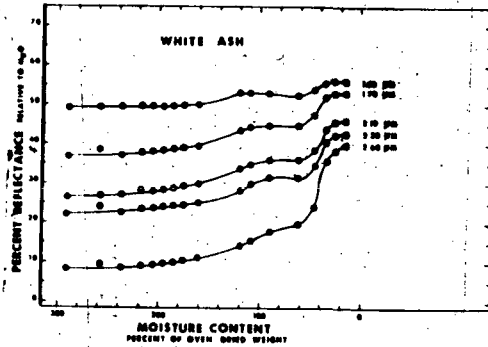
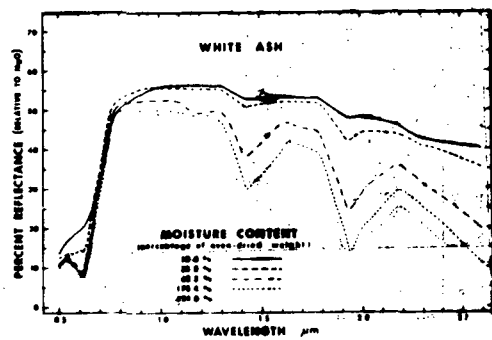
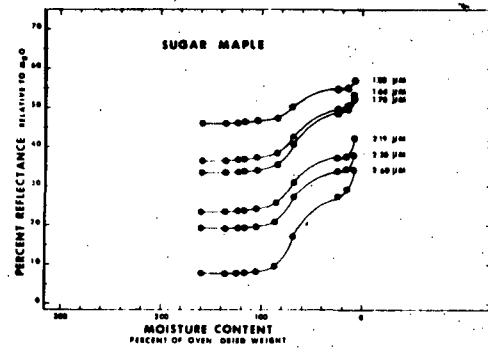
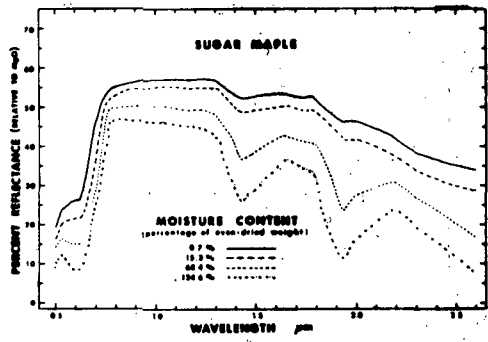
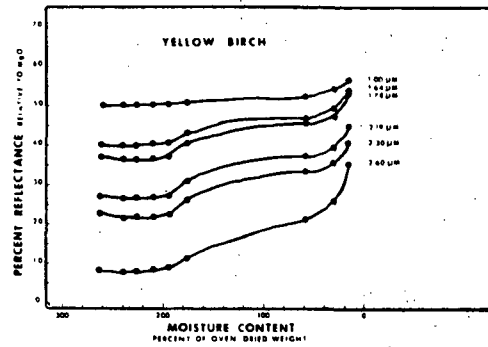
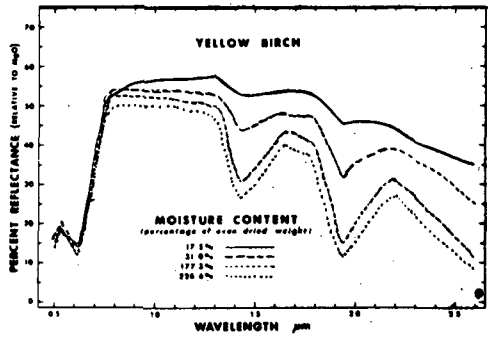


Figure 2. Variation in reflectance with changing levels of moisture content for three tree species. Reflectance between 1.3  $\mu\text{m}$  and 2.6  $\mu\text{m}$  consistently increased as foliar moisture content decreased.

Reflectance data at 1.00  $\mu\text{m}$  seem to support the hypothesis set forth above, for reflectance at this wavelength is more strongly influenced by cell structure than by leaf moisture content. Gates (1970) suggests that near infrared reflectance of mature foliage will remain relatively unchanged until severe levels of moisture stress are attained. Our data indicate that reflectance at 1.00  $\mu\text{m}$  varies with species, showing negligible change in yellow birch, a slight linear increase in sugar maple to the first critical threshold but increases sharply in a curvilinear fashion at lower moisture contents. At the second critical threshold the pattern of change in reflectance is quite similar to the reflectance change at wavelengths longer than 1.3  $\mu\text{m}$ . This may be due to increased dependence of reflectance at 1.0  $\mu\text{m}$  on cell wall structure. Leaf reflectance at wavelengths shorter than 0.80  $\mu\text{m}$  is more strongly influenced by other properties, including pigmentation, which are not necessarily related to moisture stress conditions.

#### Future Plans

This study is considered complete and work has been terminated. Testing of the moisture content prediction equation for aerial determination of moisture content of foliar materials will be one objective of any multispectral overflights completed during the 1972 growing season. This testing will be handled as a separate study.

#### STUDY III: DIFFERENCES IN RESPONSE OF RING-POROUS AND DIFFUSE-POROUS TREES TO MOISTURE STRESS (Study Leaders - W. G. Rohde and C. E. Olson, Jr.)

Work on this study began in 1969, but data analysis was not completed

until early 1971. The study was stimulated by earlier work by Weber and Olson (1967) and Olson (1969) suggesting that foliar reflectance properties of stressed seedlings are more closely related to the water status of the plants at the time the leaves are formed in the bud than to water conditions at the time reflectance measurements are made. This investigation was designed to study seasonal changes in reflectance properties of four tree species -- sugar maple (Acer saccharum Marsh.), yellow poplar (Liriodendron tulipifera L.), white ash (Fraxinus americana L.) and red oak (Quercus rubra L.) -- subjected to varying levels of moisture stress before, during and after leaves flushed and reached full size. Of the four species named, the first two have diffuse-porous xylems and the latter two have ring-porous xylems. The report that follows summarizes the work done over a two year period and is a partial duplication of fragmentary results included in previous annual reports.

#### Procedures

Nine seedlings of each species, grown in four gallon containers under normal outdoor conditions during 1968, were brought into the greenhouse late in February of 1969. The seedlings of each species were divided into three groups with the groups watered according to the schedule shown in Table 2.

Reflectance measurements were made at weekly intervals throughout one growing season from several leaves on each tree. All measurements were made without removing the leaves from the trees, allowing subsequent measurements to be made on the same leaves at approximately the same

Species/Treatment	Watered	Not Watered
Yellow Poplar 1	May 1 to Aug 16 Sept 21 to Oct 5	Aug 17 to Sept 20
Yellow Poplar 2	May 1 to June 17 Sept 21 to Oct 5	June 18 to Sept 20
Sugar Maple 1	May 1 to Aug 1	
Sugar Maple 2	May 1 to June 17 July 28 to Aug 1	June 18 to July 27
Sugar Maple 4	July 13 to Aug 1	May 1 to July 12
Red Oak 1	May 1 to Aug 20	
Red Oak 2	May 1 to June 17 July 26 to Aug 20	June 18 to July 25
Red Oak 4	July 13 to Aug 20	May 1 to July 12
White Ash 1	May 1 to Aug 1	
White Ash 2	May 1 to June 17 July 1 to Aug 1	June 18 to July 1
White Ash 4	June 18 to July 8	May 1 to June 17 July 8 to Aug 1

Table 2. Watering schedules for four species subjected to varying drought treatments.

position on the leaf. Although most measurements were made on individual leaves, reflectance data were also collected for layers of leaves.

Radiometric temperature measurements were made on several days to determine the magnitude of diurnal changes in radiometric temperature differences among treatments. Apparent temperature data were also collected from leaves illuminated at different sun angles to evaluate the effect of wilting on apparent temperatures of tree foliage, and from the sunlit and shaded portions of stressed and healthy seedlings to illustrate the magnitude of variation in apparent temperature.

Visible indicators of stress (i.e., wilting, color changes and the relative length of time before these symptoms become apparent) were recorded for each species. Leaf moisture tension data were also collected on several dates to indicate relative degrees of moisture stress and to illustrate possible differences in the water status of ring-porous and diffuse-porous species.

Reflectance and emittance data collected during this study were computerized and analyzed to determine changes in response characteristics for the species studied.

### Results

Moisture stress affects many physiologic processes in plants. Water uptake, stomatal closure, transpiration rates, growth and shrinkage of cell tissues are only a few processes affected by moisture stress but this list includes those that most strongly alter the spectral and emissive properties of plant foliage. Changes in several of these

processes may occur before any visible sign of change in plant vigor.

### Stress Indicators

The most obvious sign of moisture stress is foliar wilting which frequently occurs diurnally. Although each species studied showed characteristic wilting symptoms when subjected to high levels of moisture stress, there were differences in foliar symptoms between species. Foliage on stressed oak seedlings showed symptoms of severe marginal necrosis before any significant wilting. Marginal necrosis of oak foliage resulted in significant differences in reflectance between the necrotic and greener portion of the leaf, complicating the procedure for measuring reflectance. White ash foliage became severely wilted and chlorotic, and eventually became reddish brown with no evidence of necrosis as stress became more severe. Since the change was relatively uniform over the entire leaf surface, reflectance measurements were made at one spot on the leaf. Sugar maple leaves also became severely wilted with increasing levels of stress, but foliar symptoms of moisture stress differed from those of red oak and white ash. As the maple leaves developed increasing levels of moisture stress, the leaves exhibited a mottled chlorotic appearance which was essentially uniform over the surface of the leaf. This was followed by severe "burning" and the leaf became somewhat reddish brown in color.

Although wilting and foliar symptoms of moisture stress for these three species were quite different and readily apparent, indications of stress were obtained prior to any visible symptoms by monitoring leaf

moisture tension for each species. Because environmental conditions often cause diurnal fluctuations in leaf moisture tension, measurements made early in the morning, when the soil-plant-water system is at or near equilibrium, provide estimates of plant moisture stress little influenced by diurnal fluctuations in leaf moisture tension. Oak and ash seedlings developed extremely high leaf moisture tensions over time, and although moisture tension of stressed maple leaves increased over time, the magnitude of increase was considerably less than for the ring-porous oak and ash. Leaf moisture tension for stressed diffuse-porous seedlings seldom exceeded 180 pounds-per-square-inch while leaf moisture tension values of stressed ring-porous species frequently exceeded 300 pounds-per-square-inch (quantitative estimates of moisture stress can be expressed as negative bars of leaf moisture tension, e.g., -1 bar approximately equals -14.7 pounds-per-square-inch of moisture tension).

Leaf moisture tension values recorded on droughted and well-watered seedlings were consistently lower for diffuse-porous species than for ring-porous species. Water conducting vessels of ring-porous species are longer and larger in diameter than water conducting vessels of diffuse-porous species (Kramer and Kozlowski, 1960). This should result in increased rates of water movement and subsequent increases in moisture tension. Large vessels of ring-porous species are more susceptible to disruptions of water columns when under stress, however, and are more susceptible to mechanical injury than the smaller vessels of diffuse-porous species. This increased susceptibility to disruption of water

conducting tissues may result in a rapid loss of conductive capacity (Kramer and Kozlowski, 1960). White ash foliage consistently developed higher moisture tension values than all other species studied. Leaf moisture tension of ash leaves, watered to field capacity after prolonged periods of drought, was consistently higher than leaf moisture tension for seedlings receiving ample water, suggesting that disruptions of the water column may have occurred.

#### Apparent Foliar Temperature

Morphology, soil moisture stress and diurnal fluctuations in illumination were found to affect the apparent temperature of healthy and stressed foliage.

Apparent temperatures, ambient air temperatures and values of incoming solar radiation were recorded at hourly intervals on several days. Analyses of these data indicate that, under relatively low levels of solar illumination, ambient air temperature is slightly higher than the apparent temperature of well-watered foliage and approximately equal to the apparent temperature of stressed foliage (Figure 3). As intensity of incoming solar radiation increased, apparent temperatures of both well-watered and stressed foliage became higher than ambient air temperature (Figure 4). Temperatures of foliage were observed to increase in the morning and decrease late in the afternoon, in a pattern similar to the changing pattern of air temperature and incoming solar radiation.

Differences in apparent temperature between watered and unwatered foliage developed sooner for ring-porous species than for diffuse-porous



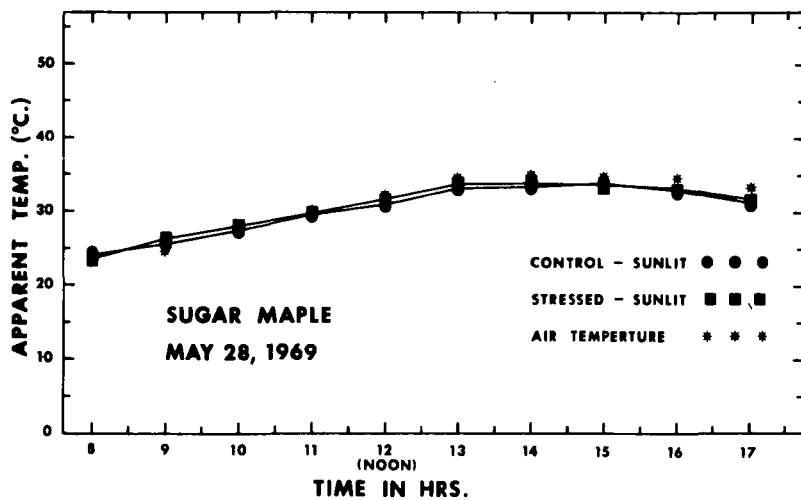
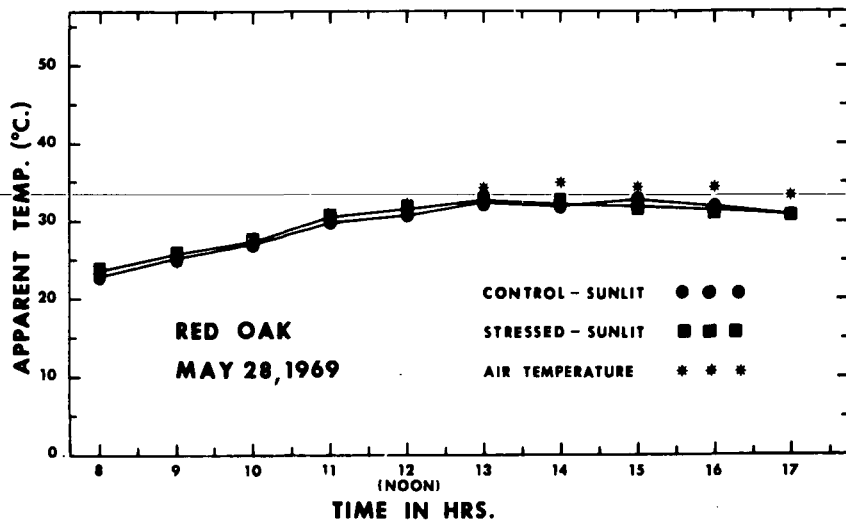
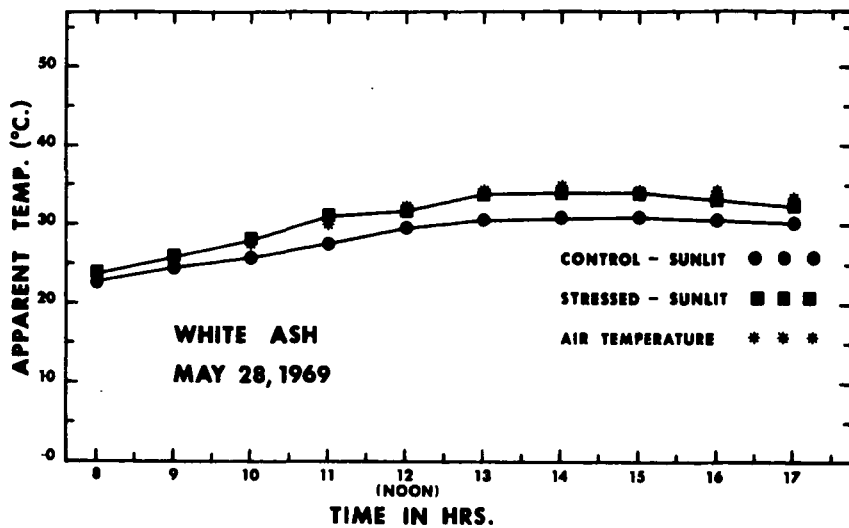


Figure 3. Apparent temperatures of three tree species two weeks after study was begun. Air temperature is warmer than temperature of control seedlings and about equal to temperature of stressed seedlings.

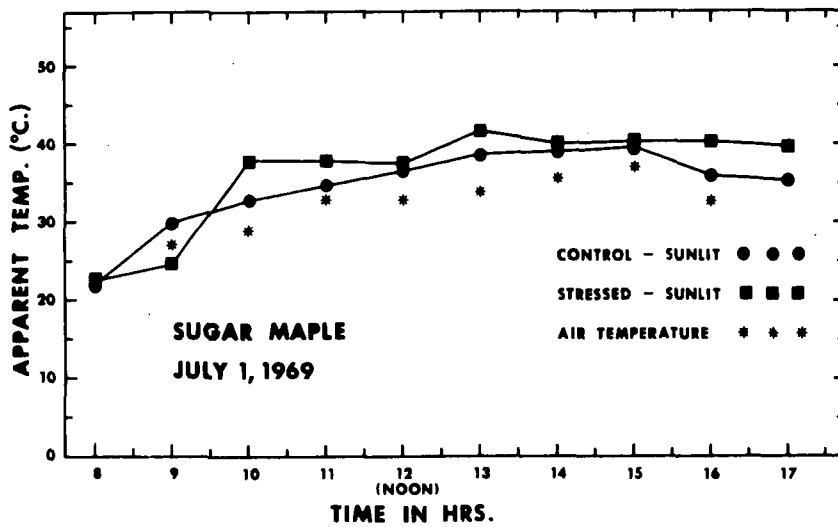
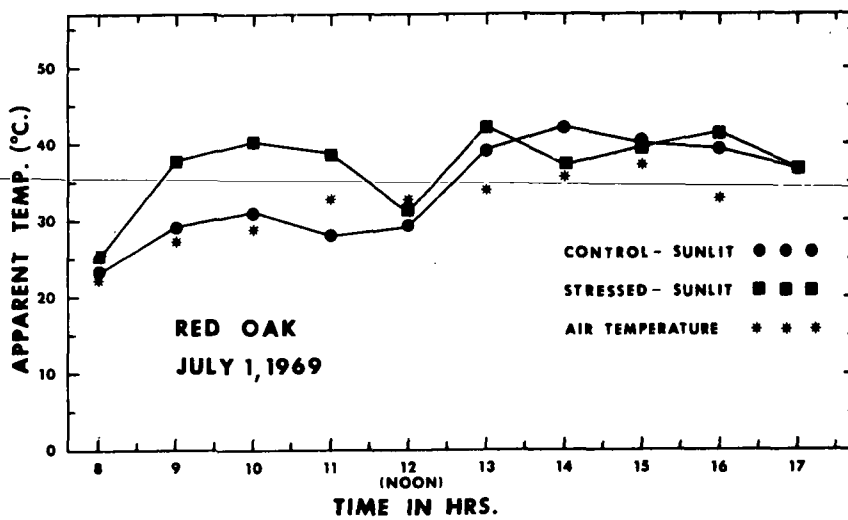
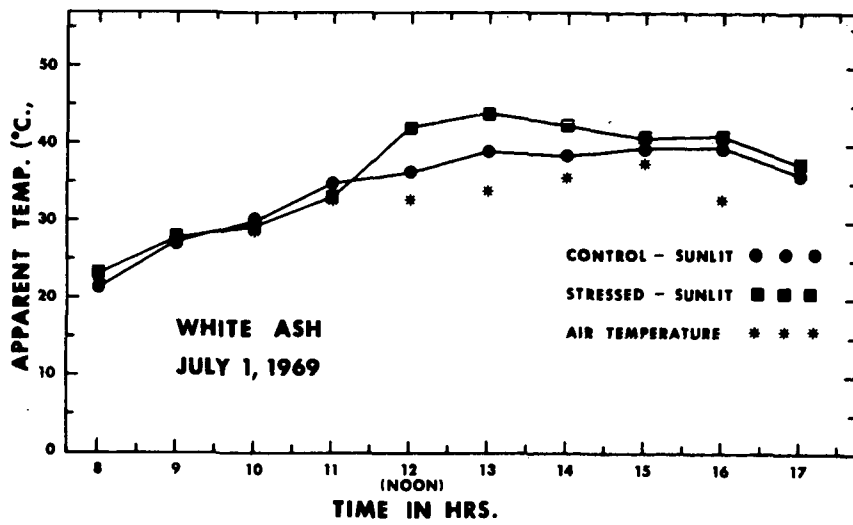


Figure 4. Apparent temperatures of control and stressed seedlings were generally higher than ambient air temperature at high levels of incoming solar radiation.

species (Figure 5). Foliage on well-watered ring-porous species was slightly warmer than foliage on well-watered diffuse-porous species from about 1000 hours to 1300 hours and foliage of stressed ring-porous species was significantly warmer by 2° to 3° C than foliage of stressed diffuse-porous species. Within the ring-porous group, white ash developed greater foliar temperature differences than red oak. Within the diffuse-porous group, yellow poplar developed greater foliar temperature differences than sugar maple. The least temperature difference between unwatered trees and those watered regularly was recorded for sugar maple, a result that may be related to wilting.

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Differences in angle of illumination resulted in large differences in apparent temperature of foliage on the same tree. Sunlit foliage was considerably warmer than shaded foliage on stressed red oak trees (Figure 6). Although shaded foliage of stressed trees was warmer than shaded foliage of well-watered trees, sunlit foliage of well-watered trees was warmer than shaded foliage of stressed trees (Figure 7).

To check the effects of wilting on apparent temperature, leaves on well-watered sugar maple seedlings were tied in a nearly vertical position. Apparent temperatures of such leaves were consistently cooler, by 3° to 6° C, than trees with leaves in a horizontal position. These results indicate that morphological response to moisture stress is important and should be more thoroughly understood if considering thermal detection of stress in vegetation.

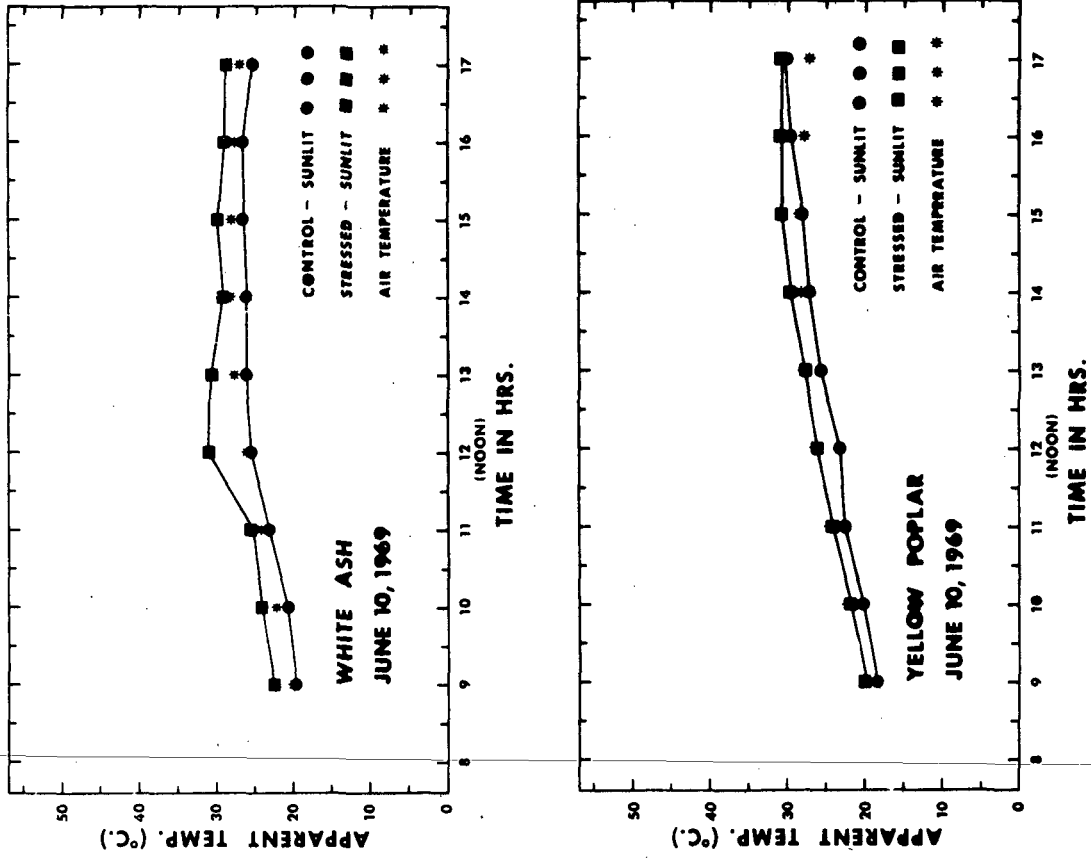


Figure 5. Temperature differences developed sooner between control and stressed ring-porous red oak and white ash than between control and stressed diffuse-porous sugar maple and yellow poplar.

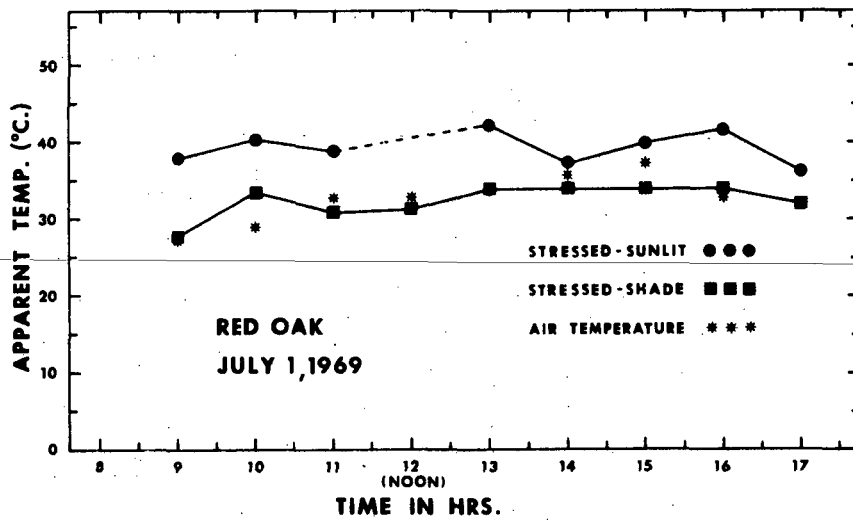


Figure 6. Sunlit foliage of stressed red oak is warmer than air temperature and considerably warmer than shaded foliage on the same tree.

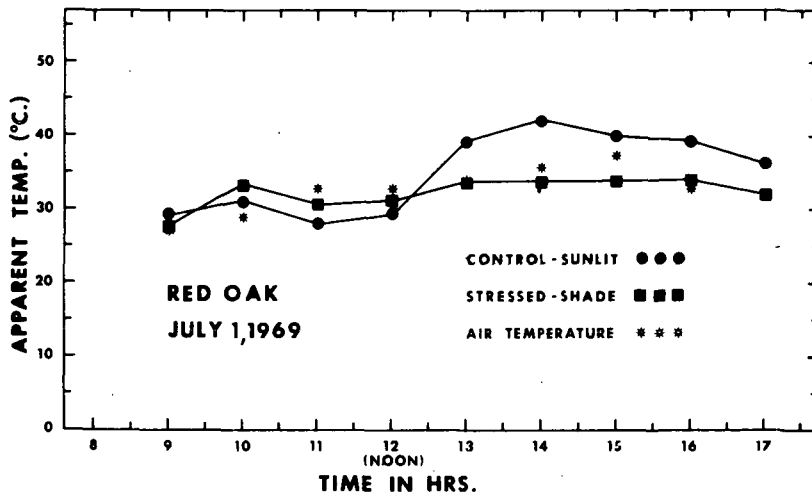
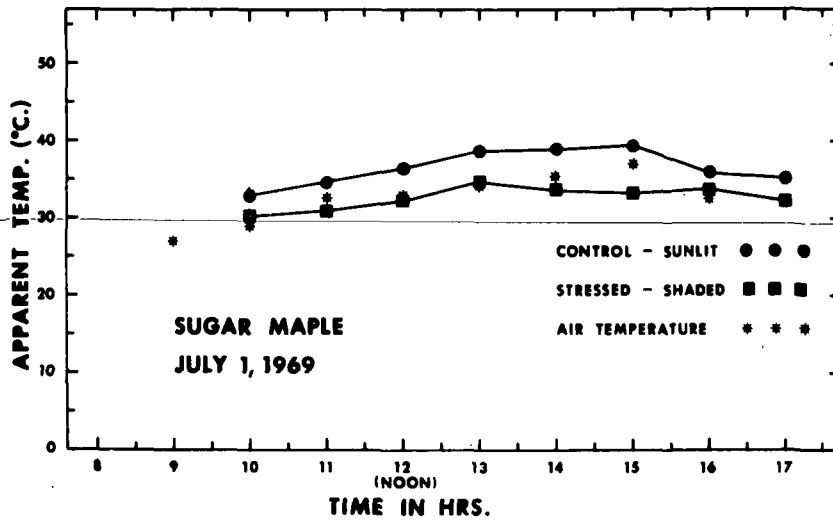
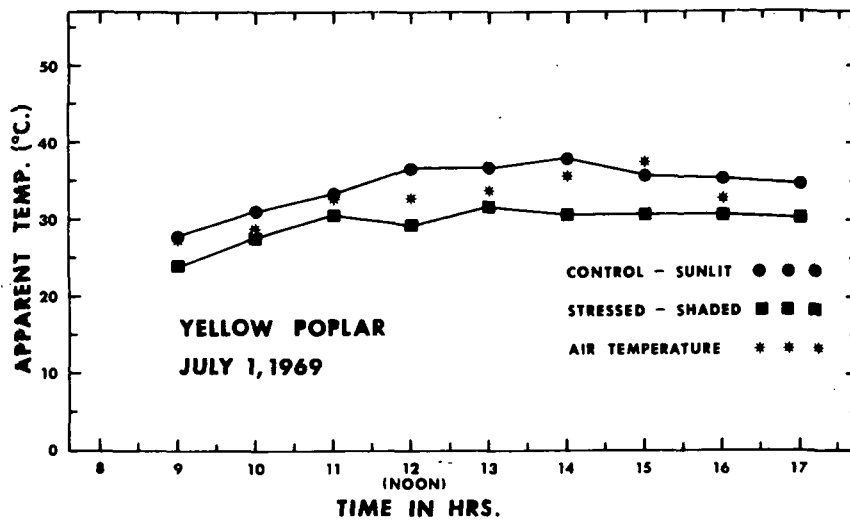


Figure 7. Apparent temperature curves for three species. Sunlit foliage of control seedlings was consistently warmer than shaded foliage of stressed seedlings.

### Reflectance of Stacked Leaves

Reflectance patterns of stacked leaves from three well-watered seedlings were similar. Reflectance increased at all wavelengths from 0.50  $\mu\text{m}$  to 2.6  $\mu\text{m}$  as more leaves were stacked. The largest increase in reflectance occurred at wavelengths from 0.80  $\mu\text{m}$  to 1.30  $\mu\text{m}$  where plant foliage is highly transparent. Small water absorption bands at 0.97  $\mu\text{m}$  and 1.20  $\mu\text{m}$  became more apparent as the number of stacked leaves increased. No reflectance changes were recorded between 0.80  $\mu\text{m}$  and 1.30  $\mu\text{m}$  for leaf samples composed of more than 10 leaves. Small increases in reflectance were recorded between 0.50  $\mu\text{m}$  and 0.80  $\mu\text{m}$  and between 1.30  $\mu\text{m}$  and 2.60  $\mu\text{m}$  as the number of stacked leaves increased to three. A fourth leaf did not significantly change the reflectance properties at these wavelengths (Figure 8).

Discrimination between species was accomplished with reflectance data at several wavelengths. Reflectance of sugar maple and red oak was higher than reflectance of white ash between 0.50  $\mu\text{m}$  and 0.80  $\mu\text{m}$  and between 1.30  $\mu\text{m}$  and 2.60  $\mu\text{m}$ . Reflectance of sugar maple foliage was lower than reflectance of white ash foliage between 0.80  $\mu\text{m}$  and 1.30  $\mu\text{m}$  for all combinations of leaves. No differences in foliar reflectance between red oak and white ash were recorded between 0.80  $\mu\text{m}$  and 1.30  $\mu\text{m}$ . Foliar reflectance was higher for sugar maple than for red oak between 0.50  $\mu\text{m}$  and 0.80  $\mu\text{m}$  but lower for red oak between 0.80  $\mu\text{m}$  and 1.30  $\mu\text{m}$ , for all combinations of leaves. There was no difference in reflectance between sugar maple and red oak at wavelengths from 1.30  $\mu\text{m}$  to 2.60  $\mu\text{m}$  (Figure 8).

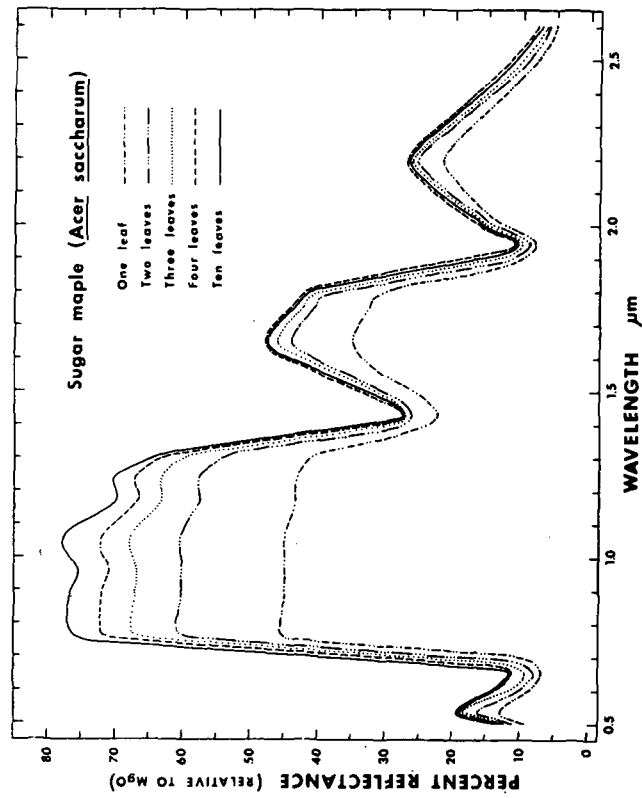
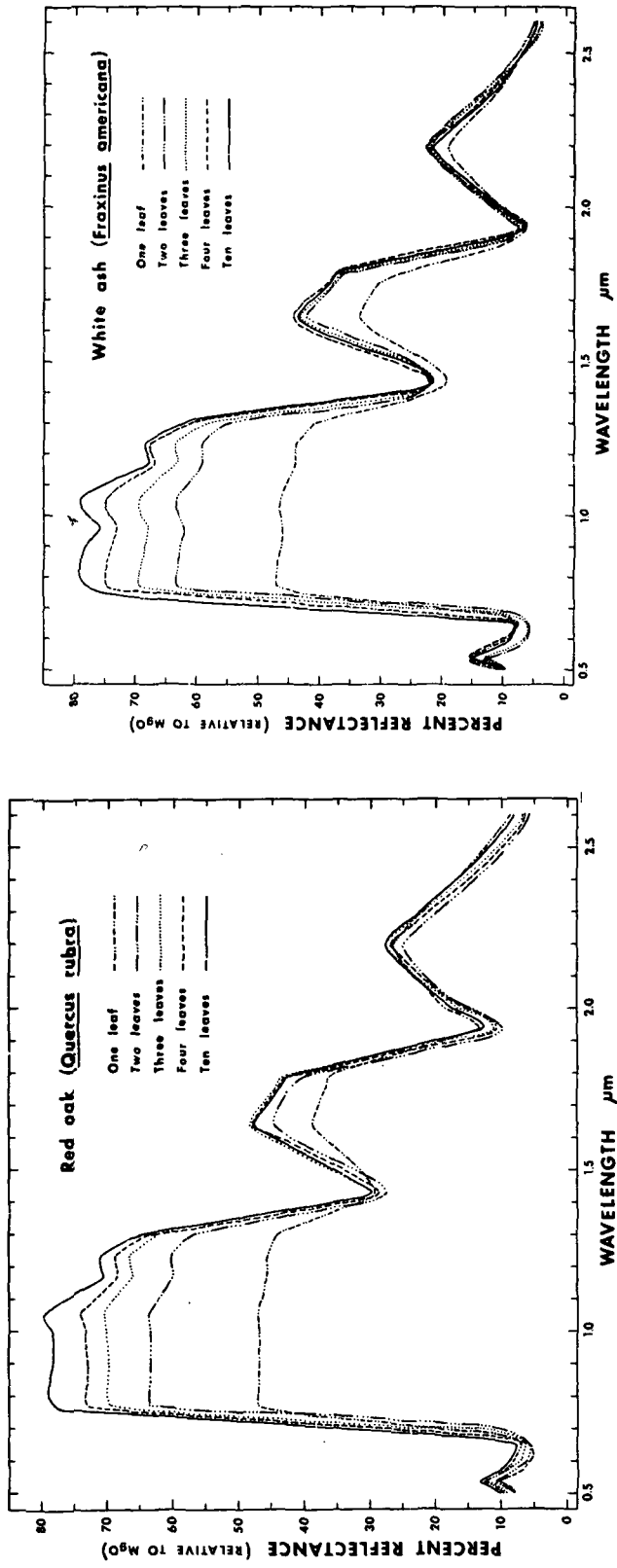


Figure 8. Free hand reflectance curves for several combinations of leaves for three species. Reflectance increased for all combinations of leaves between 0.80 and 1.30 micrometers.



### Seasonal Differences in Reflectance

Seasonal reflectance data between 0.50  $\mu\text{m}$  and 0.80  $\mu\text{m}$  for white ash, red oak and sugar maple indicate similar reflectance patterns between ring-porous and diffuse-porous species. All species leafed out in early May 1969 and continuous reflectance measurements were begun on May 19, 1969. Reflectance at wavelengths from 0.50  $\mu\text{m}$  to 0.80  $\mu\text{m}$  showed slight increases, initially, and then decreased during May and June, increased slightly in July and remained relatively unchanged after the middle of July and throughout the rest of the study (Figures 9 and 10).

Seasonal reflectance changes between 0.80  $\mu\text{m}$  and 1.2  $\mu\text{m}$  were similar for ring-porous and diffuse-porous species. Reflectance increased slightly throughout the entire growing season at all wavelengths from 0.80  $\mu\text{m}$  to 1.2  $\mu\text{m}$  (Figures 9 and 10).

Reflectance properties of all species between 1.2  $\mu\text{m}$  and 2.6  $\mu\text{m}$  are strongly affected by foliar moisture content. Reflectance of ring-porous species increased by about 2% at these longer wavelengths throughout May and remained relatively unchanged from June through August with an increase in reflectance of only about 1%. Reflectance of sugar maple, however, was observed to increase gradually over the entire growing season by about 3%. The change in magnitude of reflectance occurred only in the early part of the growing season (Figures 9 and 10).

Results of data analyses suggest that careful analysis of seasonal reflectance data at several wavelengths will improve species discrimination. Reflectance of white ash between 0.50  $\mu\text{m}$  and 0.80  $\mu\text{m}$  remained 5% to 7% higher than reflectance of red oak and sugar maple throughout the

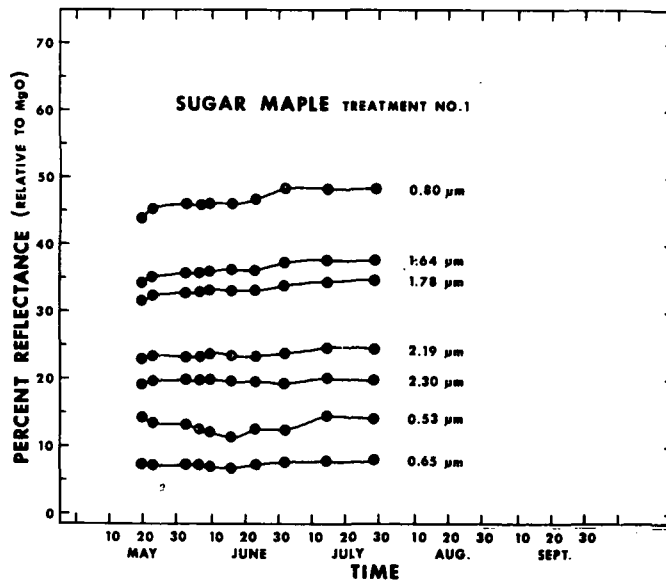
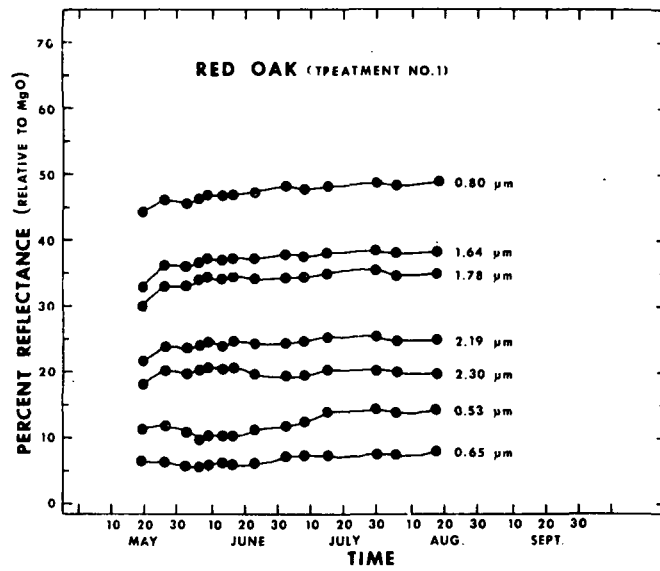
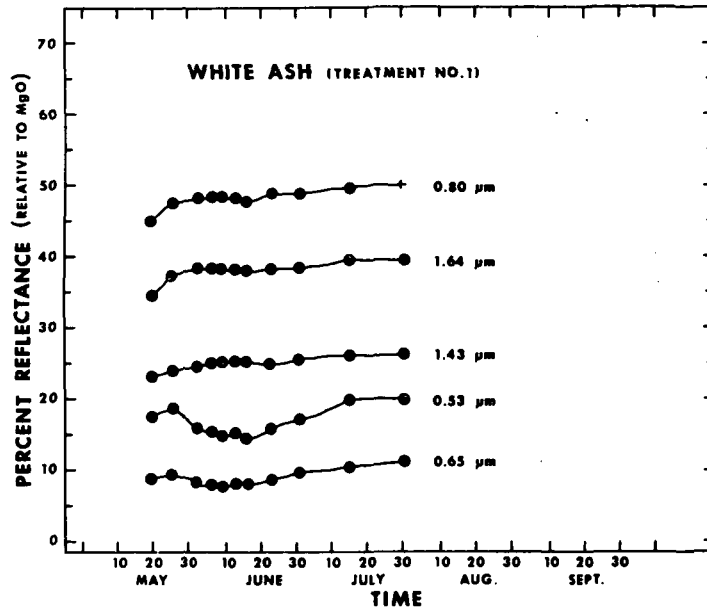


Figure 9: Free hand curves illustrating seasonal changes in reflectance at several wavelengths.

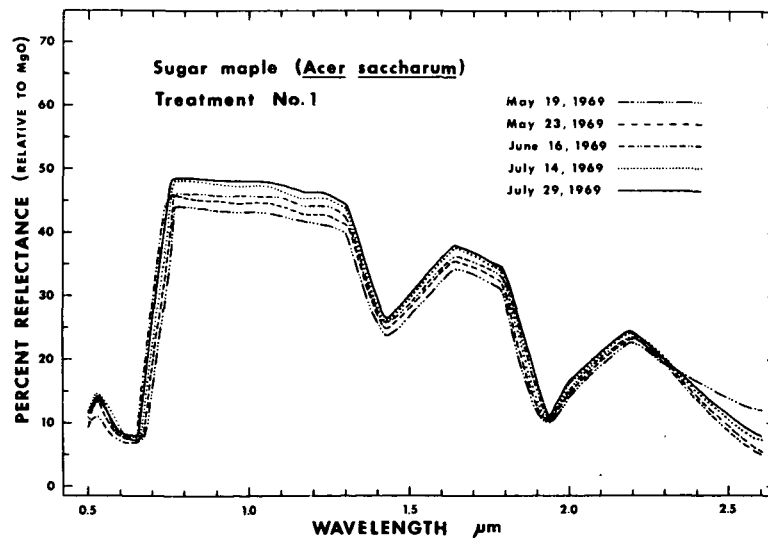
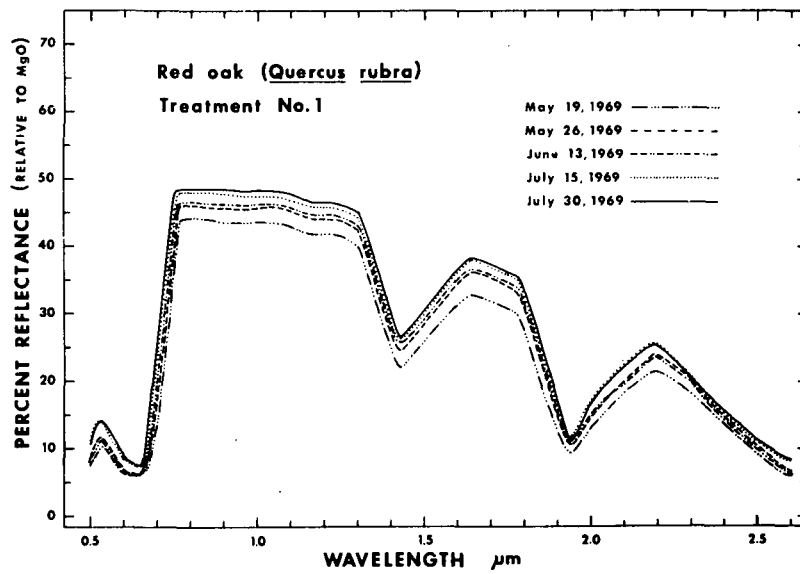
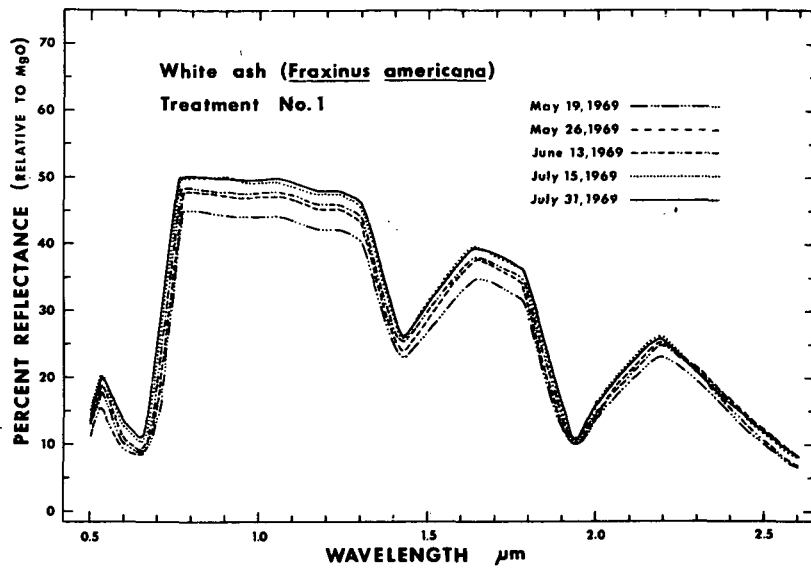


Figure 10. Free hand reflectance curves illustrating seasonal changes in reflectance at wavelengths from 0.50 to 2.6 micrometers.

entire study. Although higher reflectance between 0.50  $\mu\text{m}$  and 0.80  $\mu\text{m}$  was recorded for sugar maple than for red oak early in the growing season when reflectance for all species was generally decreasing, no difference in reflectance was recorded later in the season when reflectance for all species was increasing.

Reflectance of white ash was higher than reflectance of red oak and sugar maple at all wavelengths from 0.80  $\mu\text{m}$  to 2.6  $\mu\text{m}$  throughout the growing season. Although no differences in reflectance between 0.80  $\mu\text{m}$  and 1.2  $\mu\text{m}$  were recorded for sugar maple and red oak, sugar maple had slightly lower reflectance between 1.3  $\mu\text{m}$  and 2.6  $\mu\text{m}$  throughout the growing season.

#### Patterns of Reflectance Changes of Stressed Ring and Diffuse-porous Species

Reflectance of white ash foliage which leafed out under moisture stress and foliage which had reached full size before being under stress increased at all wavelengths from 0.50  $\mu\text{m}$  to 2.6  $\mu\text{m}$ . After reaching high levels of stress, the trees were watered and reflectance of those leaves which recovered decreased at all wavelengths from 0.50  $\mu\text{m}$  to 2.6  $\mu\text{m}$  but remained higher than reflectance of foliage on seedlings receiving ample water. Reflectance differences of 4% and 5% at wavelengths from 1.5  $\mu\text{m}$  to 1.8  $\mu\text{m}$  and 2.0  $\mu\text{m}$  to 2.6  $\mu\text{m}$ , respectively, were recorded prior to any change in visible reflectance. When reflectance differences of 3% between stressed and healthy foliage were recorded in the visible spectral region, stressed foliage had become more reflective at all wavelengths beyond 0.80  $\mu\text{m}$  with a maximum difference of 11% occurring at wavelengths

from 2.0  $\mu\text{m}$  to 2.6  $\mu\text{m}$ . Differences in reflectance between stressed and healthy foliage of ash seedlings were first detected on June 2, 1969 (Figures 11, 12 and 13).

Although sugar maple and white ash seedlings were placed under stress at the same time, differences in reflectance between foliage stressed and well-watered maple seedlings were not recorded until July 2, 1969. This suggests that sugar maple is relatively less susceptible to drought than white ash. Reflectance of sugar maple foliage which leafed out under stress and foliage which had reached full size before being placed under stress increased at all wavelengths from 0.50  $\mu\text{m}$  to 2.6  $\mu\text{m}$  (Figures 14, 15 and 16). Data are not available for foliage which received water after prolonged periods of drought.

Reflectance of severely stressed sugar maple foliage at 0.65  $\mu\text{m}$  was about 45% while reflectance of severely stressed white ash foliage at 0.65  $\mu\text{m}$  increased to only about 15%. This difference may be related to the stress symptoms of each species. Since sugar maple became highly chlorotic, the high reflectance may be due to decreased absorption by chlorophyll; severely stressed white ash foliage did not become as chlorotic; rather it gradually became pale green in color eventually changing to a fawn or brownish color.

Reflectance patterns were similar for red oak. Reflectance of foliage which leafed out under stress was less reflective than foliage on well-watered seedlings early in the growing season but as stress increased, the foliage became more reflective. Reflectance of foliage which had reached full size before being placed under stress increased at all

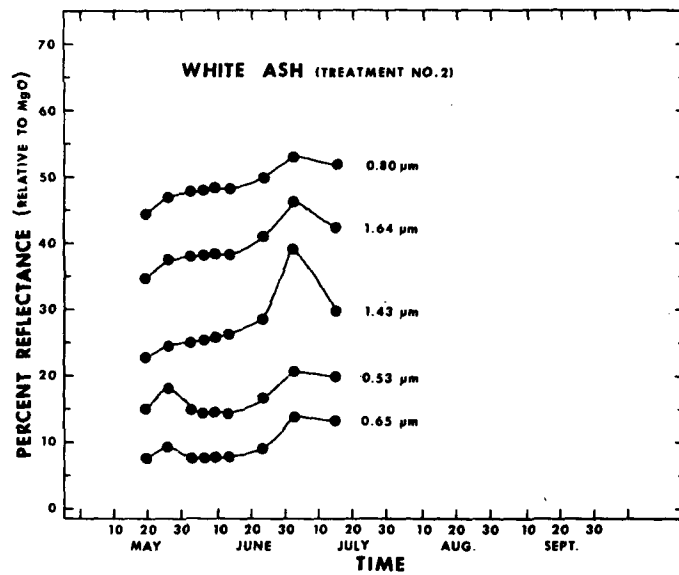
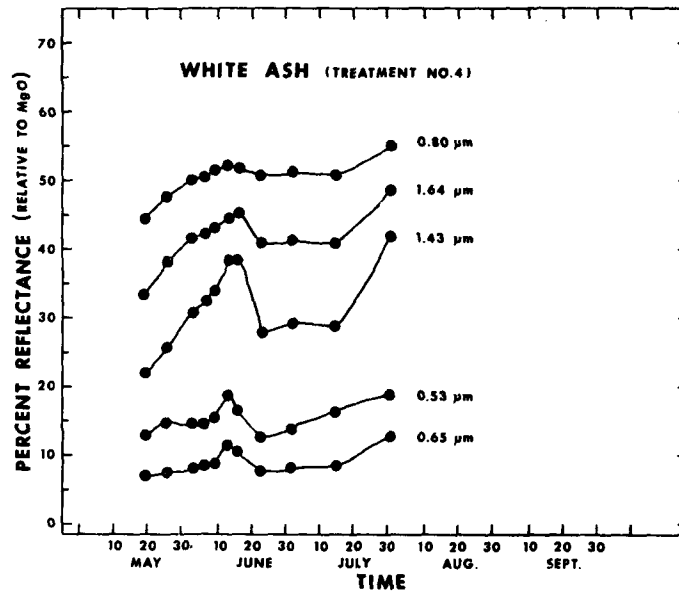


Figure 11. Free hand reflectance curves of white ash foliage illustrating increasing reflectance at several wavelengths for foliage developing under stress (treatment No. 41) and foliage developing before being placed under stress (treatment No. 21).

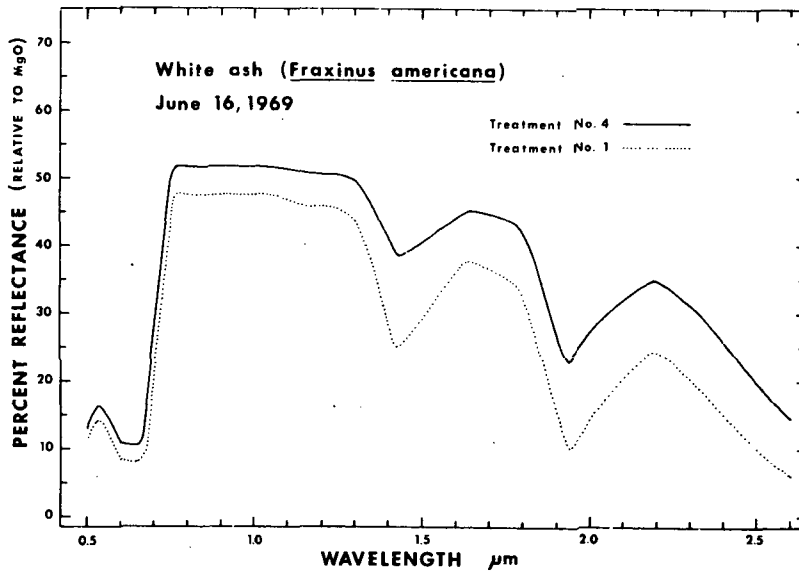
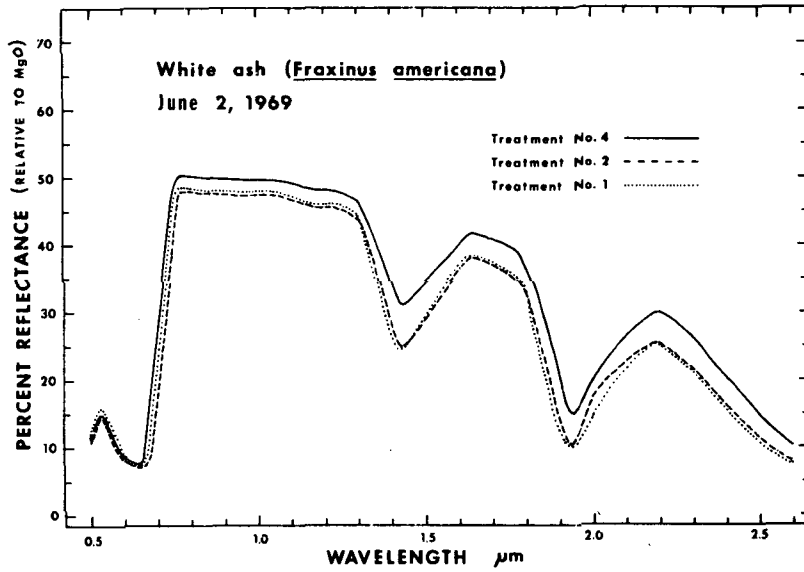
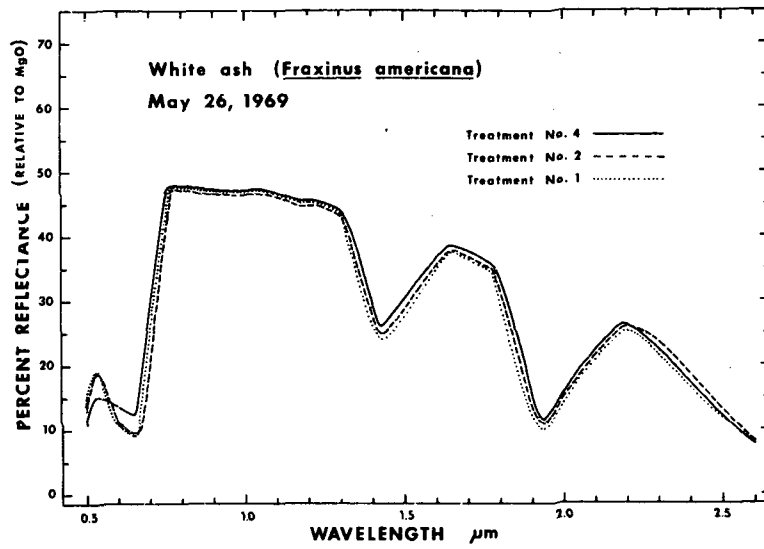


Figure 12. Free hand reflectance curves at three dates indicating changing patterns of reflectance with increasing moisture stress. (see Table 2).

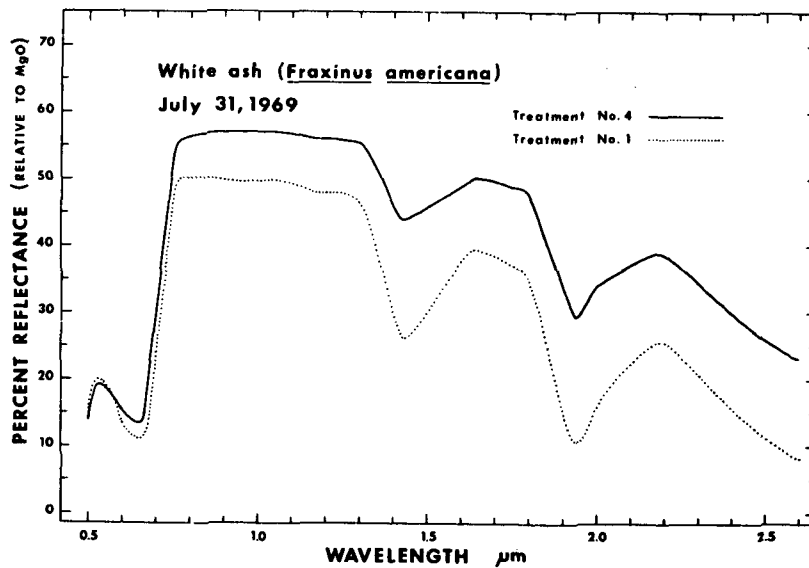
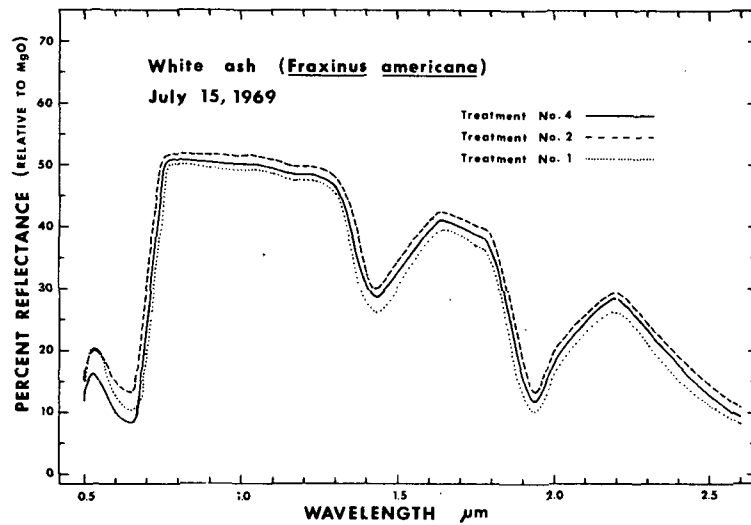
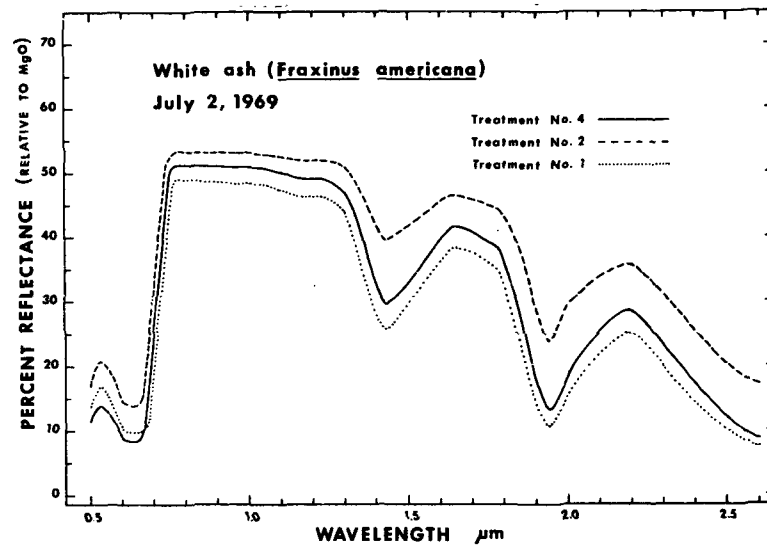


Figure 13. Free hand reflectance curves at three dates indicating changing patterns of reflectance with changing levels of moisture stress (see Table 2).



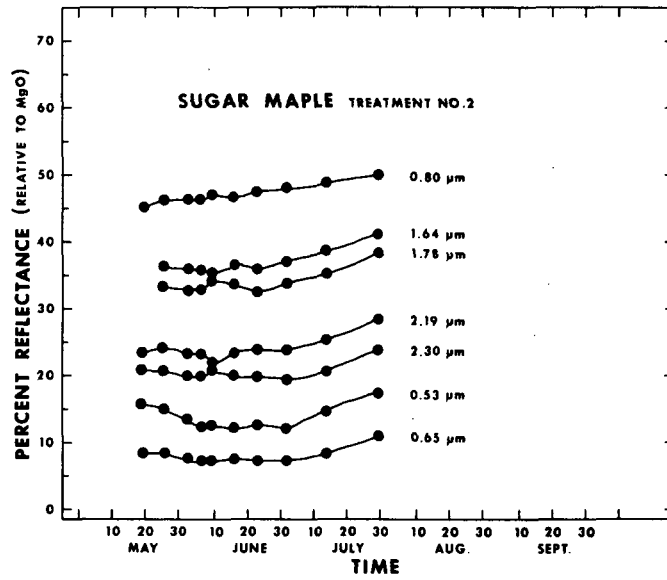
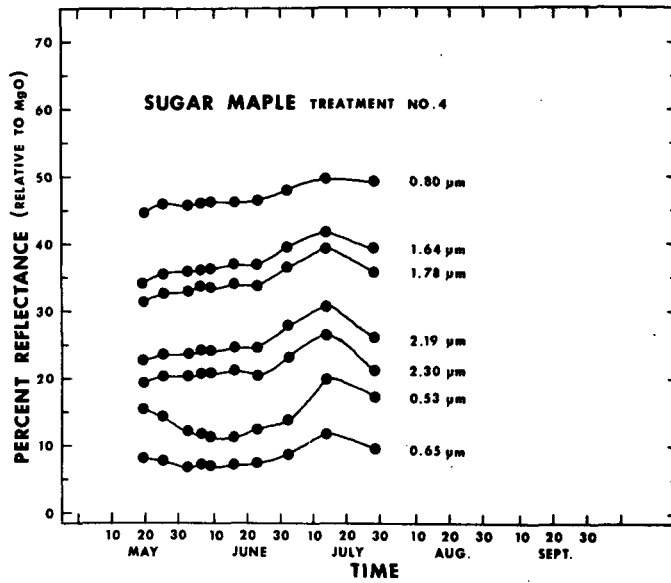


Figure 14. Free hand reflectance curves of sugar maple foliage illustrating increasing reflectance at several wavelengths for foliage developing under stress (treatment No. 4) and foliage developing before being placed under stress (treatment No. 2).

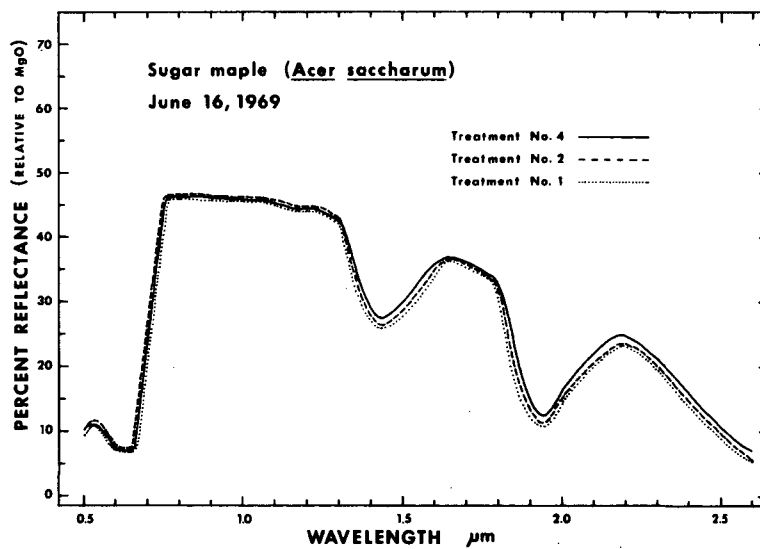
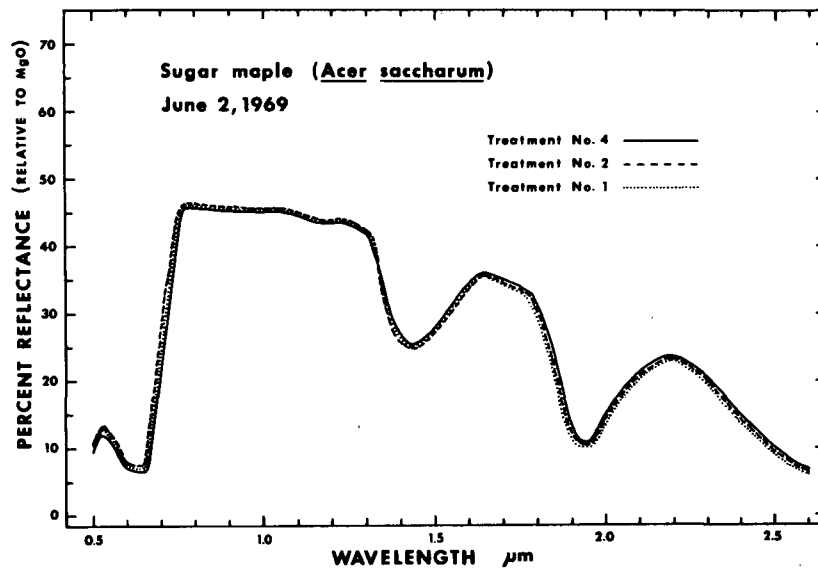
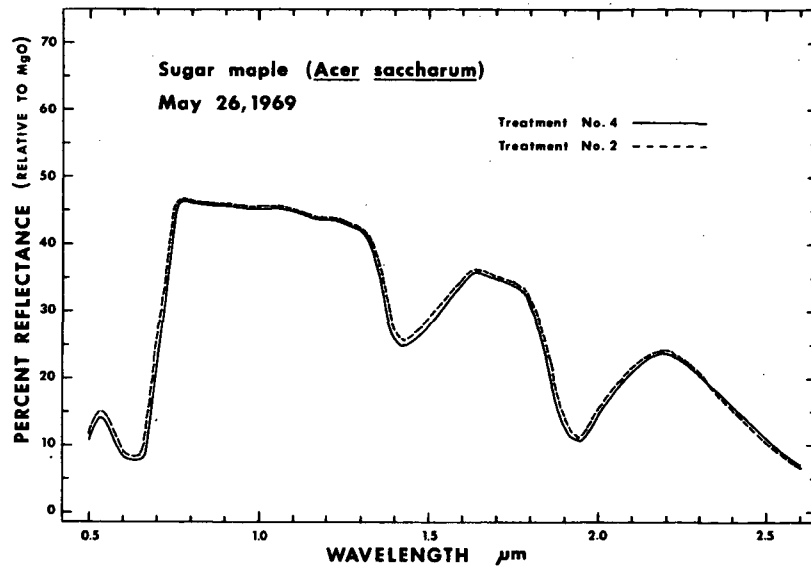


Figure 15. Free hand reflectance curves at three dates indicating changing patterns of reflectance with increasing moisture stress (see Table 2).

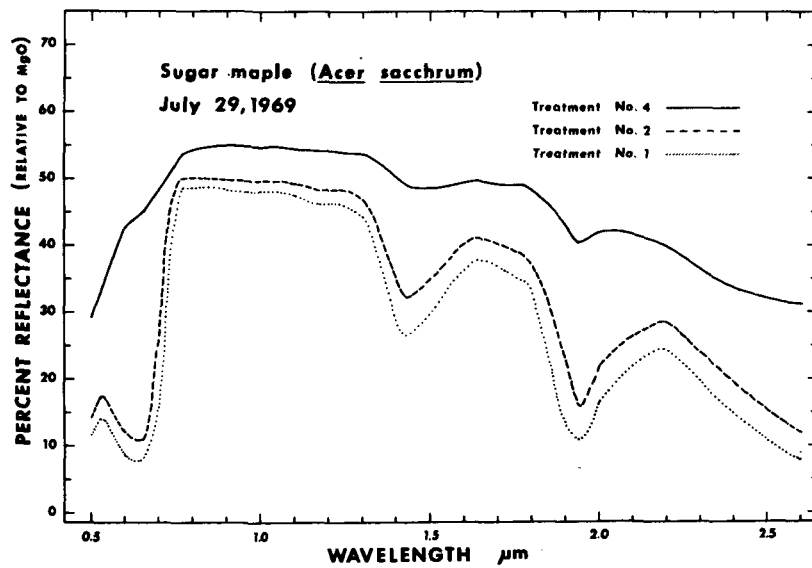
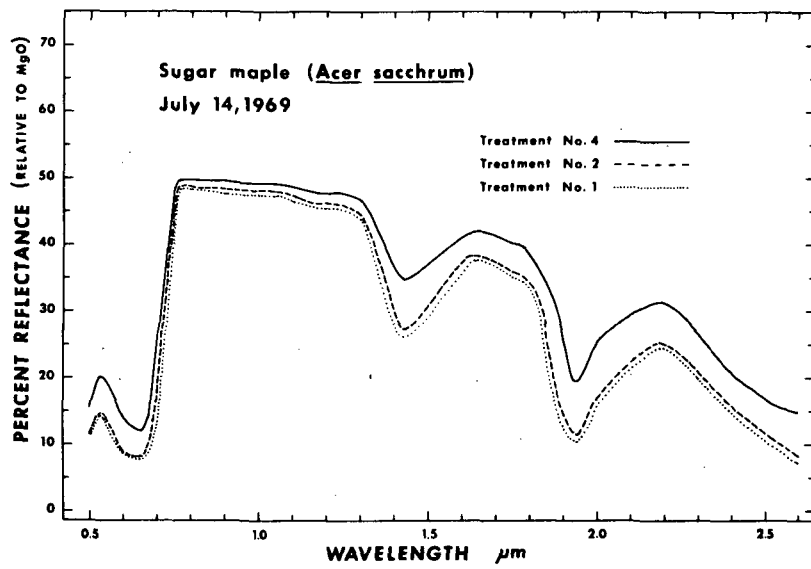
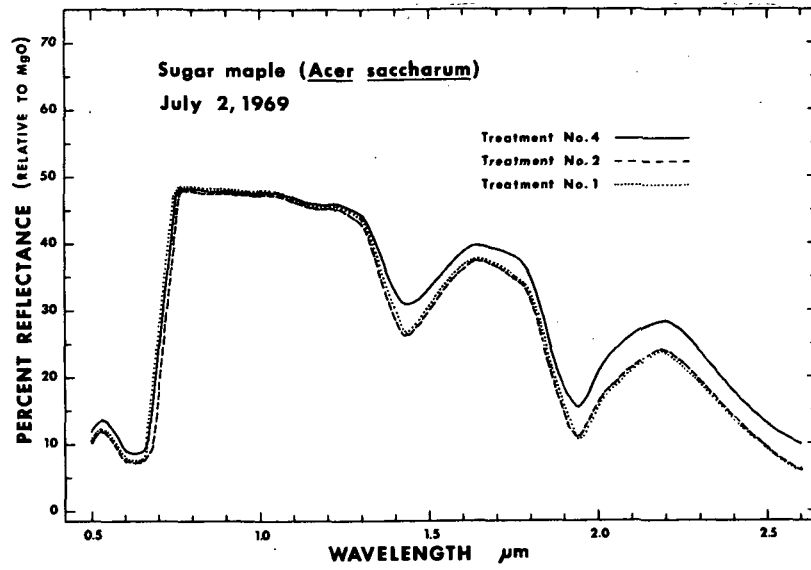


Figure 16. Free hand reflectance curves at three dates indicating changing patterns of reflectance with changing levels of moisture stress (see Table 2).

wavelengths from 0.50  $\mu\text{m}$  to 2.6  $\mu\text{m}$  as stress became more severe (Figures 17, 18 and 19). Moisture stress symptoms in oak resulted in large variation in reflectance on a single leaf. Marginal necrosis was characterized by wilting and discoloration of the outer edge of the leaf. Reflectance of the leaf's outer edge (dried portion) was higher at all wavelengths from 0.50  $\mu\text{m}$  to 2.6  $\mu\text{m}$  than reflectance from the apparently healthy center (green portion) of the leaf (Figure 20).

It must be pointed out that those trees considered to have flushed out under stress were in soil near field capacity at the beginning of the study and received no water during the course of the study. Because the soil was near field capacity, the trees may not have actually leafed out under stress.

Yellow poplar is the only species studied in this phase of the project which flushed continuously throughout the growing season. Reflectance properties of yellow poplar foliage were similar to those reported by Weber and Olson (1967). Reflectance of foliage which had flushed out and reached full size before being placed under stress was slightly higher than reflectance of foliage from seedlings receiving ample water. Leaves which flushed out and matured under stress during the growing season were less reflective at all wavelengths from 0.50  $\mu\text{m}$  to 2.6  $\mu\text{m}$  than foliage from well-watered seedlings (Figure 21). Since foliage of yellow poplar trees which were near field capacity at the start of the study but received no additional water until late in the growing season became more reflective than foliage from well-watered trees, it appears that those trees assumed to be flushing out under stress actually were

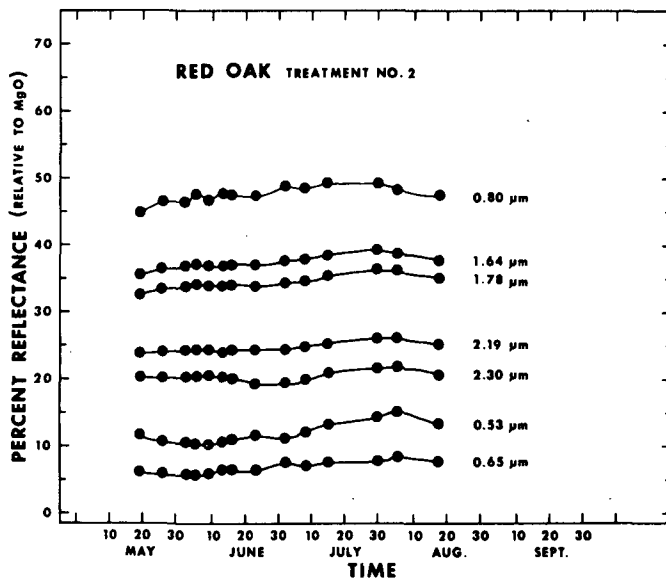
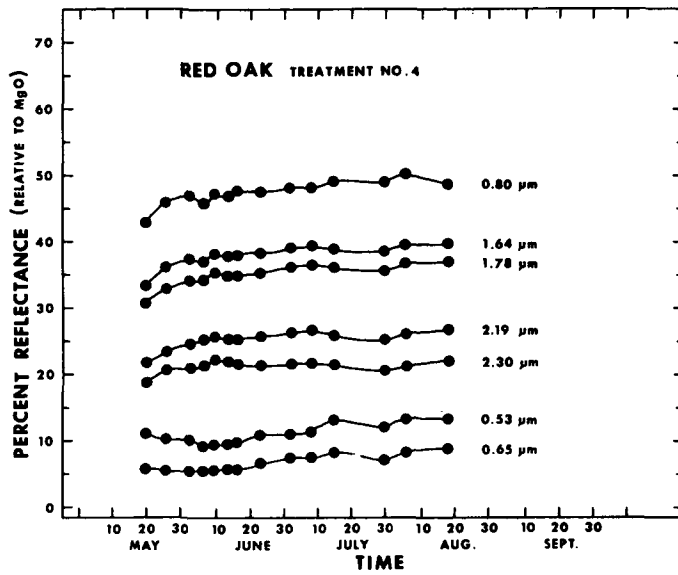


Figure 17. Free hand reflectance curves illustrating changing reflectance patterns at several wavelengths for foliage developing under stress (treatment No. 4) and foliage developing before being placed under stress (treatment No. 2).

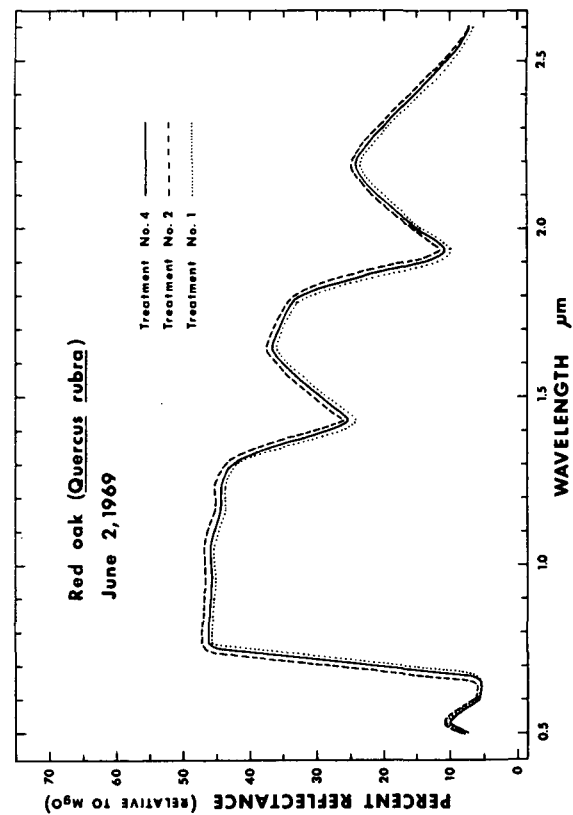
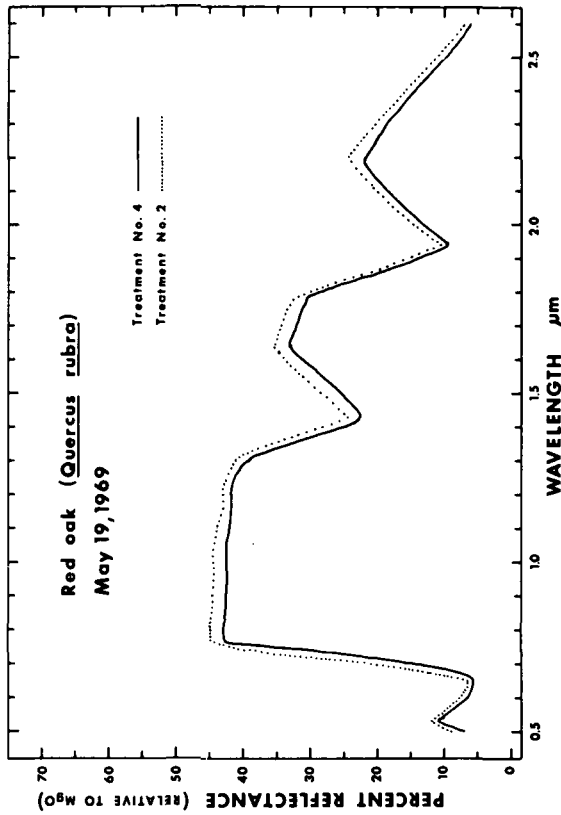
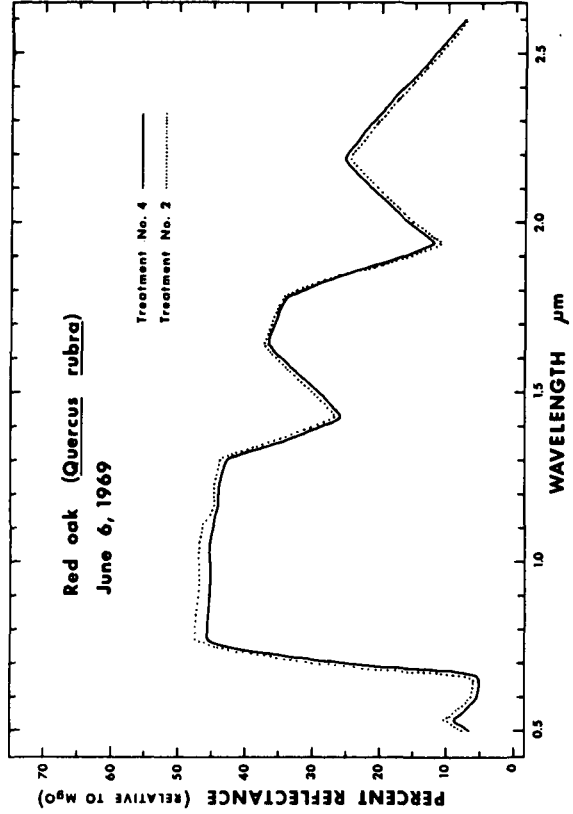
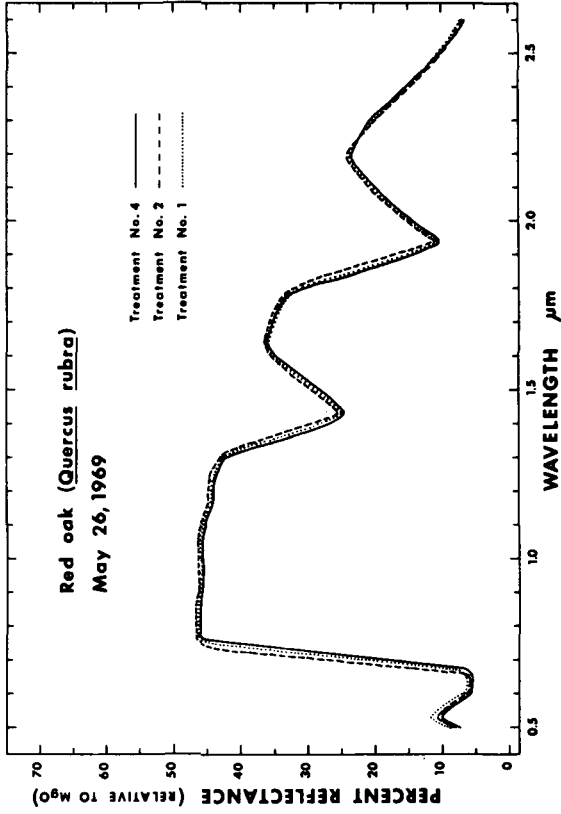


Figure 18. Free hand reflectance curves of red oak foliage at four dates indicating changing patterns of reflectance with changing levels of moisture stress (see Table 2).

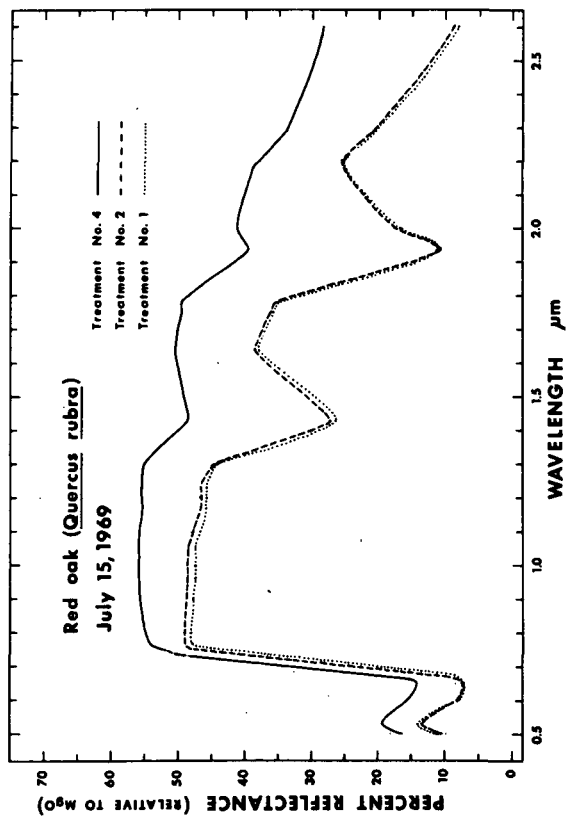
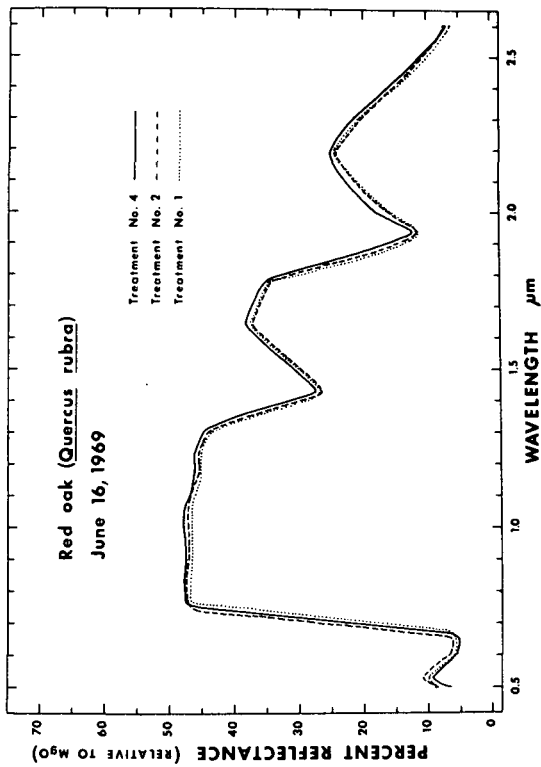
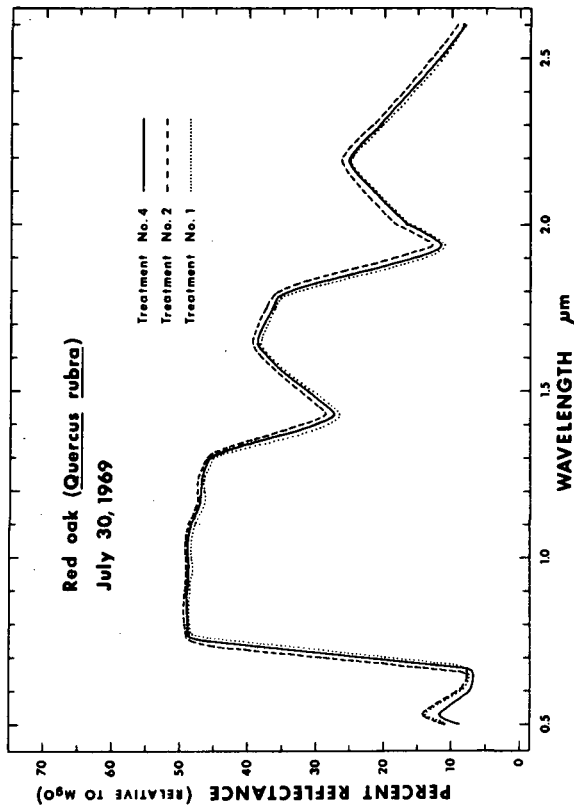
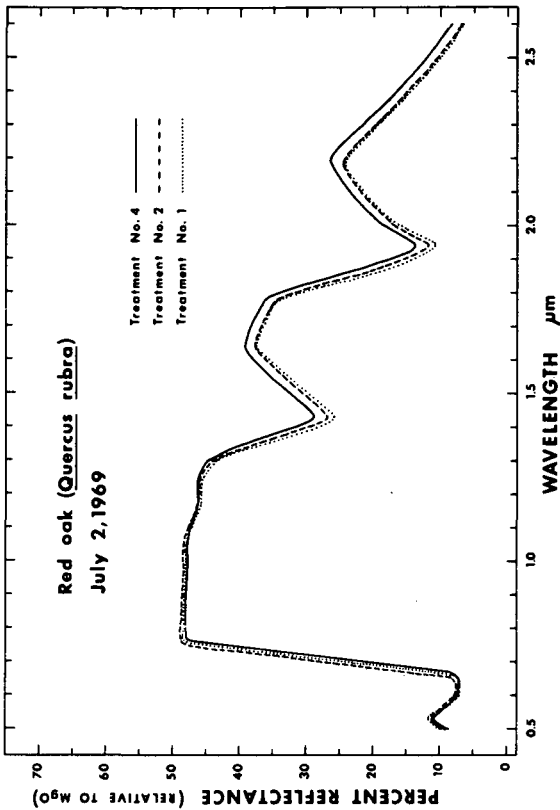


Figure 19. Free hand reflectance curves of red oak foliage at four dates indicating changing patterns of reflectance with changing levels of moisture stress (see Table 2).

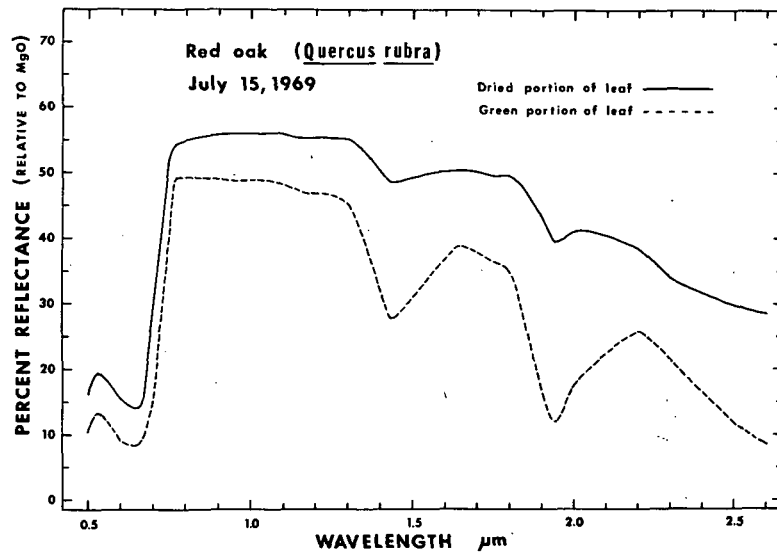
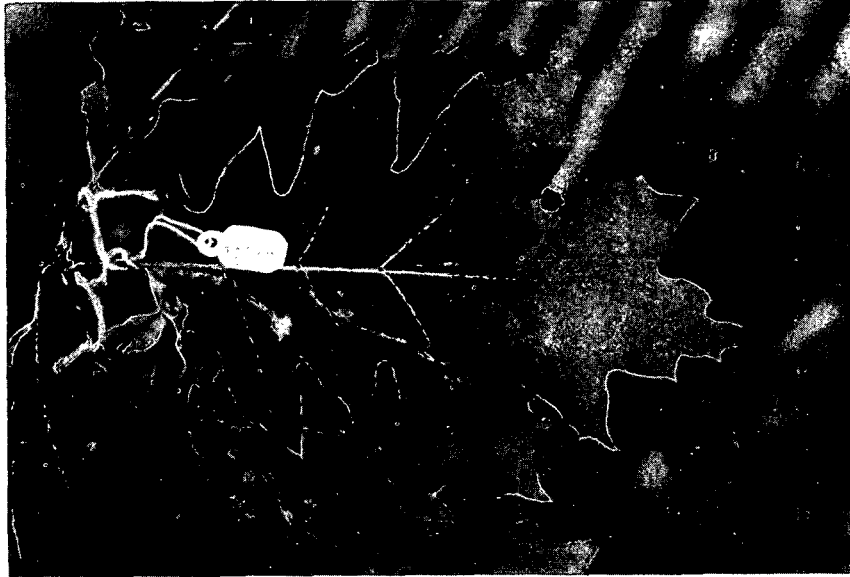


Figure 20. Free hand reflectance curves (bottom) from a stressed red oak leaf. The necrotic edges are more reflective than the apparent healthy center.



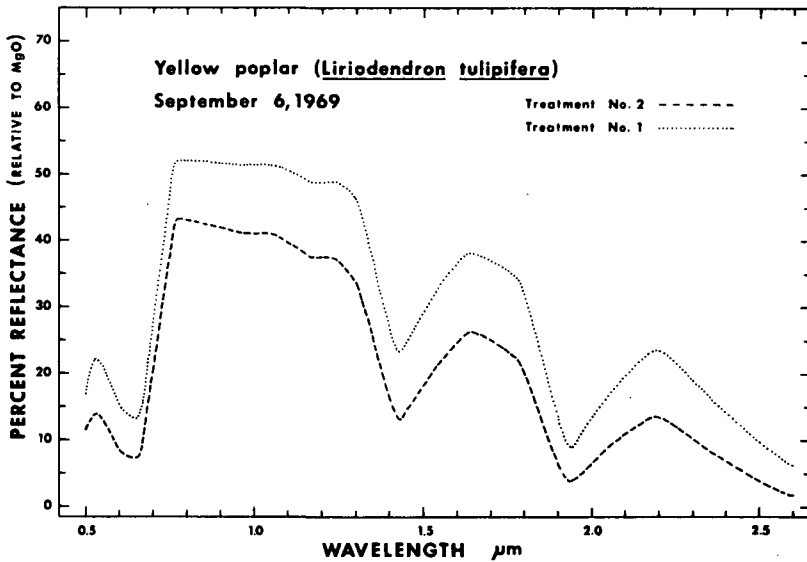
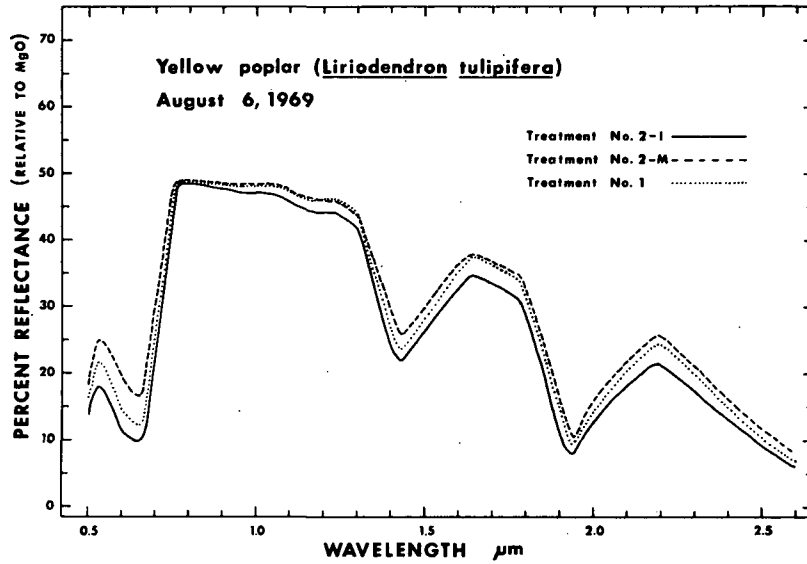


Figure 21. Free hand reflectance curves for yellow poplar foliage. Reflectance of foliage which had flushed and reached full size before being placed under stress (treatment No. 2M) was slightly higher than reflectance of foliage on well-watered seedlings (top). Reflectance of foliage preformed and enlarging under stress (treatment No. 2) was less than reflectance of watered seedlings (bottom).

receiving sufficient water early in the growing season and became stressed only after the leaves had reached full size.

## Discussion

### Apparent Foliar Temperature

Leaf temperatures are valuable indicators of the response of foliage to many dynamic environmental parameters. Temperature differences between wilted and non-wilted foliage were reported by Miller and Saunders (1923). Several other workers have reported temperature differences between healthy and stressed foliage. Gates (1964) states that leaves transpiring may be as much as 5° C cooler than non-transpiring leaves. Weber and Olson (1967) found that trees subjected to moisture stress had foliage which was consistently warmer than healthy foliage on stressed trees. Results of this study and others (Olson, Ward and Rohde, 1969) indicate that consistent temperature differences can be recorded between trees subjected to moisture stress and trees receiving ample water. Although large variations in temperature within a crown were recorded and have also been reported by Gates (1968), detection of temperature differences of 1.8° C between healthy and stressed trees with airborne thermal detectors has been reported by Weber (1965).

Leaf temperatures are strongly influenced by transpiration, an important process which cools leaves and aids in translocation of minerals. Transpiration, however, is largely controlled by stomatal activity. When stomates are open, carbon dioxide required for photosynthesis can enter, but considerable amounts of water may be lost as water evaporates from

cell walls and diffuses out through the stomates. When water absorption lags behind transpiration, leaf cells may lose turgor, resulting in stomatal closure during midday. This process results in disruption of many biochemical processes which may adversely affect plants (Kramer and Kozlowski, 1960).

Although laboratory results presented in this study indicate that significant temperature differences can be recorded throughout most of the day, it must be remembered that many factors can influence the temperature of plant foliage. Wilting, which acts to orient the leaves parallel to the sun's rays, and differences in illumination are two factors which can affect leaf temperatures possibly resulting in shaded, stressed leaves being cooler than sunlit leaves on well-watered seedlings. Combinations of high solar illumination and high ambient air temperatures may lead to water loss by transpiration exceeding that absorbed, resulting in what has been termed a "transpiration lag". The net result may be short term increases in foliar temperature, masking temperature differences between trees under prolonged stress and trees under temporary, short term stress.

The thermal data presented in this study indicate that the 8 to 14  $\mu\text{m}$  spectral band may provide early detection of moisture stress in trees. Several workers have reported detection of stress in vegetation with thermal infrared sensors (Weber, 1965; Wear and Weber, 1969; Heller, et al., 1969; Rohde and Olson, 1970). Although the available data show large differences in radiometric temperatures between stressed and unstressed trees from early morning to late afternoon, an understanding

of developing transpiration lags, wind velocities and solar illumination is essential for planning missions for optimum detection of moisture stress in vegetation.

#### Reflectance of Stacked Leaves

Many workers have studied reflectance properties of vegetation primarily by examining spectra from a single leaf. This is done because of the ease of making measurements and because of a specific interest in the reflectance response of an individual leaf. Predicting tonal contrasts on aerial photography or multispectral imagery has been attempted many times. Because leaves have been shown to transmit incident light at many wavelengths, the reflectance of light from foliage within a tree crown that had been transmitted through an upper layer of leaves will also affect tonal properties of vegetation on photographs and imagery. It is imperative, however, that reflectance properties of individual mature and immature leaves and leaves at various positions in the crown be thoroughly understood. Many workers have demonstrated that immature leaves have different reflectance properties than mature leaves and that reflectance of shade leaves is different than reflectance of sun leaves.

Results of this phase of the study show the possible importance of transmittance through upper leaves and back reflection from lower leaves. Myers, et al., (1966) reported a similar study where no increase in visible reflectance of cotton leaves was recorded from stacks of up to six leaves. Their conclusion was "...reflectance of visible light from leaf surfaces is from the top-most exposed leaves". Reflectance spectra from stacked

tree leaves show increasing visible reflectance for combinations of up to four leaves. This is not in keeping with results of similar studies with agricultural crops. Since the cuticle and epidermis of plant leaves have been shown to be nearly transparent to radiation in the visible and near infrared spectral regions (Clark, 1946), reflectance properties must be affected by internal scattering of light and multiple reflections from cells and cell walls. Reflectance at wavelengths from 0.50  $\mu\text{m}$  to 0.80  $\mu\text{m}$  does not increase as much as reflectance at wavelengths from 0.80  $\mu\text{m}$  to 1.30  $\mu\text{m}$  as the number of leaves is increased because of the increased amount of chlorophyll and other pigments which absorb a large amount of radiation in the shorter of these two wavelength regions.

Reflectance characteristics of vegetation at wavelengths from 1.30  $\mu\text{m}$  to 2.60  $\mu\text{m}$  are strongly influenced by foliar moisture content. Reflectance was increased by about 10% between 1.60  $\mu\text{m}$  and 1.80  $\mu\text{m}$  and by about 5% between 2.10  $\mu\text{m}$  and 2.30  $\mu\text{m}$  when two and three layers of leaves were sampled. Additional layers of leaves did not significantly increase the reflectance of any wavelength from 1.30  $\mu\text{m}$  to 2.60  $\mu\text{m}$ .

Data presented by Myers, et al. (1966), and results of this study, indicate that tonal characteristics of plant foliage on aerial photographs may be more related to multiple reflectance of leaf layers than from individual leaves. These two data do not preclude the possibility, however, of obtaining detectable tonal contrasts from reflectance of individual leaves or a single layer of leaves. Results of this study do, however, indicate the importance of understanding reflectance properties of individual leaves of forest trees.

An understanding of the reflectance properties of immature and mature foliage, shaded and sunlit foliage and differences in reflectance between stressed and healthy foliage is necessary before attempting to explain expected tonal contrasts using the multiple reflectance of leaf layers concept.

#### Seasonal Difference in Reflectance

Seasonal reflectance patterns for ash, oak and maple were found in the present study to be similar to those reported by Olson (1969). The recorded initial increase in visible reflectance has been attributed to cell enlargement and cell differentiation. After the leaf reached full size, visible reflectance generally decreased with age until about mid-June when reflectance remained relatively constant. Production and accumulation of chlorophyll within a leaf increases throughout the early part of the growing season and remains constant or decreases slightly later in the growing season, up until the time of fall color change (Gates, 1965; Olson, 1969) indicating that the changing patterns of visible reflectance are apparently due in part to chlorophyll production. It should be pointed out that the width of the absorption curve between 0.60  $\mu\text{m}$  and 0.68  $\mu\text{m}$  became wider as foliage matured. This phenomenon is in keeping with Heath (1969) who attributes this effect to increasing amount of other pigments and particles within a leaf as the leaf matures. Gates (1965) reports similar results and notes that the "absorption edge of the reflectance curves at 0.70  $\mu\text{m}$ " shifted toward longer wavelengths as the leaf matured. Analysis of the raw reflectance curves generated in this study reveals similar shifts.

Near infrared reflectance between 0.80  $\mu\text{m}$  and 1.20  $\mu\text{m}$  increased rapidly during the early part of the growing season for ring-porous red oak and white ash and remained fairly constant after the leaves had reached full size. Near infrared reflectance of diffuse-porous species increased gradually but became relatively stable as the leaves matured. These results are in keeping with results of studies reported by other workers (Gausman, et al., 1969a; Olson, 1969). As a leaf enlarges and matures, more intercellular air spaces are formed increasing the number of cell-wall-air interfaces, which tends to increase internal scattering and results in an increase in infrared reflectance. This is well demonstrated by Gausman, et al. (1969a), and supports the theory discussed in Clark (1945).

Leaves of ring-porous species are generally preformed in the bud the previous year, and when leaves flush out there is an initial, rapid period of leaf development and growth. After the leaves enlarge and reach full size no new leaves are formed during that growing season except under unusual conditions. Since leaves of diffuse-porous species are not preformed in the buds, there is a somewhat slower rate of leaf development early in the growing season. Leaves of diffuse-porous species generally mature later in the growing season than leaves of ring-porous species, and diffuse-porous species continue to put forth new foliage through most of the growing season. The rapid, short increase in infrared reflectance of ring-porous species and the more gradual increase in infrared reflectance of diffuse-porous species may be attributed to the differences in leaf flushing characteristics and

seasonal development of foliage between these two groups of broadleaved species.

The increase in reflectance at wavelengths from 1.3  $\mu\text{m}$  to 2.6  $\mu\text{m}$  is strongly related to moisture content. Increasing oven-dried weights of leaves may result in an increase in reflectance (Olson and Weber, 1967; Gausman, et al., 1970). The data suggest that leaf maturation, changes in cellular structure and changes in leaf moisture content all affect reflectance at these wavelengths.

#### Patterns of Reflectance Changes of Stressed Ring and Diffuse-porous Species

The time of leaf flushing associated with plant moisture relationships appears critical to the characteristic patterns of reflectance from foliage. Reflectance data for yellow poplar support the findings of Weber and Olson (1967) who were working with a diffuse-porous species which flushed continuously throughout the growing season. It is important to note that not only do leaves of this type enlarge under stress but the leaves are pre-formed in the buds under stress. Although the ring-porous species used in this study were near field capacity prior to leaf flushing, the leaves enlarged under progressively more severe levels of moisture stress. Reflectance of these species, however, increased at all wavelengths rather than decreasing. If the decreasing reflectance reported here for yellow poplar and also reported by Weber and Olson (1967) and Olson (1969) can be attributed to the plant moisture relationships at the time of leaf formation in the bud, the results obtained in this study for ring-porous species are as expected, since the leaves were pre-formed in buds the previous year when the trees received water



regularly.

Leaves of xeromorphic plants are well adapted to their environment with small leaves and compact structural features. Esau (1966) states that moisture deficiency may induce a degree of xeromorphy in normally mesomorphic leaves. Evidence presented by Zahner (1968) supports the hypothesis that development and growth of foliage pre-formed in the buds is closely related to environmental conditions at the time the leaf primordia are being formed. Leaves forming under severe stress would tend to have a thickened mesophyll structure, small intercellular-air spaces and generally smaller cells than leaves developing with an adequate water supply. These characteristics, reported by other workers, result in reduced reflectance at all wavelengths (Gausman, et al., 1969a; Gausman, et al., 1969b) and would account for the lower reflectance of yellow poplar foliage developing under stress.

Near infrared reflectance of leaves which developed and enlarged before being placed under stress increased in all cases as stress became more severe. This is not in keeping with the theory of internal reflectance discussed by Clark (1946) and Colwell (1956). They reported that near infrared reflectance decreased as plant foliage became severely stressed. Cells are larger and cell walls are thinner for leaves developing with adequate moisture than for leaves developing under stress. Moisture stress of mature leaves which enlarge with adequate moisture, are characterized by reduced cell turgor and increased shrinkage of cell walls which may result in an increase in reflecting surfaces. Hypotheses presented by Clark and Colwell are explained on

the basis of the theory first proposed by Willstatter and Stoll (1913) and subsequently discussed by many authors (Clark, 1946; Colwell, 1956; Murtha, 1968; Howard, 1969; Sinclair, 1968; Olson, 1969). Their theory, briefly, is that light passes through the leaf and is critically reflected at cell-wall-air interfaces. Decreased infrared reflectance of diseased foliage has been explained by decreasing cell-wall-air interfaces. Knipling (1967) states that shrinkage and collapsing of cells could result in more reflecting surfaces and hence higher near infrared reflectance. Until recently, most hypotheses explaining near infrared reflectance were based on the sole assumption that number and size of cell-wall-air interfaces were the controlling factor. Recent work has shown that diffuse reflectance of cell walls may be an important factor controlling near infrared reflectance (Sinclair, 1968). Sinclair proposed that the mat of cell walls of dehydrated leaves resulted in an increase in thickness of cell walls resulting in an increase in diffuse reflectance. While the theories presented by Willstatter and Stoll (1913) and Sinclair (1968) may explain the reflectance properties to which decreasing and increasing near infrared reflectance of diseased or stressed foliage may be attributed, they do not explain why some workers have recorded decreasing near infrared reflectance and other workers have recorded increasing near infrared reflectance from stressed foliage. A closer look at patterns of leaf development may help to explain these conflicting results.

The conflicting results reported in the literature by several authors suggest that both theories may be correct and which one is most

applicable may be related to the patterns of leaf development and the time at which the leaf is placed under stress. It is known that immature leaves have a compact structure with few intercellular spaces and as the leaf develops there is an increase in the size of mesophyll cells and intercellular air spaces (Maximov, 1929; Esau, 1966). This presumably would result in more or larger cell-wall-air interfaces and thicker cell walls for maturing leaves, accounting for the increasing near infrared reflectance. Leaves developing under stress (in bud and during enlargement) tend to exhibit xeromorphic characteristics (i.e., small, compact leaves with thickened cuticles, epidermis, compact mesophyll structure and fewer or smaller intercellular-air spaces) and are less reflective at all wavelengths than leaves developing with ample water. Leaves which have enlarged to full size have relatively large cells and intercellular spaces, and when placed under stress the generally more lacunose structure may result in many dehydrated cell walls being oriented perpendicular to incident radiation resulting in increased near infrared reflectance. Evidence by Sinclair (1968) supports this hypothesis. As a leaf reaches late maturity several workers have reported a decrease in leaf volume (Allen, et al., 1970; Gausman, et al., 1969b). According to Gausman (1969b), cells are enlarged longitudinally before an increase in thickness occurs and longitudinal growth continues after growth in leaf thickness has stopped. If cells continue to expand longitudinally after growth in thickness stops, a decrease in leaf thickness would be expected. In any case, this pattern would result in fewer intercellular spaces and

possibly account for reduced near infrared reflectance when subjected to stress.

Visible reflectance between 0.50  $\mu\text{m}$  and 0.70  $\mu\text{m}$  is strongly influenced by changes in pigmentation, particularly chlorophyll. The increasing reflectance of mature leaves when placed under stress is attributed to breakdown of chlorophyll production, reduced absorption, and increased internal scattering of incident light (Gates, 1965; Loomis, 1965; Moss and Loomis, 1952). It must be remembered that this increase in reflectance was not apparent until large changes in leaf water content had occurred. The decreased reflectance of foliage developing under stress is probably related to the dense, more compact leaf structure and greater absorption properties due to a greater concentration of chloroplasts indicating that although visible reflectance from leaves is strongly related to pigmentation, cell structure is also an important factor.

Sinclair (1968) used moisture content as a relative indicator of chlorophyll presence in a leaf. Results of this study suggest that small changes in water content do not change visible reflectance until high levels of stress are reached, indicating that moisture content is not necessarily an accurate indicator of the presence of chlorophyll in a leaf. The discussion by Gates, et al. (1965), and Heath (1969) indicates that scattering of light, by changing amounts of pigments in foliage, results in a shift toward longer wavelengths. This phenomenon is not well shown on the reflectance curves in the present study because the inflection point of the shoulder of the infrared plateau was

nominally placed at 0.77  $\mu\text{m}$ . An examination of the raw reflectance curves, however, indicates a shifting pattern similar to that described by Gates, et al. (1965). The location of this edge appears to be a better indicator of chlorophyll content than does leaf water content.

Reflectance at wavelengths from approximately 1.3  $\mu\text{m}$  to 2.6  $\mu\text{m}$  is strongly related to moisture content and to cell structure. Many workers have reported increasing reflectance with decreasing moisture content. This is apparently true for mature leaves as evidenced by results of this study. Leaves which developed and enlarged under stress were less reflective than leaves which developed with ample water, presumably because of compact structure and increased amount of water within the leaf. As stress became more severe, causing morphological and physiological damage, the reflectance increased for all leaves.

#### Future Plans

This study has been completed. No further work is planned.

#### STUDY IV: MULTISPECTRAL REMOTE SENSING OF VEGETATION STRESS (Study Leader - W. G. Rohde)

As indicated in our 1970 Annual Report, several combinations of multispectral scanner (MSS) data give promise of improved detection and possible previsual detection, of disease and stress in forest stands. Work during 1970-71 was to include "thermal contouring" of infected stands, and preparation of various types of "false-color" imagery assembled from a wide range of spectral bands, including reflective infrared channels in the 1.5 to 2.0 micrometer ( $\mu\text{m}$ ) region. The early results of this work were reported (Rohde, 1971) just before the study

leader received his military draft notice. The material below is a summary of the work done during the 1970-71 contract year, extracted from the paper just mentioned.

### Procedures

Multispectral data were collected in thirteen spectral bands in the wavelength region between 0.40 and 2.60  $\mu\text{m}$ . Data from selected channels were color-coded and combined to simulate normal color, color IR and a series of "false-color" photographic images (Table 3). While single bands from the multispectral scanner do not have sensitivity characteristics which match the sensitivity characteristics of the three emulsions in commercially available color films, two or more bands can be weighted, combined and color-coded as a single "emulsion" which closely approximates the sensitivity of any of the separate emulsions of commercial color films.

Normal color and color IR aerial photographs were obtained in conjunction with the multispectral scanner data. These photographs were compared with the simulated color and color IR imagery assembled from the scanner data.

### Results

The color rendition of normal color and infrared color photography was quite similar to the simulated color and color IR imagery assembled from the scanner data. Better color saturation was obtained with the "weighted channel" simulations, than with the single channel simulations, and this provided closer duplication of the color renditions of

Film Type	Spectral Band	Color Coding
Simulated Normal Color Film (single channel)	0.45 to 0.48 $\mu\text{m}$	Blue
	0.55 to 0.58 $\mu\text{m}$	Green
	0.62 to 0.66 $\mu\text{m}$	Red
Simulated Color IR Film (single channel)	0.55 to 0.58 $\mu\text{m}$	Blue
	0.66 to 0.72 $\mu\text{m}$	Green
	0.80 to 1.00 $\mu\text{m}$	Red
Simulated False Color Film (A)	0.66 to 0.72 $\mu\text{m}$ or	Blue
	0.62 to 0.66 $\mu\text{m}$	
	0.80 to 1.0 $\mu\text{m}$	Green Red
	2.0 to 2.6 $\mu\text{m}$	
Simulated False Color Film (B)	0.80 to 1.0 $\mu\text{m}$ or	Blue
	0.66 to 0.72 $\mu\text{m}$	
	1.5 to 1.8 $\mu\text{m}$	Green Red
	2.0 to 2.6 $\mu\text{m}$	
Simulated Color IR Film (weighted channels)	0.093 x (0.50 to 0.52 $\mu\text{m}$ )	Blue
	+0.504 x (0.52 to 0.55 $\mu\text{m}$ )	
	+0.404 x (0.55 to 0.58 $\mu\text{m}$ )	
	0.102 x (0.52 to 0.55 $\mu\text{m}$ )	Green
	+0.160 x (0.55 to 0.58 $\mu\text{m}$ )	
	+0.475 x (0.58 to 0.62 $\mu\text{m}$ )	
	+0.173 x (0.62 to 0.66 $\mu\text{m}$ )	
	+0.090 x (0.66 to 0.72 $\mu\text{m}$ )	
	0.015 x (0.50 to 0.52 $\mu\text{m}$ )	Red
	+0.043 x (0.52 to 0.55 $\mu\text{m}$ )	
	+0.033 x (0.55 to 0.58 $\mu\text{m}$ )	
	+0.066 x (0.58 to 0.62 $\mu\text{m}$ )	
	+0.142 x (0.62 to 0.66 $\mu\text{m}$ )	
	+0.221 x (0.66 to 0.72 $\mu\text{m}$ )	
	+0.472 x (0.72 to 0.80 $\mu\text{m}$ )	
+0.386 x (0.80 to 1.00 $\mu\text{m}$ )		

Table 3. Spectral characteristics of four simulated color films.

the actual photography.

Although conifers are readily distinguishable from broadleaved species on high altitude color IR photography, no apparent difference in color was detected between these two broad groups of trees on color IR imagery obtained from altitudes below 9,000 feet. Simulated color IR imagery assembled from single channels provided better broadleaved/conifer discrimination than the "weighted channel" simulated color IR imagery. The single channel simulation of color IR photography also provided better broadleaved/conifer discrimination than either the normal color or color IR photography obtained at the same time.

Red pine (Pinus resinosa Ait.) trees attacked by Fomes annosus were detected on color and color IR photography. Similar but enhanced results were obtained with simulated color and color IR imagery. Old killed trees imaged blue to blue green and faded attacked trees imaged orange or yellowish on both simulated and normal color IR imagery. The increased enhancement of Fomes annosus damage in the simulations may be due to edge enhancement or to more sensitive recording of differences in moisture stress.

Repetitive multispectral sensing, supplemented with color and color infrared photography during 1969 and 1970, was used to monitor increasing levels of ozone damage on white pine (P. strobus L.) and needle cast infestations on Scots pine (P. sylvestris L.). The needle cast disease, readily apparent on low altitude color photography in 1969, was not apparent on color or color IR photography obtained in July 1970. Ground truth efforts during 1970 confirmed that the disease was still present,



although less severe than in 1969. Needle cast damage was easily detected on all simulated color images prepared from 1969 data, but only those false color images simulated with non-photographic data (1.50  $\mu\text{m}$  to 2.60  $\mu\text{m}$ ) provided discrimination of needle cast damage in 1970.

### Discussion

Color imagery assembled from multiband black-and-white photography with an additive color processing technique can provide as much information as either color or color IR photography; and, in some cases, provides information of interest to foresters and agriculturists that is not readily apparent on conventional photography (Yost and Wenderoth, 1968; Yost and Wenderoth, 1969; Lent and Thorley, 1969).

The simulated color imagery obtained during this study indicates that normal color and color IR photography can be reconstructed from multispectral imagery and that false color imagery prepared from imagery in non-photographic spectral regions can aid in detecting stress in forest trees. Although considerable research is still needed to determine patterns of change in reflectance for various tree species under stress, work by many authors indicates a strong relationship between increasing moisture stress and changes in reflectance at wavelengths from 1.30  $\mu\text{m}$  to 2.60  $\mu\text{m}$ . Several workers have found that significant changes in reflectance at these longer wavelengths can be detected prior to any change in visible reflectance.

Detection of moisture stress in vegetation has been accomplished with black-and-white multispectral imagery in the spectral region from

1.0  $\mu\text{m}$  to 2.6  $\mu\text{m}$ , but the limit of discernible shades of gray often restricts detection of subtle changes in moisture stress (Heller, et al., 1969). The ability to combine imagery from these spectral regions into false color images should provide improved detection capabilities.

This technique offers considerable promise to the plant sciences since multispectral data can be collected in narrow spectral regions and combined to form broad spectral bands; or the equivalent of a false color film can be prepared using any three spectral channels. A major advantage of this system is that non-photographic spectral regions can be combined to a color image which yields detailed information about parameters not recorded directly with photographic systems.

#### Future Plans

This study has been terminated. We hope to continue investigating color combining techniques for enhancement of stress symptoms, but future work will be treated as a "new" study.

#### STUDY V: AERIAL DETECTION OF FOMES ANNOSUS IN PINE PLANTATIONS (Study Leader - C. E. Olson, Jr.)

In our 1970 Annual Report we wrote in part: "Analysis of the MSS imagery indicated that infection centers resulting from Fomes annosus are easily located in virtually any spectral region ...".

The analyses leading to this statement were based upon imagery obtained during calendar year 1969. Results of preliminary analyses of imagery from 1970 did not agree with the results from 1969. Since different interpreters were being utilized, it is possible that the poorer results with 1970 imagery were due to less ability of the

interpreters rather than poorer detection capabilities of the spectral bands.

A detailed study of the detection accuracy of several interpreters with different levels of training was begun in the spring of 1971. Personnel requirements in support of the University of Michigan participation in the 1971 "Corn Blight Watch" necessitated transfer of interpreters from our project to the "Corn Blight Watch". This prevented significant progress during the current reporting period.

#### Future Plans

This study will be continued and completion is expected in the spring of 1972.

#### STUDY VI: FOLIAR REFLECTANCE CHANGES DURING OAK WILT PATHOGENESIS (Study Leader - J. A. Bruno)

Since leaf moisture is sensitive to the presence of some tree pathogens, any anomalous changes in reflectance due to leaf moisture disruption may provide a means of early detection of some diseased condition. The purpose of this study was to determine the relationship between leaf reflectance and transmittance and leaf moisture content during the onset of one important forest disease, oak wilt, caused by the fungus Ceratocystis fagacearum (Bretz) Hunt.

#### The Fungus

The oak wilt fungus is heterothallic and hermaphroditic, and attacks both red and white oaks; but, due to the differences in physiology and vascular system anatomy between the two oak groups, the

fungus is more pathogenic on the red oak group. The fungus travels locally by means of root grafts, while long distance spread is accomplished through insect vectors, particularly the nitidulid beetles.

Oak wilt is a vascular wilt disease. Once inside the host the fungus gains its nutrients primarily from the parenchyma cells surrounding the conducting elements of the xylem (Wilson, 1961). This network of parenchyma cells serves as a breeding place for the fungus, with the transpirational stream in the xylem vessels as its transportational mode. Differences in opinion exist as to how the fungus induces tyloses, or the rupturing of infected parenchyma cells into the vessels, thereby disseminating the fungus internally while gradually constricting the transpirational stream. Because the symptoms displayed in oak wilt cannot be fully explained by simple wilting and subsequent dehydration, many investigators have speculated on a variety of responsible mechanisms.

One line of reasoning implies that there must be toxins involved in the symptoms, such as bronzing, chlorosis, necrosis, dehydrated green leaves and rapid leaf fall. The idea that toxins are solely responsible for all oak wilt symptoms is probably supported by very few credible researchers today. It is the degree of toxin influence which is in question.

Investigators, noting that white oaks frequently recover from the disease, have found that the white oaks have the ability to lay down a new layer of wood, thereby burying the pathogen. From this it was concluded that the red oak's particular ring-porous characteristics and

its inability to confine the pathogen to the initially infected annual ring while providing a new ring for transport, as does white oak, are the sole biological factors contributing to the susceptibility of red oak (Schoeneweiss, 1959; Struckmeyer, et al., 1954). Linked with the above reasoning, the observation that cambial activity is normal until several days after wilt further substantiated the mechanical wilt theory, that is, the idea that wilt occurs because of occlusion caused by tyloses and associated gums and slimes (Kozlowski, et al., 1962).

A more comprehensive and widely accepted explanation of the pathogenesis stresses both the mechanical plugging effect of tyloses and the toxic effect of the associated gums and slimes and the by-products of the general host:pathogen reaction (Beckman, et al., 1953; Kozlowski, 1968). After infection, the fungus requires time in which to establish itself within the parenchyma cells. It may use tyloses, a possible host response, to get back into the vessels to spread internally. This establishment period is probably an accelerative process in which no significant changes occur until the last stages of the pathogenesis. Pathogenesis, or the interim from infection to symptom development, varies in length from two to five weeks, with extreme wiltings, radical marginal necrosis, bronzing and leaf fall occurring rapidly at the end. This almost overnight expression of symptoms is probably caused when the accelerative establishment reaches some threshold of occlusion.

#### Oak Wilt Detection

True, et al. (1963) and Roth, et al. (1963) have described past attempts to detect the disease from aircraft, using both photographic

and visual coverage. The common finding from these attempts was, in general, that reliable detection of the disease was possible only after the infection centers were extensive and in the advanced stages of pathogenesis. In most cases, simple visual detection was more reliable than photographic detection with either normal color or color IR emulsions. Lack of success with photographic and non-photographic disease detection systems can, in most cases, be attributed to a lack of understanding of the biological chain of events leading to symptom development.

#### Procedures

This study was conducted at the University of Michigan Matthaei Botanical Gardens near Ann Arbor, Michigan. Three-foot-high red oak (Quercus rubra L.) seedlings were flushed in the last week of May 1971, the average flush consisting of about ten leaves. Prior to flushing, and throughout the entire experiment, the seedlings were watered every other day and the soil water in their pots kept near field capacity. All measurements were made on the days when no watering occurred. Relative humidity and air temperature were typical of temperate greenhouse conditions and were reasonably constant.

Twelve seedlings out of twenty-four were randomly selected and inoculated with a conidial spore solution of approximately 13,500 spores per milliliter of sterile distilled water. All spores were from a single mating type of the fungus. The reservoir method of inoculation was employed with each reservoir having a capacity of about four milliliters. Incisions were made below the surface of the solution,

girdling the stem by approximately 25 percent. The remaining twelve seedlings were inoculated similarly with sterile distilled water.

#### Reflectance and Transmittance Measurements

Spectral analysis of foliar materials was performed with a Beckman DK-2a spectrophotometer over the wavelength range from 0.9 to 2.6 micrometers. All reflectance and transmittance curves were run relative to barium sulfate standards which were periodically checked against desiccated bond paper samples. (This is a departure from our normal procedure of using magnesium oxide reference plates . . . C. E. Olson, Jr.)

Two diseased and two control seedlings were sampled every other day in sequential fashion such that any one seedling was selected for sampling every twelfth day. Reflectance and transmittance measurements were made on two leaves from each plant on each sampling day.

#### Leaf Moisture Determination

The most reliable tests of leaf water content are those requiring destruction of the leaf (Kramer, 1969). Of the several measures of leaf water content available, relative turgidity seemed most appropriate for this study and was calculated as:

$$\text{Relative turgidity (\%)} = \frac{\text{fresh weight} - \text{oven dry weight}}{\text{saturated weight} - \text{oven dry weight}} \times 100$$

Oven-dry weight was determined by drying the leaf for 24 hours at 75° C, while saturated weight was measured after sealing the leaf in a jar for 24 hours with its petiole submerged in water, as recommended by Hewlett and Kramer (1963).

Relative turgidity was determined for one leaf from one diseased, and one leaf from one control, seedling on each sampling day.

### Results

The small sample size and high variability encountered in disease symptoms prevented satisfactory statistical analysis of changes in reflectance and transmittance occurring during oak wilt pathogenesis. It was observed, however, that significant differences in leaf moisture developed only seven days after infection, and 23 days after infection 75 percent of the foliage showed visual symptoms. The work of Strobel and Mathre (1970) on cell permeability changes during the later stages of pathogenesis suggests that relative turgidity determinations, as made in this study, are probably inaccurate after 75 percent visual symptoms develop.

Reflectance and transmittance data reveal no detectable differences between diseased and control seedlings until the thirteenth day after infection. Differences developed rapidly after the thirteenth day, and were both severe and variable. Qualitative analysis of the data led to the following observations:

1. Reflectance is inversely related to relative turgidity. As relative turgidity increases, foliar reflectance decreases.
2. Reflectance in the 1.43 and 1.93 micrometer bands seems to be more sensitive to changes in leaf moisture than at other wavelengths. Since these two bands are water absorption bands, this result is to be expected.



3. Transmittance at 0.9 micrometer is directly related to leaf moisture, with transmittance decreasing as relative turgidity decreases.

4. Transmittance at 1.43 and 1.93 micrometers is inversely related to leaf moisture levels.

5. Transmittance at 1.65 and 2.20 micrometers seems to have little, if any, relation to changing levels of leaf moisture.

A few measurements of foliar reflectance were made for the wavelength region from 0.5 to 0.9 micrometer. Although the number of samples is small, it appears that, at least as early as 18 days after infection, and perhaps earlier, seemingly healthy foliage from plants infected with oak wilt reflects more strongly at 0.7 micrometer than does foliage from control plants.

#### Discussion

The typical sequence of symptoms began with a wilting of the petiole and/or leaf margin, followed by paling and greater wilting, ultimately resulting in patch or marginal chlorosis and necrosis. This sequence of events is similar to that observed in a second control group subjected to high water stress. However, another sequence of symptom development observed in the infected seedlings was an initial copper colored necrosis or bronzing of the leaf tip region, with the remainder of the leaf having a dark green, fully turgid appearance. The bronzing proceeded from the tip toward the base of the leaf, with leaf fall usually occurring before total bronzing. This second sequence was peculiar to the diseased group and is similar to sequences observed by Kozlowski (1968), White (1955) and Beckman (1953). It was also

noted that the smaller, younger branches displayed symptoms first, but whether this was common to the controls under stress was not noted. Although the rapid bronzing phenomenon was not as prevalent as the progression of wilting ultimately ending with necrosis, the fact that a radical difference in symptoms occurs undoubtedly indicates variation in fungal attack strategies. Furthermore, the peculiarities of the bronzing system imply the influence of an alien chemical or toxin; while the general wilting symptoms probably occur because of the simple mechanical obstruction of the vascular system. An interesting observation was that on any given seedling, leaves bronzed only after other leaves were already wilted. Apparently, the fungus first differentially occludes the seedling's vascular system, causing the initial wilting, after which it somehow instigates the bronzing of other wilt-free leaves. It is most probable that there exists a cause and effect relationship between these two phenomena. It might be that the host-pathogen reactions in the occluded vessels manufacture substances which somehow find their way into other free transpirational streams, causing malfunctioning of the pigment synthesis apparatus of the leaves connected to these free streams.

The finding that reflectance in all regions investigated increased with a decrease in leaf moisture is in general agreement with the literature. When leaf moisture is diminished, the intensity of photosynthesis and corresponding pigment synthesis decreases, resulting in less absorption and more internal scattering and escape of visible radiation (Dadykin, et al., 1960; Hubbard, 1962). The increase in infrared

reflectance caused by the decrease in leaf moisture is primarily due to the sensitivity of infrared radiation to the presence of water. The explanation for the increase in near infrared reflectance due to decrease in leaf moisture is more speculative than those for the other two regions, and hence will not be discussed here.

Transmittance in the water absorption bands is more sensitive to changes in leaf moisture than is transmittance in the regions between these bands, a fact substantiated by Thomas, et al. (1966).

The visible portion of the electromagnetic spectrum probably holds the most promise in early detection of oak wilt. This contention is based on the fact that leaf pigments are sensitive to changes in water content and to alien chemical substances suspected to be present during the oak wilt pathogenesis. Using panchromatic film and common tri-color cut-off filters, an attempt was made to further investigate this pigment change. However, because pigment deviations are not exclusive to any single band in the visible, but are probably combinations of many bands, results were negative. Although Roth, et al. (1963) experienced only very limited success in detecting the disease from the air with both standard and false color films, the fragmentary data for the visible derived in this study justify further investigation.

### Summary and Conclusions

#### Pathology

Given the age of the seedlings and the inoculum density, the fungus requires approximately three weeks to induce symptom development of 75 percent of the foliage. The variable nature of the wilting implies

differential occlusion of the vascular system. Bronzing seems to be a secondary symptom expression occurring on seemingly healthy, fully turgid leaves only after other adjacent leaves have already wilted. There is a strong possibility of the existence of toxins and a cause and effect relationship between the initial wilting through occlusion and the bronzing phenomenon.

### Remote Sensing

Trees under simple water stress undergo uniform foliar desiccation. Leaf moisture levels for foliage on trees infected with oak wilt vary radically, however. For this reason, and because of the relatively short time between changes in leaf moisture and the appearance of visible symptoms, it becomes obvious that non-visible early detection of oak wilt is less feasible than non-visible early detection of trees under drought conditions. Although significant non-visible responses were noted for individuals infected leaves under laboratory conditions, aerial sensing of entire tree crowns will undoubtedly be hindered by the aforementioned symptom variations. Even if near infrared and infrared aerial sensors were capable of detecting the differential wilting characteristic of the early stages of pathogenesis, remote sensing systems to date do not have the sophistication to determine whether oak wilt or simple water stress was the cause. Our failure in these regions lies in our inability to predict the leaf-structural and biochemical responses to environmental stimuli.

The possibility exists that leaf pigments are rapidly and peculiarly affected by the presence of Ceratocystis fagacearum, resulting in a

unique detectable spectral signature. Future investigation in this region should be based on a firm understanding of the pathological effects of the fungus on pigment synthesis and the means of multi-spectrally discriminating the visible responses brought about by these effects.

#### Future Plans

This study has been concluded. No additional work is currently planned.

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