REMOTE SENSING IMPLICATIONS OF CHANGES IN PHYSIOLOGIC STRUCTURE AND FUNCTION OF TREE SEEDLINGS UNDER MOISTURE STRESS

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ABSTRACT

This is the first annual progress report of a new study which seeks to assess physiologic and structural changes which accompany loss of vigor in forest trees. Particular interest is paid to associated changes in spectral reflectance and thermal emittance characteristics under closely controlled laboratory/greenhouse conditions usings potted seedlings, where moisture stress is introduced at several intensities.

Instrumentation of physiologic responses to various levels of moisture stress was an important part of this study. A Scholander hydrostatic pressure chamber was used to measure moisture stress in the foliage. This device measures the tension with which moisture is held in the leaf cells, and is a very sensitive indicator of temporary or permanent moisture stress in plants. A heater source probe and detector, inserted into the active xylem, was used to measure the rate that water was translocated upward in trees. When rate of water transport and the foliage moisture tension were measured together with the weight loss of the plant due to transpirational water loss for a particular controlled level of soil moisture stress, a great deal was learned about the interaction of solar energy and its effect on the energy budget of a tree. Simultaneous measures of apparent temperatures with an infrared filtered radiometer gave information about the changes in - thermal response under various solar energy imputs in light of the adjustments of physiologic functions.

Spectral reflectance curves generated regularly during the study showed the changes in spectral response at various wavelengths due to the buildup of moisture stress in the foliage and resultant change in moisture content. In the case of broadleaved species, attempts were made to identify the changes in spectral response related with leaf maturity as differing from the change associated solely with the buildup of moisture stress. It was found in all cases that the level of water stress at the time of leaf formation and development appeared to exert a greater influence on foliar reflectance than did the level of water stress at the time the reflectance measurements were made.

Special effort was made to study structural or anatomical changes, microscopically, that take place during the long-term buildup of moisture stress and the resulting loss of vigor. Observations and measurements indicate that the foliage of pine trees affected by moisture shortage does in fact undergo some structural changes as a result of physiologic stress. However, evidence to date indicates that these changes are not enough to alter the spectral signature of foliage to permit pre-visual detection of moisture stress at the waveband 0.72-1.2 microns.

Water loss does not affect reflectance directly except in the region of the infrared water absorption bands. Microexamination of transections of foliage from conifers that had grown under moisture stress of at least -15 bars (e.g., 220 pounds per square inch of tension) revealed that the epidermal cells were tightly packed in comparison to cells of healthy foliage. There is evidence of nonfunctional stomata where subsidiary cells and guard cells are disoriented and the sub-stomatal cavity has broken apart. This would strongly indicate the tree's inability to readjust unfavorable heat loads through transpiration. Other notable differences in high-stress needles are thickened mesophyll cell walls and reduction in the area occupied by the cytoplasm of the mesophyll cells. This results in decreased contact of the cytoplasm with the cell wall interfaces.

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TABLE OF CONTENTS

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INTRODUCTION

Early detection of insect and disease attacks is one of the keys to preventing epidemic conditions. In the early stages, infestations of many organisms are difficult to detect and rapid surveys of large areas are virtually impossible using ground techniques.

Aerial survey techniques are rapid and permit coverage of large areas at relatively low cost. Attempts have been made to utilize aerial photography, or aerial observers, for such surveys and some have been successful (Colwell, 1956; West, 1956; Heller et al, 1959; Meyer and French, 1967) while others have not. Results of unsuccessful attempts are seldom published and a cursory review of the literature indicates a need for a continuing effort to update the state-of-the-art techniques to permit ready detection and mapping of many, if not most, insect and disease attacks.

Recent advances in aerial reconnaissance techniques, particularly in simultaneous recording of several spectral bands (i.e., bands differing in wavelengths recorded), appear to provide a more potent detection system. Work with agricultural crops has shown that differences exist between diseased and undiseased, and fertilized and unfertilized crops (Hoffer and Miller, 1965; Hoffer, Holmes, and Shay, 1966; LARS, 1967) that permit separation of these conditions when the correct spectral bands are used. Similar techniques may permit detection of early physiologic disturbances in forest trees, (Heller et al, 1966, 1967) and for tree species identification (Weber, 1966).

Many insect and disease attacks produce a disruption of the water metabolism of host trees by plugging or severing of the water and solute conducting tissues. Trees subjected to such attacks become less vigorous and their foliage develops higher moisture tensions than unaffected trees. The reduced rate of water uptake through the disrupted tissues of the infected trees makes them less efficient at replacing water lost through transpiration. As moisture stress increases, at least two changes occur: (1) the foliage becomes less turgid, possibly altering the internal geometry of the foliage as a reflector; and (2) foliage temperature increases. These changes undoubtedly contribute to the change in leaf reflectance relative to moisture content reported by Thomas et al, (1966), and to the difference in foliar temperatures of pine needles observed by Weber (1965). Such differences may be detectable through multi-spectral reconnaissance.

Since reduced vigor and increasing moisture stress are found in trees subjected to drought as well as in trees attacked by a number of organisms, these symptoms do not necessarily indicate the presence of an insect or disease attack. Careful analysis of the pattern in which the symptoms occur may permit at least partial interpretation of their cause when recent weather conditions in the area are known. However, Olson et al (1964) showed that reflectance characteristics of tree foliage change dynamically during the growing season and Gates (1965) points out several factors affecting leaf reflectance and emission characteristics which are imperfectly understood. It seems likely that at least some of the unsuccessful attempts at aerial inventory of insect and disease conditions did not attain their potential because of inadequate understanding of the basic patterns of change in reflectance and emission from tree foliage and the factors producing change.

This report presents results of a study of changes in reflection and emission characteristics of coniferous and broadleaved foliage on tree seedlings subjected to varying degrees of moisture stress under greenhouse conditions. To make most effective use of available personnel and equipment we devoted primary effort to conifers and lesser effort to deciduous seedlings. Since previous work has shown predictions of photographic tone from reflectance data to be more accurate for broadleaved than for needled trees (Olson, 1964), and because several of the more important insect problems in the forest are associated with species of pine, primary effort was devoted to the study of changes in reflection and emission characteristics of pine needles.

LOCATION OF THE STUDY

The University of Michigan Botanical Gardens, with greenhouses, environmental control chambers, and laboratories served as the active research site for this study. The Botanical Gardens provided closely controlled environmental conditions for this research, and its proximity to supporting scientists and facilities was also desireable.

METHODS

Under this section treatments to coniferous seedlings will be considered first and to hardwood seedlings second.

CONIFERS

In anticipation of this greenhouse study, three-year-old red pine, Pinus resinosa, Aiton, and white pine, Pinus strobus, Linnaeus were planted the summer of 1966 in seven-inch standard clay pots and left out-of-doors until the first week in January 1967. To complete environmental preconditioning, the pine seedlings were brought into a cool room where they were subjected to slowly increasing temperature and daylength. After they had been brought up to 65°F. and 20-hour daylength, considered optimum for growth of these pine, they were transferred to the greenhouse where these environmental parameters were maintained throughout the remainder of the study.

From a total of twenty-five seedlings of each species, individuals were selected at random to be subjected to one of five levels of soil moisture tension. The design was created such that there were two groups (species), five treatments to each group (soil moisture tension levels), and five individuals per treatment.

Prior to the greenhouse study an analysis was made of the soil (homogeneous for all seedlings) to determine the soil moisture release characteristics. Thereafter, by applying gravimetric analysis techniques, moisture content could be rapidly related to any desired level of soil moisture tension. Water was added as needed to maintain the desired levels throughout the study.

At the beginning of the greenhouse experiment, after each potted seedling was brought to the desired treatment level, the clay pots were placed in heavy plastic bags which were secured around the root collar to prevent water loss other than through transpiration.

Throughout the greenhouse study, the following factors were measured on a routine schedule:

1. Solar and thermal radiation.
2. Soil moisture.
3. Rate of water movement in the xylem.
4. Leaf moisture tensions.
5. Transpiration.
6. Absolute and apparent needle temperatures.
7. Spectral reflectance of foliage.

At regular intervals during the study, needle samples of both pine species were taken from an individual in each treatment and prepared for microscopic examination and for making photomicrographs.

Greenhouse Procedures

Solar Radiation

In a study of this kind, one of the most important quantities to measure is the solar input to the plant system. The amount of transpiration of an individual plant is dependent on an external energy source to satisfy the latent heat demand. The other factors governing transpiration rate--water vapor concentration gradient and diffusive resistances--are interelated with solar energy. Thus, a change in the energy input will greatly influence the above two factors and an entirely new energy balance is created governing transpiration, (Slatyer, 1967).

Short-wave radiation incident at plant growing levels was collected with a recording pyrheliometer. It provided excellent records of daily accumulation of incoming energy and was also useful for determining the level of incoming radiation at any particular time of day. Total energy values were determined at any point in time by combining the short wave radiation values from the recorder with instantaneous long wavelength measurements made with a Stoll-Hardy infrared radiometer.

Soil Moisture

As a check on the relative values of soil moisture, Coleman soil moisture transducers were placed within the root zone in the soil of each seedling. Lead wires from each transducer were hooked individually to a potentiometer for getting daily soil moisture values. This technique served as a check on the weighing method for maintaining soil moisture values within each treatment.

Xylem Transport

Sap velocity in the active xylem was measured by the heat-pulse techniques of Marshall (1958) as modified by Weber (1965). Additional refinements in the instrumentation to permit recording of the heat pulse dissipation resulted from personal communication with Robert H. Swanson, Rocky Mountain Forest and Range Experiment Station (1966) and from Swanson (1965).

The equipment used to measure the rate of transport in the xylem consists of a portable micro-voltmeter for detecting small temperature differences between thermocouples inserted into the active xylem on either side of a heat source. A timer was built for measuring the desired heat load introduced into the xylem stream which automatically monitors the time for down-stream dissipation of the heat pulse.

The operating principles is that a small heat source is placed into the xylem stream, and thermocouples inserted into the xylem above and below the heat source. A spacing of 4 mm. below and 7 mm. above the heat source allowed a minimum detectable velocity of 1.8 cm/hr with good accuracy.

The purpose of measuring sap flow in the xylem was to add quantitative information about the relationship between solar energy, soil moisture conditions, the buildup of moisture tensions in the foliage and their effect on relative rates of transpiration. The comparative rates of water movement in the xylem of trees growing under different soil moisture conditions is an excellent indicator of transpirational activity.

Leaf Moisture Tension

Leaf moisture tension was an additional parameter measured on a regular schedule to help determine early vigor loss. The technique was first reported by Dixon (1914), and the apparatus improved and described by Scholander (1965). Briefly, the method is as follows: the twig end of a freshly cut foliage sample (3 to 4 inches long) is inserted through a rubber "O" ring which is fitted to the top side of a pressure chamber. The proximal end of the twig is exposed to atmospheric pressure. The needle portion of the sample is then placed inside the lower part of the container and the two parts are screwed together. Inert nitrogen gas is introduced slowly to the container until free water begins to exude from the cut end of the twig (observed with a 10X hand lens). Normal foliage requires less pressure to force out the water column which retreats towards the moisture sink (in the foliage)when the water column is severed. The pressure value required to force the water column to the cut end of the stem is called the hydrostatic pressure value. It is the same value, though opposite in sign, to the negative hydrostatic pressure or leaf tension by which water is held in the leaf cells. This quantitative value of moisture stress in the foliage is expressed as a negative bar of leaf tension, e.g., -1 bar being approximately equal to -14.7 pounds-per-square-inch of tension.

Leaf moisture tension values were recorded within 90 seconds after removing a sample from a tree, and were obtained several times a day as required to account for daily variations due to varying solar conditions, soil moisture available, rate of water transport in the xylem and relative state of tree vigor.

Transpiration

Consideration of transpiration rates of trees at various soil moisture conditions is important to the understanding of differential energy transfer and thermal patterns of trees at various stages of vigor decline. Transpiration from plant leaves involves water vapor transfer from the evaporating sufaces within the leaf to the leaf surface and then from the leaf surface to the surrounding atmosphere. This transfer of energy is responsible for a heat flux in the plant and is a major mechanism for heat dissipation, thus creating a cooling effect. This action is thought to be important to plants in maintaining tolerable temperatures in sunlight conditions, and thus, an interruption of this cooling process through a disruption of the water transfer can be harmful. Differential heating of plants is the basis for thermal detection of moisture stressed vegetation.

Recognizing the variability of plant material and the effect of differing environmental conditions, the best method for measuring transpiration of individual trees appeared to be by the measurement of weight loss from the whole system. This was done with a load cell (i.e., a stain gauge) lysimeter which was used to measure the instantaneous weight of an individual seedling, or track the weight loss over time, which could be recorded on an analog chart. The load cell transducer gave a linear electrical output of 0 to 12 millivolts which was calibrated for weights from 0 to 7900 grams full scale output, accurate to ± 2.5 grams. The precision of this measurement was about 2% of the normal daily transpirational weight loss.

Foliage Temperatures

Foliage temperature provides a direct means to measure tree vigor as varying environmental conditions affect physiological functions when related to the energy flow. This includes a measure of both the absolute temperature and the apparent or emitted temperature. These kinds of temperature data permit us to evaluate the expected effectiveness of a thermal detection system in discriminating between healthy and moisture stressed seedlings as a function of time of day, physical environment and level of tree vigor.

Absolute temperature. Leaf temperatures were obtained by inserting copper-constantan thermocouples, approximately 2.5 mm. long and sharpened to a point, into living cell tissue of individual needles. These thermocouples were placed inside needles of one individual per treatment. Lead wires joined the thermocouples to a multipoint strip chart recorder.

The recording device was programmed to automatically record temperatures during the first 15 minutes of each daylight hour and 15 minutes every two hours at night.

Apparent temperature. Emitted temperatures were measured periodically with a Stoll-Hardy infrared radiometer (Figure 1). Incoming energy to the radiometer was sampled at various bandwidths in the spectrum, e.g., energy from wavelengths longer than 3.5 microns, between 4.5 and 5.5 microns, and between 8.1 and 13.2 microns. The useful output of the infrared radiometer was to give ΔT (difference in temperature) values in tenths of degrees centigrade between foliage of trees subjected to various levels of moisture stress at various times of the day and under varying solar input conditions.

Reflectance

The reflectance curves generated during this study were measured with a Beckman DK-2a spectrophotometer. This instrument measures the ratio of monochromatic energy reflected from the sample foliage to that reflected from a magnesium oxide coated aluminum plate, which is a nearly perfect (0.98) diffuse reflector. This ratio, expressed as a percentage, is plotted against the wavelength of incident radiation. The effective region for this instrument with the lead sulfide and photo-multiplier detectors is 0.38 to 2.70 microns.

Foliage reflectance responds to changes in leaf structure, pigmentation and moisture content as affected by changes in tree vigor.

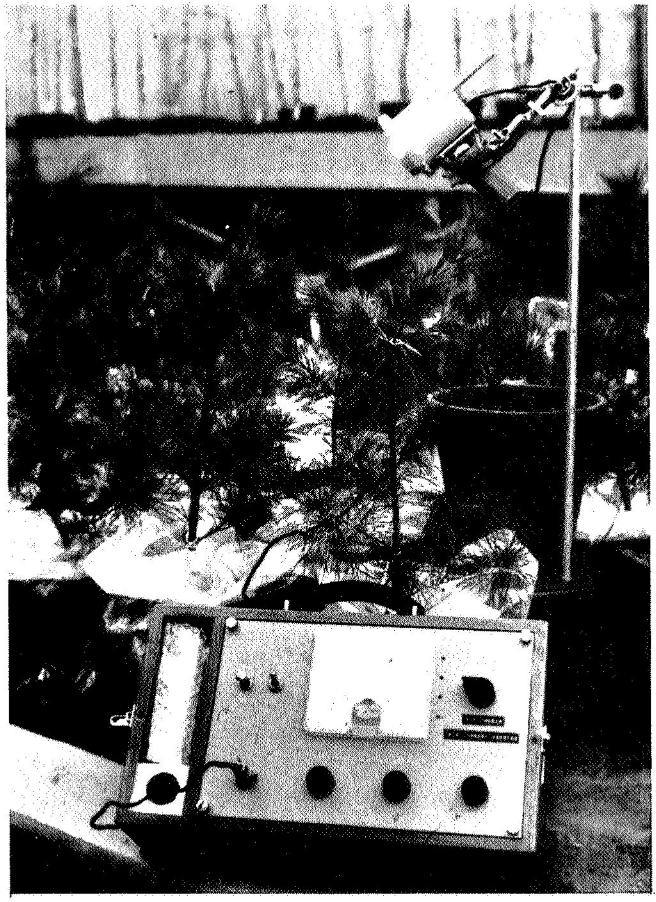

Figure 1. --Stoll-Hardy infrared radiometer being used to measure emitted temperature of pine seedlings used in the study. The radiometer head, in the upper right-hand corner, houses the thermistor detectors and bandpass filters.

The spectrophotometer was located in a stable environment room within a few steps of the experimental greenhouse. The convenience of this setup permitted reflectance measurements to be made on individuals from each treatment every few days, thereby showing patterns of reflectance change as a function of wavelength throughout the length of the study.

Spectral reflectance curves were run at least once each week throughout the study covering the bandwidth 0.5 to 2.6 microns for the conifer test seedlings. All spectral data presented in this report have been corrected for errors due to deviations of the zero and 100% setting of the spectrophotometer, and have also been corrected for deterioration of the magnesium oxide standard. Spectral reflectance data for conifers have been corrected for scaling and run differences, and reflectance values as a function of wavelength are thus comparable between dates.

Laboratory Procedures

Previous observations and measurements made on another forest remote sensing study indicated that the foliage of pine trees affected by moisture stress undergoes structural changes that substantially affect reflectance characteristics. There is considerable speculation in the literature which attempts to explain changes in reflectance of decidous foliage as it is affected by moisture stress and loss of turgor; however, little work appears to have been done in that area with conifers. Consequently, it was made a prime objective of this study to investigate structural changes that might be evidenced through microscopic examination which would effect changes in the spectral reflectance characteristics of foliage grown under varying levels of moisture stress.

Slide Preparation

Coincidental with physiological and physical/environmental measurements, needle samples were taken from each individual at regular intervals throughout the study. After considerable practice good freehand transections were achieved at about 20-micron thickness. These sections were immediately viewed under a microscope, and in most cases were photographed.

At the end of the study, foliage samples from each group were killed and fixed in Bouin's fluid, dehydrated in tertiary butyl alcohol, and embedded in 58°C. Bioloid. Transections were obtained at 15 and 20-micron thicknesses on a rotary microtome. After being mounted on glass slides the sections were stained with two coal tar dyes, fast green, which highlighted lignified cells and safranin which highlighted non-lignified substances.

Photomicrography

The most useful photomicrographs taken of these slides were made with Ansco D-200 color film. Subtle differences that were present in cell walls and other structures simply did not show up as well on other photographic media. The taking of photomicrographs was a simple matter with a spot-matic type camera attached directly to the optical viewing system of a high-power microscope.

HARDWOODS

Work with broadleaved species began in January 1967. The main emphasis of the work was placed on the effects of moisture content and moisture stress on foliage reflectance characteristics. Several economies were accomplished by combining work on this project with work being performed in connection with Forest Service Contract 13-220. Although most of the results of this combined effort were included in the final report on that contract (Olson, 1967), they are repeated here because of the important bearing which these data have on the conclusions stated later in this report.

GREENHOUSE PROCEDURES

In late February eight trees were moved indoors and induced to break dormancy in the manner described for pine. Four were watered regularly and four received no water until the evening of May 7; otherwise all eight seedlings were grown under comparable environmental conditions in a single area in the greenhouse. On March 28 one large mature leaf and one small immature leaf were selected on each tree and tagged for identification. A second, and then a third, immature leaf were selected on each tree on April 5 and April 18, respectively. Leaf size at the time of selection ranged from 6 to 41 sq. cm. for immature leaves, and from 147 to 254 sq. cm. for mature leaves.

Light reflectance measurements were made on each leaf without removing the leaf from the plant. Instead, the entire seedling was moved into the spectrophotometer room and then returned to its original greenhouse location after the measurements were completed. This process was repeated at intervals from March 28 through May 17 and on each occasion the leaf was placed in the spectrophotometer in as nearly the same position as possible. The resulting data provide a sequence of reflectance measurements over time for leaves that appeared to function normally throughout the study. Water stress was monitored for each tree using the Scholander pressure cell, and the size of each tagged leaf was determined from leaf prints obtained on photographic proof paper at intervals throughout the study.

Another group of seedlings was moved indoors in mid-March and induced to break dormancy. As soon as leaf flushing began, all ten trees in this group were moved to the greenhouse and water regularly for four weeks. On April 25, plastic bags were placed over the "pots" of five of these seedlings and they received no further water until measurements were discontinued on May 19. The remaining five trees continued to receive water regularly. Data collection from this group of seedlings was accomplished in the same manner as for the first group of trees.

A final series of measurements was obtained in September. The seedlings used previously had been left in the greenhouse and watered at least every second day from May 19 through September 2. In mid-June all trees were fertilized with a balanced liquid fertilizer whose exact nutrient content is unknown. This promoted vigorous vegetative growth of all trees. On September 2, plastic bags were placed around the "pots" of seven of the trees which had been subjected to moisture stress in the spring. These seven received no further water until data collection was concluded on September 20. The most severe water stress in the seedlings occurred in September. Water stress was so severe in September that several leaves and terminal buds withered and turned black. This condition had not developed during April or May, although water tensions at 0800 in excess of -20 bars were also recorded in May.

Reflectance v. moisture content

Leaves from one-year old seedlings of sycamore (Platanus occidentalis L.) and yellow poplar (Liriodendron tulipifera L.) were used to determine the effect of decreasing moisture content on foliage reflectance. Three cottonwood (Populus deltoides Bartr.) leaves were also used. Leaves picked from the seedlings were weighed immediately (nearest 0.0001 gram) and then placed in the Beckman DK-2a spectrophotometer. After a reflectance curve for the wavelength range from 0.5 to 2.6 microns had been obtained, the leaf was weighed again. When the leaf had air-dried for 15 to 30 minutes, it was weighed, another reflectance curve obtained from as nearly the same spot on the leaf as possible, and the leaf reweighed and allowed to air-dry for another 15 to 30 minutes. This procedure was repeated until the leaf became so brittle that it could not be placed in the spectrophotometer without breaking. The leaf was then oven-dried and weighed for the last time, and this oven-dry-weight used to calculate leaf moisture content at each previous weighing. Leaf moisture content decreased by approximately 8 percent in the time required to obtain the reflectance curve, and the average of moisture content before and after any reflectance curve has been used as the moisture content of the leaf at the time the curve was run.

Subsequently, measurements of moisture content were obtained for leaves taken from seedlings subjected to severe moisture stress. In no case was the moisture content at the time the leaf was picked less the 218 percent of its oven-dry-weight, and this value was observed for a leaf taken from a seedling for which moisture tension measurements exceeded -20 atmospheres at 8 a.m.

For a small part of the data, the photo-multiplier detector was used with the DK-2a, extending the wavelength range of the instrument to 0.38 micron on the short wavelength end.

Reflectance v. moisture stress

Two-year-old yellow poplar seedlings were used to investigate relationship between moisture stress and leaf reflectance. These seedlings had been obtained from the Jonesboro, Illinois, State Tree

Nursery and planted in individual four-gallon cans in May 1966. The trees were watered daily and kept outdoors in full sunlight through the 1966 growing season. Soil moisture was near field capacity in the cans when they were "heeled-in" during November.

RESULTS

CHANGES OBSERVED IN CONIFER SPECIES

Needle Moisture Tension

The hydrostatic pressure necessary to force the water column in the xylem back to its original level, (at the cut edge of the stem), is a very sensitive indicator of moisture stress in foliage and can be related to other measures of tree vigor. During the three months this study was active, a great number of leaf moisture tension measurements were made with our modified Scholander pressure cell. There was found to be great consistency in the tension measurements between individuals of the same treatment, and the mean value of tension for each treatment was usually very close to those values checked by other measurements of soil moisture. In every case, a deviation from a treatment value showed a measured tension value of greater stress than that set by the established soil moisture conditions. This is due primarily to the transpirational lag which occurred during sunny conditions and was an expression of a tree's inability to supply water to the foliage as rapidly as it was depleted through transpiration.

The inter-dependency of physiologic functions on physical environment cannot be expressed too strongly. Within treatments, moisture availability to the treatment trees was closely controlled at five levels. Two important factors which were not measured are vapor pressure and carbon dioxide concentration. These factors are mentioned here merely in recognition of their importance as possible controls or limiting factors in the gas exchange mechanism involved with transpiration/respiration processes. In a study of this sort where soil moisture availability is controlled at various levels, the most important physical input is that from solar energy.

The relation between soil moisture tension, solar energy input and the buildup of moisture tension in the foliage as measured with the hydrostatic pressure cell was particularly consistent. Table 1 shows an example on 28 April, 1967, of the daily variation of needle moisture tension as affected solar energy. Also shown in the table are values for the rate of transport in the xylem, because of its inseparable relationship to soil moisture availability, solar energy, and the buildup of leaf moisture tension. The same types of measurements were made for these same factors throughout the study. The only deviation from this pattern showed up near the end of the study for individuals in the high soil moisture tension (-15 bars) treatment. In this case, there was no measureable recovery from the high moisture tension in the foliage; that is, the foliage remained above -21 bars of tension regardless of the time of day or the solar input conditions.

Table 1. --Daily variation of leaf moisture tensions and rate of xylem transport as a function of solar input at three controlled soil moisture availability conditions on April 28, 1967.

Factors		Time of Day 0900 1/	 1200 2/	 1500 3/
Leaf Moisture Tension (bars)				
	-1 bar	-2.5	-1.2	-1.6
Treatment	-7 bar	-7.6	-10.0	-8.5
Levels	-15 bar	-18.4	>-20.5	>-20.5
Xylem Transport (cm./hr.)				
	-1 bar	7.1	12.7	8.6
Treatment	-7 bar	5.9	5.3	6.1
Levels	-15 bar	1.8	<1.5	2.1

1/ Solar Radiation 0.45 Cal.cm.$^{-2}$ min^{-1}

2/ Solar Radiation 1.22 Cal.cm.$^{-2}$ min^{-1}

3/ Solar Radiation 0.77 Cal.cm.$^{-2}$ min^{-1}

Significantly, about the same time, the rate of xylem transport dropped below the minimum measureable rate. Micro-examination of foliage sectioned at this stage showed an apparent breakdown in the function of the stomata--an indication that the trees had died from the standpoint of not being able to recover from the effects of the long-term physiologic stress.

Xylem Transport

Prior mention was made of the relationship between the rate of xylem transport and such physical conditions as soil moisture availability and incoming solar radiation. As might be inferred from Table 1, for seedlings growing under favorable soil moisture conditions, e.g., 1 bar of tension (or 1 negative atmosphere), the rate of water transport in the xylem (and the rate of transpirational water loss) is directly related to incoming solar radiation. That is, the greater the solar radiation, the greater the rate of transport in the xylem. Occasionally, in spite of the favorable soil moisture and sun conditions, there is evidence of the effects of a mid-day transpirational lag when xylem transport is reduced by an unfavorable hydrostatic gradient. An example of this is shown in Table 2 at 1200 on April 21 in the -15 bars treatment where xylem transport rate dropped to less than 1.5 cm./hr.

In considering the case of less favorable soil moisture conditions, e.g., -12 or -15 bars of tension, solar radiation does not always increase xylem transport. The rate of xylem transport is effected by

Table 2.--Summary of physical influences and physiologic responses measured at four periods during the study, for all soil moisture levels.

Factors		Date:	28 February			27 March			21 April			16 May	
		Time: 1000	1200	1500	1000	1200	1500	1000	1200	1500	1000	1200	1500
Solar radiation (cal/cm2/min)		0.35	1.20	0.25	0.38	0.92	0.48	0.44	1.05	0.70	0.65	1.35	0.86
Leaf moisture tension (bars)													
Treatment Levels	-1 bar	not available	-2.1	not available	-0.8	-1.9	-0.9	-2.3	-1.9	-1.2	-3.5	-5.2	-2.4
	-3 bar		-4.3		-1.8	-3.7	-2.2	-4.0	-7.4	-6.3	-4.2	-6.6	-3.6
	-7 bar		-6.2		-7.3	-9.8	-8.8	-8.0	-11.5	-8.9	-7.3	-13.7	-9.3
	-12 bar		-19.6		-10.3	-13.5	-11.1	-14.1	>-20.5	-18.2	-12.6	>-20.5	-15.8
	-15 bar		-15.8		-19.6	-19.4	-15.4	-17.3	>-20.5	>-20.5	>-20.5	>-20.5	>-20.5
Xylem Transport (cm/hr)													
Treatment Levels	-1 bar		not available		4.6	10.1	3.8	5.3	11.0	7.0	8.1	14.5	11.0
	-3 bar				-	-	-	4.2	7.9	6.8	7.1	12.0	8.1
	-7 bar				-	-	-	3.5	3.1	3.2	7.8	4.2	6.7
	-12 bar				-	-	-	3.1	2.8	2.6	3.3	3.5	2.9
	-15 bar				7.6	6.3	8.1	2.0	< 1.5	1.8	< 1.5	< 1.5	< 1.5
Transpiration (gm/hr)													
Treatment Levels	-1 bar		not available		4	21	6	13	62	18	10	47	14
	-3 bar				5	25	7	7	35	11	7	32	9
	-7 bar				3	13	5	5	26	7	4	21	6
	-12 bar				2	12	5	4	20	5	7	9	13
	-15 bar				4	3	4	0	0	0	0	0	0
Foliage emission temp. (°C., > 3.5μ)													
Treatment Levels	-1 bar	23.8	26.8	20.4	26.1	26.8	26.5	24.4	28.7	26.8	23.6	28.3	30.5
	-3 bar	-	-	-	-	-	-	24.6	28.1	27.1	23.5	28.7	31.4
	-7 bar	-	-	-	-	-	-	24.7	29.2	27.1	23.5	28.4	31.0
	-12 bar	-	-	-	-	-	-	24.5	29.8	26.7	24.3	29.8	32.9
	-15 bar	24.2	28.3	19.7	26.2	26.7	26.4	24.7	30.1	27.0	24.8	32.5	34.3

(1) solar radiation which provides the energy necessary for transpirational processes, which in turn creates a moisture sink [1] in the foliage due to evaporation of moisture to the atmosphere; and (2) by soil moisture availability. According to the cohesive-tension theory of water movement in woody plants, there is a source of moisture in the soil and a sink for moisture in the foliage. In order for water to move to the top of a tree there must be a hydrostatic pressure gradient with increasing tensions from the site of removal of moisture from soil particles by the active absorbing roots, through the length of the unbroken water columns in the xylem, to the transpirational sites in the foliage.

Transpiration

The design of this portion of the study was conceived so as to determine the rate of transpirational water loss as measured gravimetrically with the load cell lysimeter. These data are very important to the interpretability of the other physiologic data - and vice versa - - and were most useful in considering transpiration rates over short periods of time, e.g., 30 minutes.

As shown in Table 2, which considers physical and physiologic factors simultaneously, at four sampling periods during the study, transpiration is another factor which is inseparably related to solar input, soil moisture, xylem transport and moisture tension in the foliage.

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[1] A sink is defined as a site of temporary excessive moisture loss.

For example, in Table 2, at 1200 on April 21, treatment trees at -1 bar soil moisture tension had low values (1.8 bar) for leaf tension while the rate of xylem transport was high (11.0 cm./hr.). The relatively high value for rate of transpiration (62 gm./hr.) indicates that trees in this condition were able to maintain the transpiration load at that level of solar energy input (1.05 Langleys). However, at the same date and time, the -12 bar treatment trees responded to the same energy load with a low level of xylem transport (2.8 cm./hr.) and of transpiration (20 gm/hr.). Also, the leaf moisture tension values built up beyond the measuring capabilities of the hydrostatic pressure cell (300+ psi). This indicates an unfavorable hydrostatic tension gradient throughout these seedlings.

Prior mention was made of the fact that transpirational activity was not measureable in the high tension treatment towards the end of the study. This is shown in Table 2 on 16 May when there was no measure of transpiration. At that same time xylem transport activity had also dropped below the measureable level, and leaf tensions were uniformally high regardless of time of day.

Emitted Temperatures

Thermal measurements made with the Stoll-Hardy infrared radiometer throughout the study are summarized in Table 2. Although measurements were made regularly, it is most useful here to consider apparent temperatures simultaneously with other physical and physiologic measures.

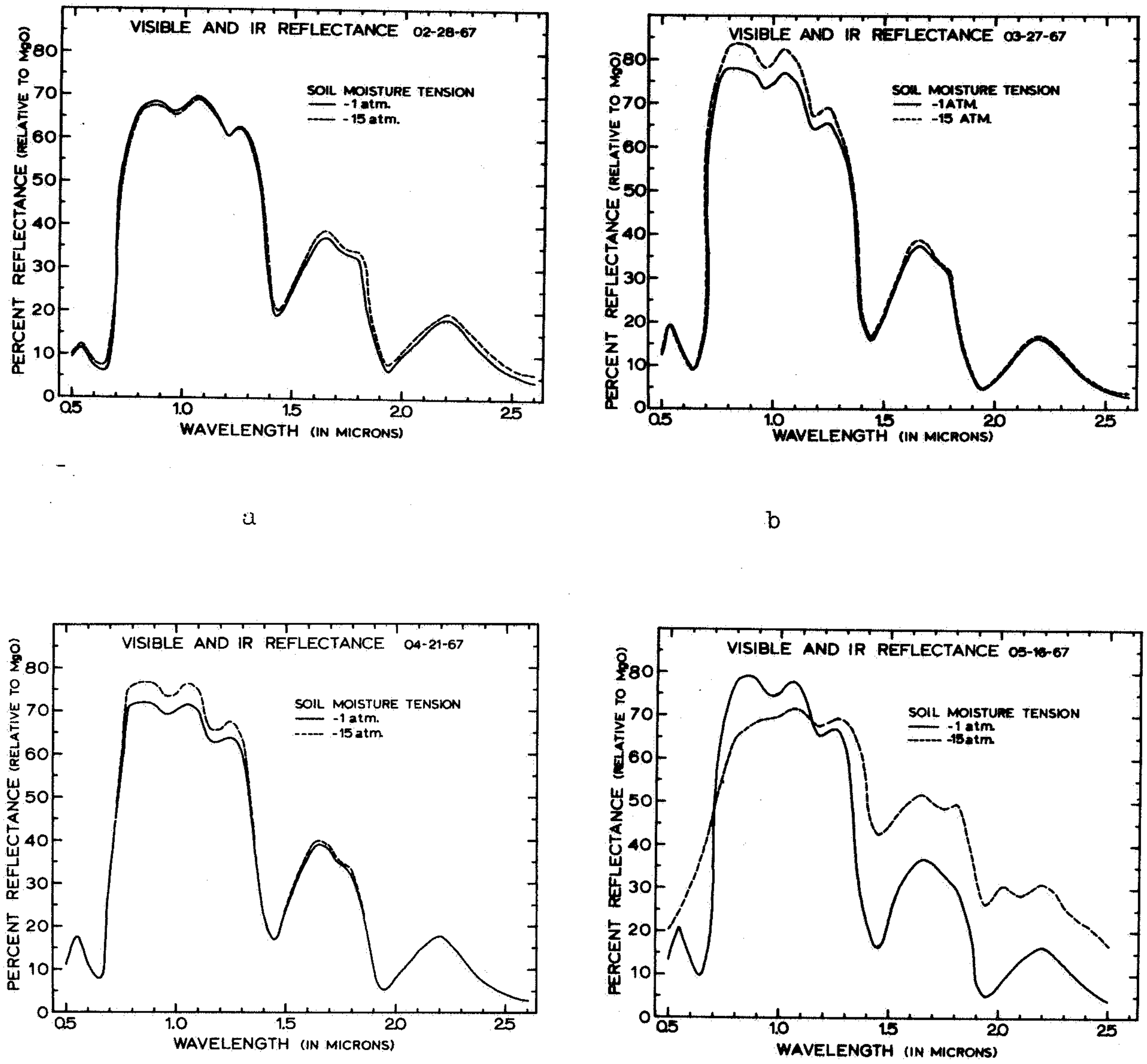
The primary purpose for gathering thermal date was to see if in fact under closely controlled moisture stress condition, and with careful measurement of other factors, there were differential emitted temperatures between individuals of various treatment levels.

There were times during the study when greater thermal differences were recorded than those shown in Table 2; however, the temperature differences recorded on the afternoon of May 16 are shown to be significantly different when subjected to a statistical test. That is, due to low variation between individuals in treatment groups, the temperatures of the highest moisture stress group (-15 bar) were different from those in the lowest three treatments (-1, -3 and -7 bar). In addition, the -12 bar treatment temperatures were found to be different from the lowest stress group (-1 bar).

Spectral Reflectance

The same four representative sampling dates - 28 February, 27 March, 21 April, and 16 May - were selected for presenting spectral data, because they are in distinct periods of change in the response of study trees, and they coincide with dates where other physical and physiologic data have been presented.

Figure 2, a composite of the four sampling dates, presents spectral reflectance curves of individuals sampled from the two extreme treatment groups, -1 bar and -15 bars of soil moisture tension. Although the data presented in Figure 2 are generated from one randomly selected individual in the two treatment groups, curves were run for each individual in the study at each of the sampling periods. A compari-



a

b

c

d

Figure 2. --Spectral reflectance curves generated from individual white pine foliage samples at four periods throughout the study. Solid line curve represents response of low moisture stress foliage and broken line curve represents response of high moisture stress foliage.

son of corrected spectral curves showed very little difference in reflectance values as a function of wavelength between individuals of the same groups.

At the beginning of the study, just after the soil moisture levels had been established, there was virtually no difference in the spectral values between treatment groups as shown in Figure 2a. The first change in the spectral response appeared one month after the beginning of the study (Figure 2b). This change was expressed as an increase in reflectance in the waveband 0.75 to 1.3 microns. Noteworthy is the fact that no change is shown in the visible portion of the spectrum shown, (0.5 to 0.7 microns). The same increase in reflectance was evidenced each time during the next five weeks as shown in Figure 2c. It was at least interesting, if not unexpected, that this difference in reflectance in the near infrared region of the spectrum showed up on infrared color film in April.

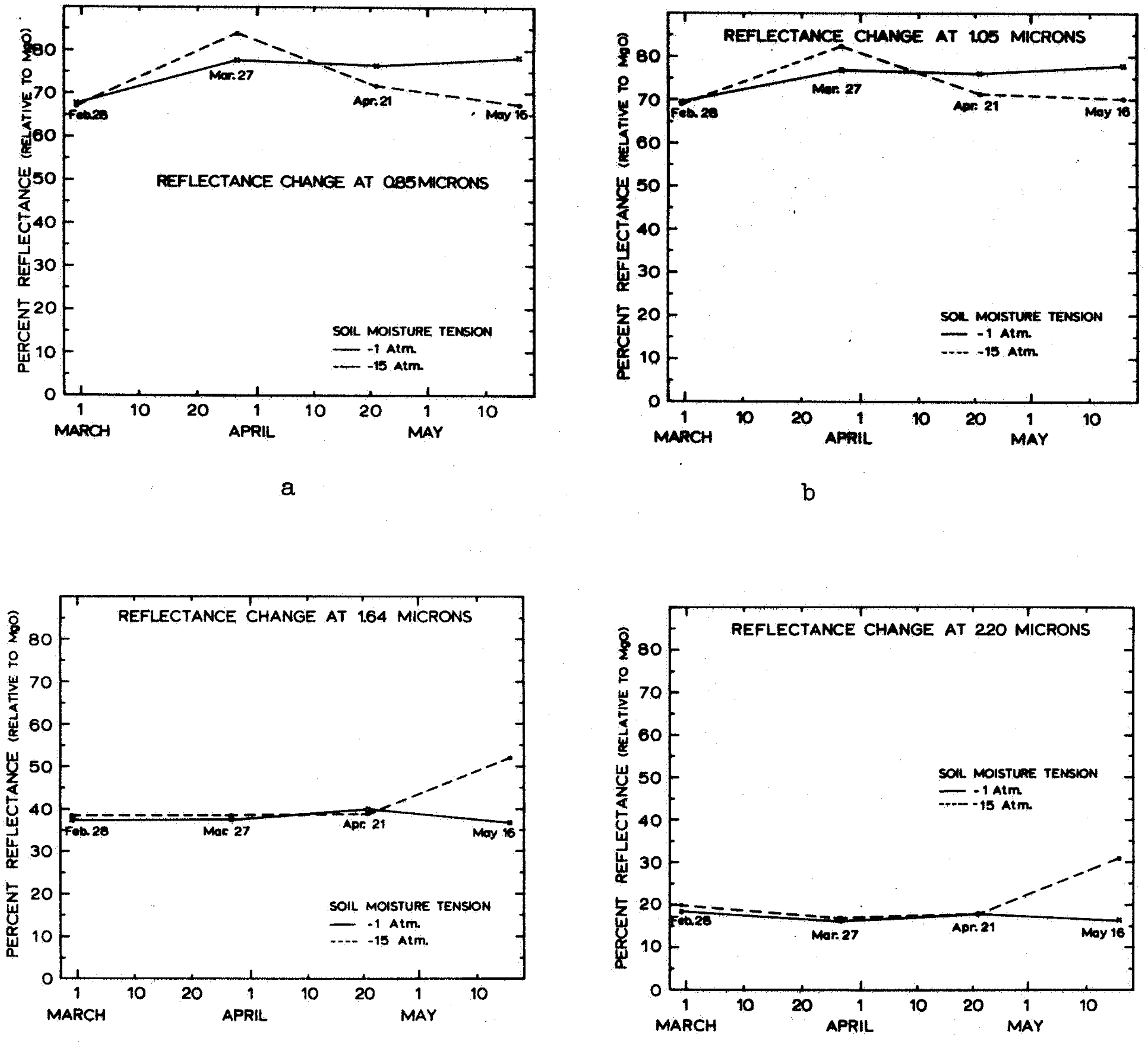
Figure 2d shows the significant and predictable change in the spectral response as a function of wavelength at the close of the greenhouse study. Most notable are the loss of the red (visible) chlorophyll absorption band 0.63 microns, the change in slope and amplitude of the bandwidth 0.70 to 0.85 microns, the distinctly lower reflectance values between 0.70 and 1.17 microns, and the rather large increase in reflectance values between 1.17 and 2.5 microns. In conifers, the cross-over in the relationship of reflectance for healthy and moisture stressed foliage between 0.70 and 1.17 microns is a phenomenon of of considerable interest.

Although the total spectral response is interesting, some wavelengths are of particular interest. Reflectance values for four wavelengths (0.85, 1.05, 1.64 and 2.20 microns) are shown in Figure 3, plotted over sampling date. Although it is apparent that the line graphs were constructed by connecting straight lines between sampling dates, the rate of change depicted is realistic in light of all data collected.

It is significant to note that the spectral responses, and particularly the change in response at various wavelengths, occurred in April coincident with major changes in the physiologic responses shown in Table 2.

Structural Influences on Leaf Reflectance

The relatively low reflection of visible radiation in conifers is attributed in part to the high absorption by leaf pigments, primarily the chlorophylls. However, these pigments are transparent to infrared radiation and the high reflectance at these wavelengths appears to be determined by the internal structure of the leaf. In addition to the absorption by chlorophyll of the mesophyll cells, the relatively low reflectance of visible light by conifer needles as compared to broadleaved species is due to compact internal structure and the orientation of the needles with respect to the illumination and the viewing angle. The reflectance minima at 1.45, 1.95 and 2.60 microns (see Figure 2) are due to the strong absorption bands of water.



a

b

c

d

Figure 3. --Spectral reflectance values for individual white pine foliage samples plotted by four sampling dates. Each set of curves depicts reflectance values at different wavelengths, e.g. (a) 0.85 microns (b) 1.05 microns, (c) 1.64 microns, and (d) 2.20 microns. Solid line connects reflectance values of low moisture stress foliage, and broken line connects reflectance values of high moisture stress.

Observations and measurements made on this study indicate that the foliage of pine seedlings affected by moisture shortage undergo structural changes as a result of physiological stress, which substantially affect reflectance characteristics.

Individual pine needles are composed of an ordered distribution of cells, each bounded by a cell wall which in turn usually holds living cytoplasm. The cytoplasm itself is highly organized and is a membranous system containing many subcellular bodies and a wide variety of chemicals dissolved in an aqueous medium (Esau, 1965). Consider a transection of a healthy pine needle, e.g., a highly magnified portion of a needle is shown in Figure 4; the outer circumference (two to three cells thick) consists of a layer of cells called the epidermis and the hypodermis. A stressed needle is shown in transection in Figure 5 for comparison. These cells are thought to lack chlorophyll. The next tightly packed multi-layer of cells is the mesophyll, which occupies by far the greatest volume within conifer foliage. The irregular shaped mesophyll cells contain the chloroplasts which are high in chlorophyll content.

A single layer of cells ringing the inside of the mesophyll (not pictured) constitute the endodermis. To the center of the needle from the endodermis are found the transfusion tissue, tracheids and the vascular bundle.

There are several examples in the literature of models depicting the hypothetical path of visible and near infrared radiation through

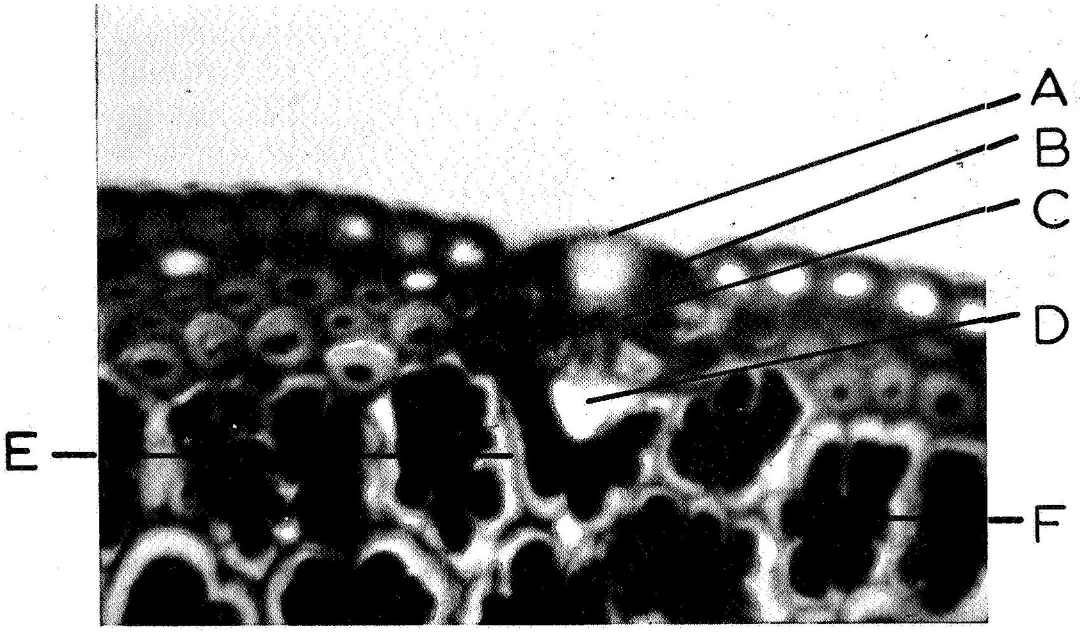


Figure 4. --Photomicrograph (430X) of a 15 micron thick section of the outer perimeter of a transection through a healthy red pine needle. Central portion (A) shows stomata in closed position, with subsidiary cells (B) and guard cells (C) and intact sub-stomatal cavity (D). Mesophyll cell walls (E) appear rigid from lignification, and cytoplasm (F) appears in contact with mesophyll ridges.

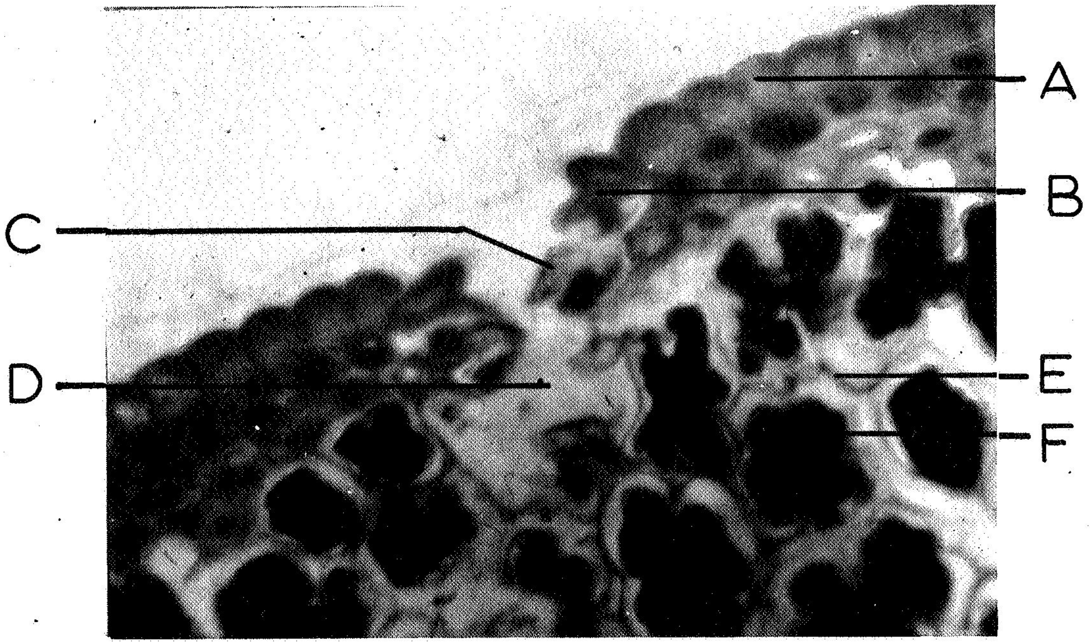


Figure 5. --Photomicrograph (430X) of a portion of the outer perimeter of a transection through a needle suffering from long-term moisture stress (-15 atm.). Notably, the epidermal cells are tightly packed (A), except for subsidiary cells (B) of the stomata which are drawn open. Also, guard cells (C) are non-functional and sub-stomatal cavity (D) is broken apart. Mesophyll cell walls (E) appear slightly thickened but not collapsed, while cytoplasm (F) occupies relatively less space within each mesophyll cell.

deciduous leaves, but apparently none for conifers. Very little radiation (approximately 4%) is reflected directly by the external surface of a healthy needle as the epidermis is nearly transparent to radiation. A portion of the incident radiation is transmitted directly through the needle, while the remainder is scattered, refracted and diffused internally. An indication that reflectivity of conifer foliage is influenced by internal structure is that the reflection and transmission spectra are the same shape and are complementary to the absorption spectra. The close relationship of reflectance to transmission (and absorption) indicates that reflectance characteristics are also determined, at least in part, within the needle structure.

In this study, reflectance curves were run for two species of pine in comparison with two species of hardwoods and in all cases in the waveband between 0.5 and 2.6 microns (except .75 to 1.25 microns); hardwood reflectance values were higher. Reflectance values for newly developing conifer foliage were considerably higher than those from old foliage, but were still lower than the curves for hardwoods.

This much discussed phenomenon of internal light scattering in leaves, likely occurs most frequently due to reflection at cell wall-air cavity interfaces within the mesophyll. The interfaces represent refractive index changes in the order of 1.0 for air to 1.4 for hydrated cell walls. Some botanists have been able to show for deciduous leaves that these interfaces contribute to the reflectance by infiltrating the air spaces with water, in which case the reflectance is reduced considerably.

It is not really sufficient to leave this subject by merely saying that reflectance from conifer foliage is similar to deciduous, as it is still not known exactly where the major reflecting surfaces are or to what extent these surfaces or structures contribute to the total reflectance. Although controversial and as yet incomplete, the above suggested mechanisms of reflectance will be referred to in an attempt to explain the reflectance changes associated with physiological and structural changes in leaves.

Effect of Moisture Stress

Different types and levels of moisture stress (insect attack, pathogens, soil salinity, etc. in conifers apparently affect the reflectance mechanism differently in different situations. It appears likely that water loss does not affect the reflectance directly, except in the region of the infrared water absorption bands where the moisture loss increases reflectance values (Figure 2d). In general, the reflectance changes appear to be caused by internal structural or chemical changes associated with the water loss. The first type of moisture stress was observed in the trees that were grown for a long period of time at the high soil moisture tension (-12 atm.). The new foliage from these trees was short and had a larger than normal transectional area. In comparison to a transection of foliage from a healthy tree (Figure 4) grown at low moisture tension, transections of foliage from trees grown at -12 bars of moisture tension revealed stoma that were so tightly closed, and in some cases degenerate, that they were difficult to differentiate from the rest of the epidermal cells under medium magnification (X100). It seems likely that these needles gave higher than normal surface reflectance.

Micro-examination of transections of foliage from trees that had grown under moisture stress of at least -15 bars (Figure 5) showed again that the epidermal cells were tightly packed in comparison to healthy foliage (Figure 4). However, now there is evidence of nonfunctional stomata where subsidiary cells and guard cells are disoriented and the sub-stomatal cavity has broken apart. It was not apparent in micro-evaluation that there was any significant collapse of the mesophyll cells as has been offered by some authors as an explanation for both increased and decreased infrared reflectance. However, there does appear to be some mesophyll cell wall thickening associated with the high stress treatment, and some distinct changes in the ridges of the mesophyll cells. It is not likely that this apparent shrinkage effect is caused by a change in the rigid lignified mesophyll cell walls, but rather is caused by shrinkage of the cytoplasm and the accompanying withdrawal from the cell walls. This phenomenon could be achieved with a very minute transfer of solute from the cytoplasm to the mesophyll cell walls. It seems reasonable that this minute movement of liquid out of the cytoplasm causes an increasing concentration of solute and subcellular particles (such as starch grains). In addition, the mesophyll calls decrease in the water while the cell walls remain the same size; this may cause or contribute to the increased internal scattering and reflectance at some wavelengths. It is also possible that increased reflectance may be the consequence of decreased absorption by compounds in the leaf other than water, at some wavelengths, and by the same reason may result in decreased reflectance at other wavebands (notably .75 to 1.15 microns).

CHANGES OBSERVED IN BROADLEAVED SEEDLINGS

Spectral Reflectance

Representative curves obtained from one sycamore leaf are shown in Figure 6 and illustrate the basic pattern of spectral reflectance changes observed.

A better view of the changes which occurred can be realized when reflectance at one wavelength is plotted over moisture content. Changes observed in the visible portion of the spectrum (0.38 to 0.72 microns) were erratic or non-existent (Figure 7) and changes in the photographic infrared (0.7 to 1.2 microns) were too small to be photographically significant (Figure 8 and 9). At longer wavelengths, however, a distinct curvilinear trend was observed with reflectance increasing as moisture content decreased (Figure 10, 11 and 12).

Leaf Reflectance Under Low Moisture Stress

Reflectance characteristics of leaves which formed and developed under low levels of moisture stress did not change radically when moisture stress increased after the leaves had matured. This was true for

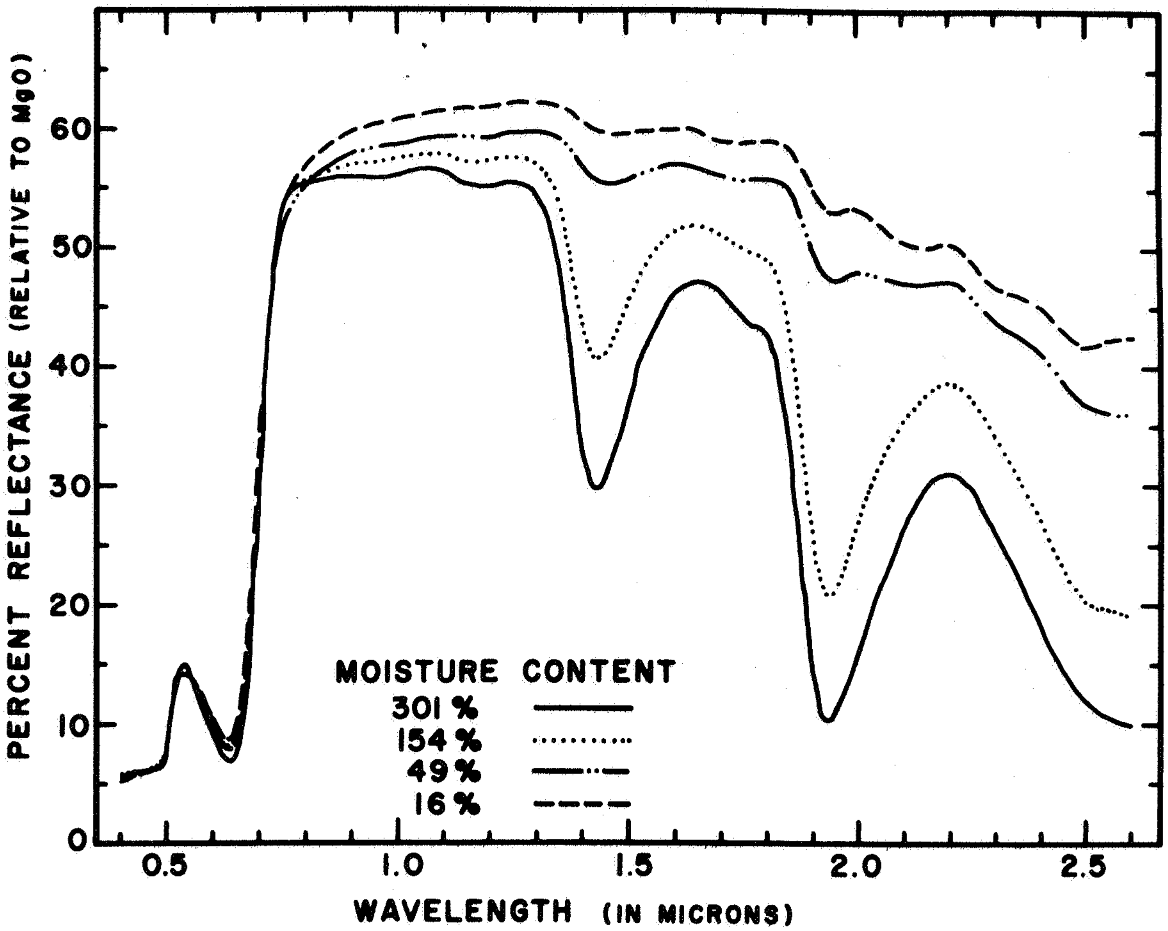


Figure 6. --Spectral reflectance curves for a sycamore leaf with varying moisture content. The leaf was allowed to air dry between successive measurements.

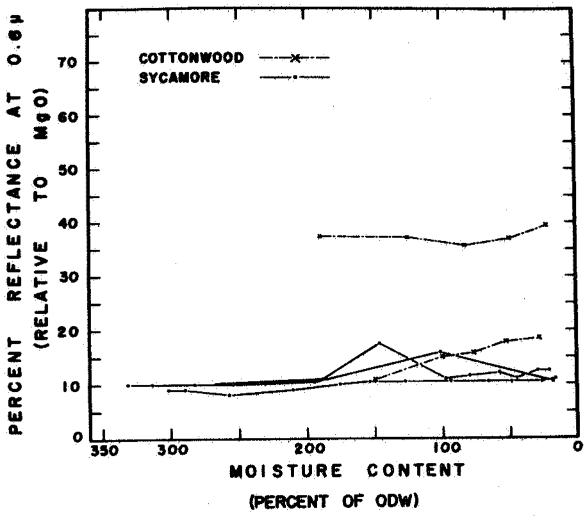


Figure 7. --Spectral reflectance at 0.6 microns as a function of moisture for five leaves.

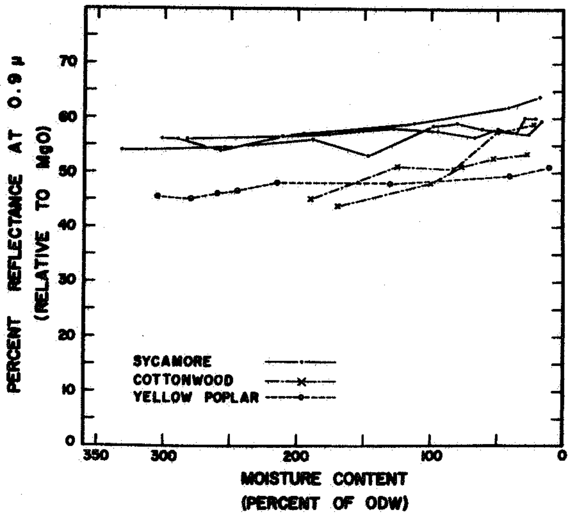


Figure 8. --Spectral reflectance at 0.9 microns as a function of moisture content for six leaves.

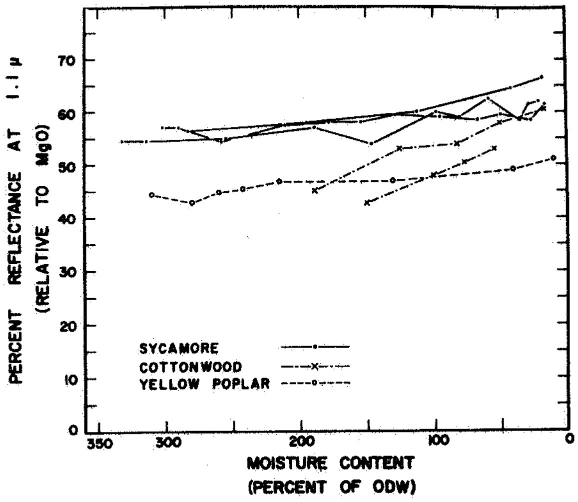


Figure 9. -- Spectral reflectance at 1.1 microns as a function of moisture content for six leaves.

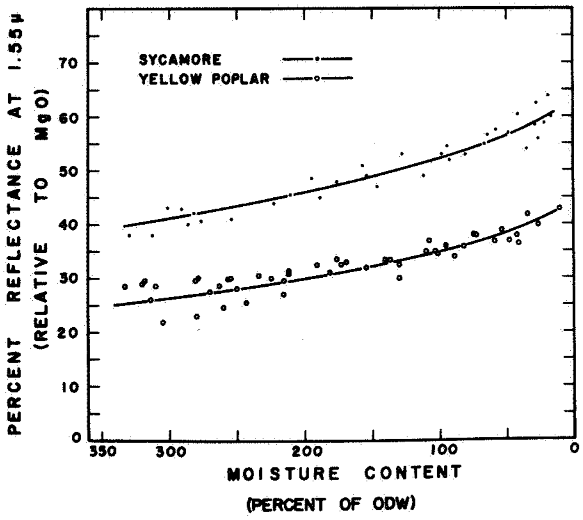


Figure 10. --Free-hand average curves of spectral reflectance at 1.55 microns as a function of moisture content for two species.

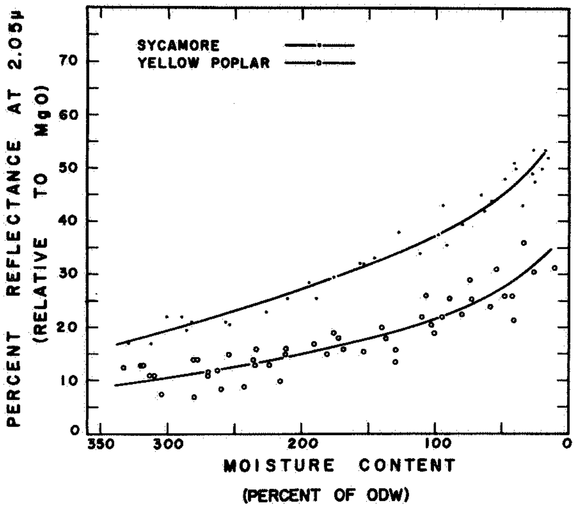


Figure 11. --Free-hand average curves of spectral reflectance at 2.05 microns as a function of moisture content for two species.

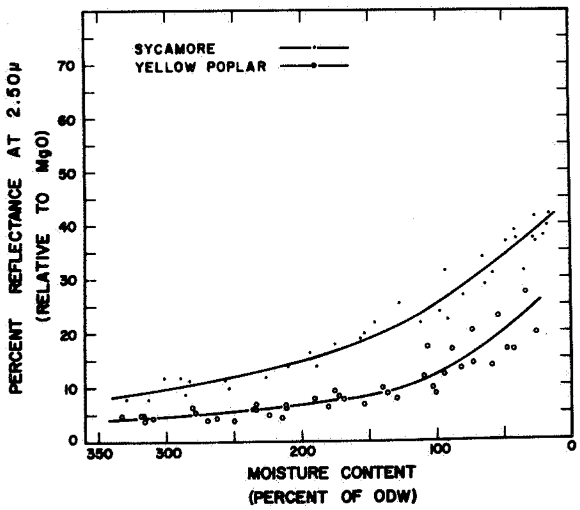


Figure 12. --Free-hand average curves of spectral reflectance at 2.50 microns as a function of moisture content for two species.

all of the mature leaves selected from the first group of trees on March 28 and from the second group of trees on May 9. The reflectance curves in Figure 13 are typical of the data obtained from mature leaves on watered and unwatered plants on March 28. By early May moisture tension had increased to -20.9 bars in the unwatered, as compared to -6.2 bars in the watered plant, but reflectance of the mature leaves had not changed greatly (Figure 14). Somewhat greater differences in reflectance were observed between the mature leaves on trees 3W and 3NW. These differences were quite small in March but grew progressively larger as moisture stress increased in 3NW. Change in reflectance over time is shown in Figure 15 for eight wavelengths. The difference between the watered and unwatered trees is most noticeable at 0.80 and 1.65 microns and is essentially nil at wavelengths below 0.80 or above 1.65 microns. Data for other mature leaves show the same pattern of change over time as the data for mature leaves from trees 3W and 3NW.

Leaf Reflectance Under High Moisture Stress

Reflectance characteristics of leaves which formed and developed under high levels of moisture stress changed markedly as the leaves matured but remained quite stable once maturity had been reached. Changes observed in the second immature leaves selected on trees 2W and 2NW are typical, and are illustrated in Figure 16. In contrast to results with mature leaves, reflectance from immature leaves is lower for the unwatered than for the watered plant at all wavelengths.

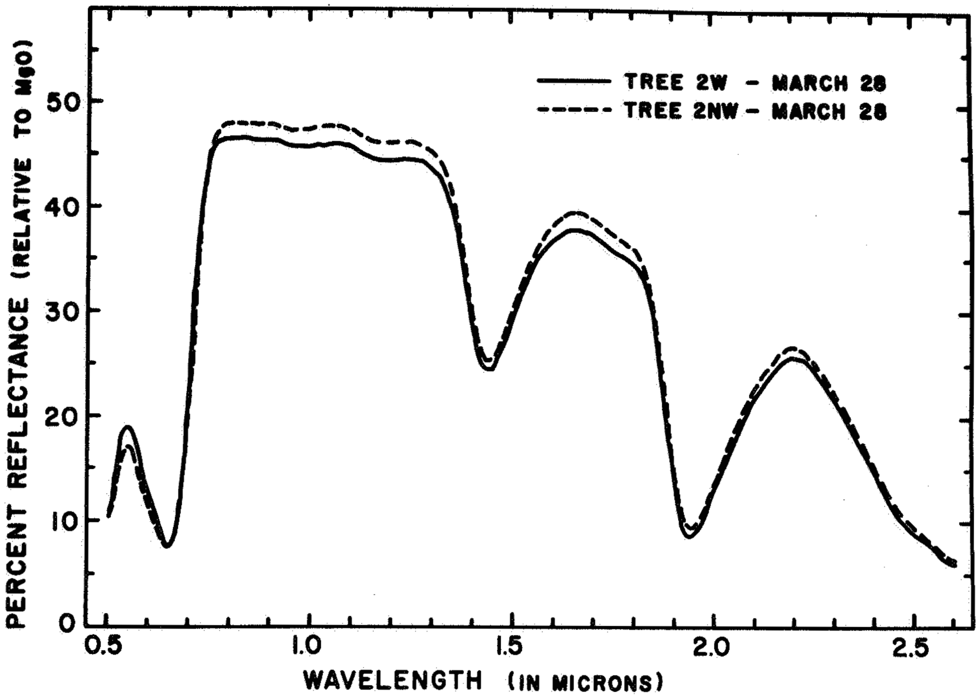


Figure 13. --Spectral reflectance curves obtained on March 28, 1967, from the upper surfaces of single mature leaves on two yellow poplar seedlings that had been watered (2W) and unwatered (2NW) as the plants leafed out at the beginning of their third growing season.

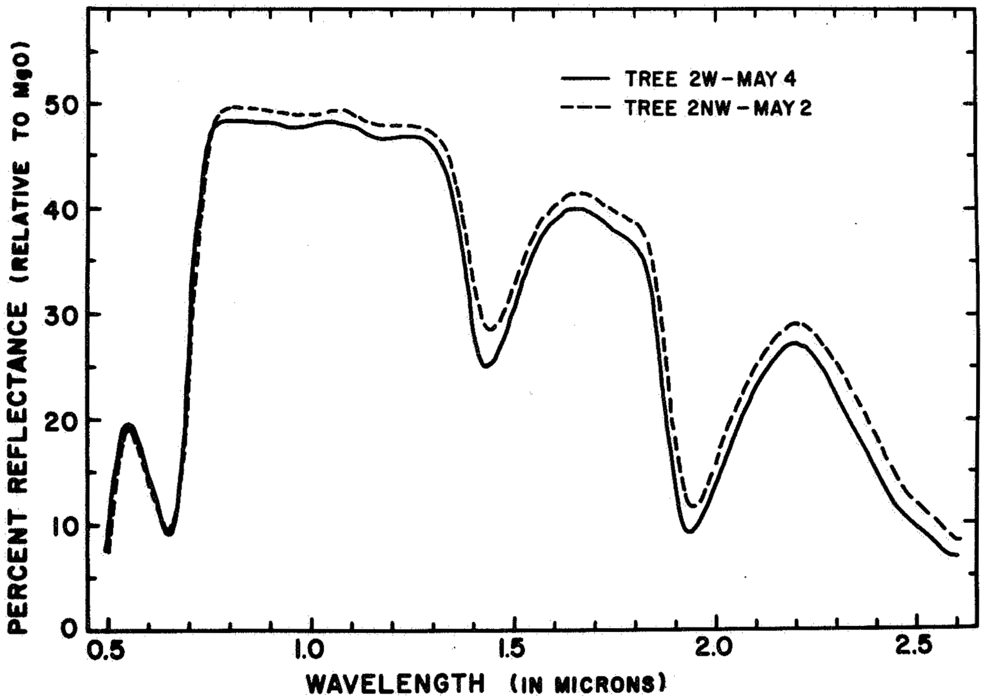


Figure 14. --Spectral reflectance curves obtained on May 2 and 4, 1967, from the upper surfaces of the same mature leaves used to obtain the curves in Figure 13. Both leaves remained on their respective plants and appeared to function normally throughout the period.

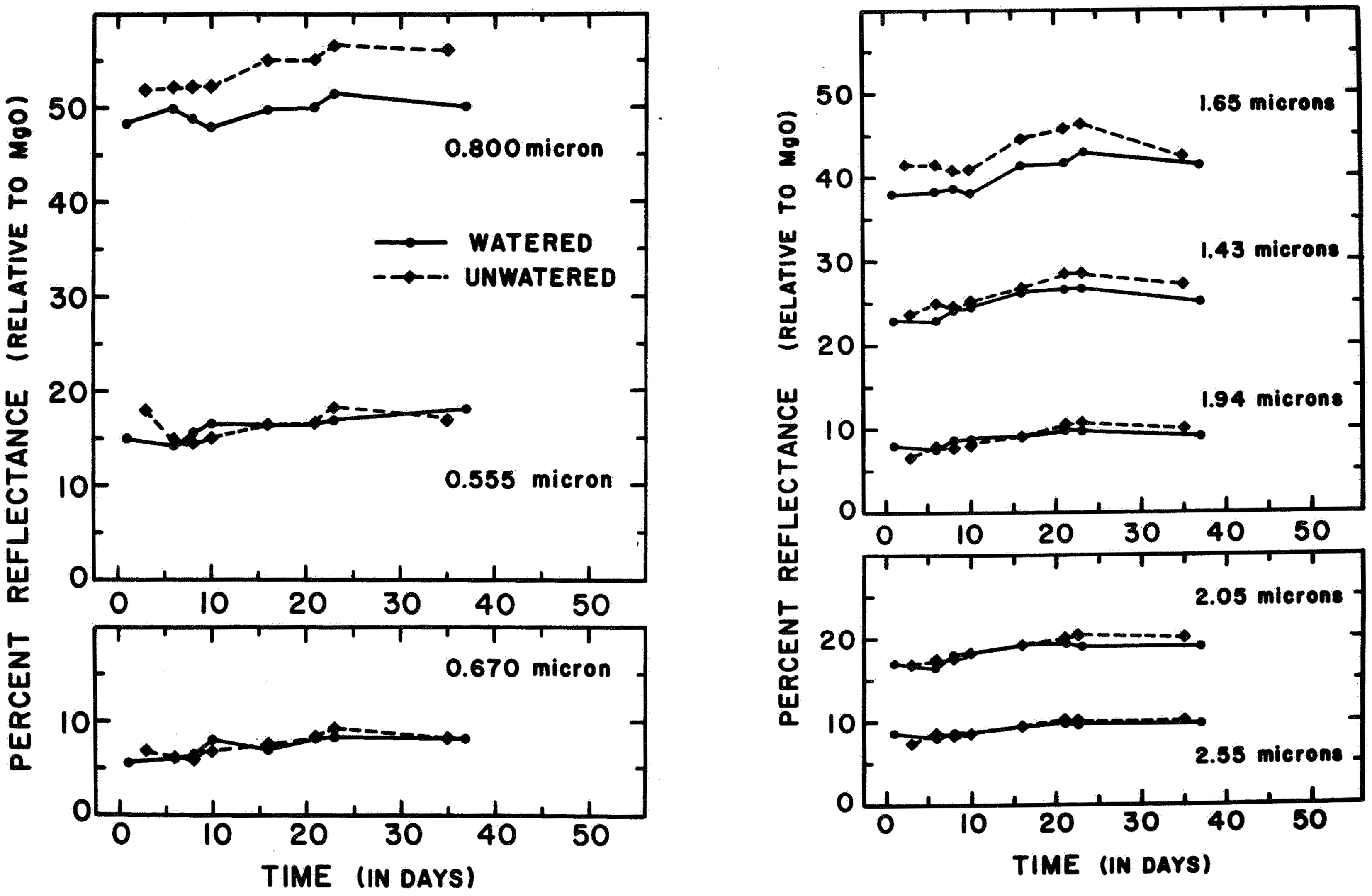


Figure 15. --Change in reflectance at eight wavelengths as a function of time for the upper surfaces of single mature leaves attached to watered (3W) and unwatered (3NW) yellow poplar seedlings. March 28, 1967, the day the first reflectance measurements were obtained, is shown as day one.

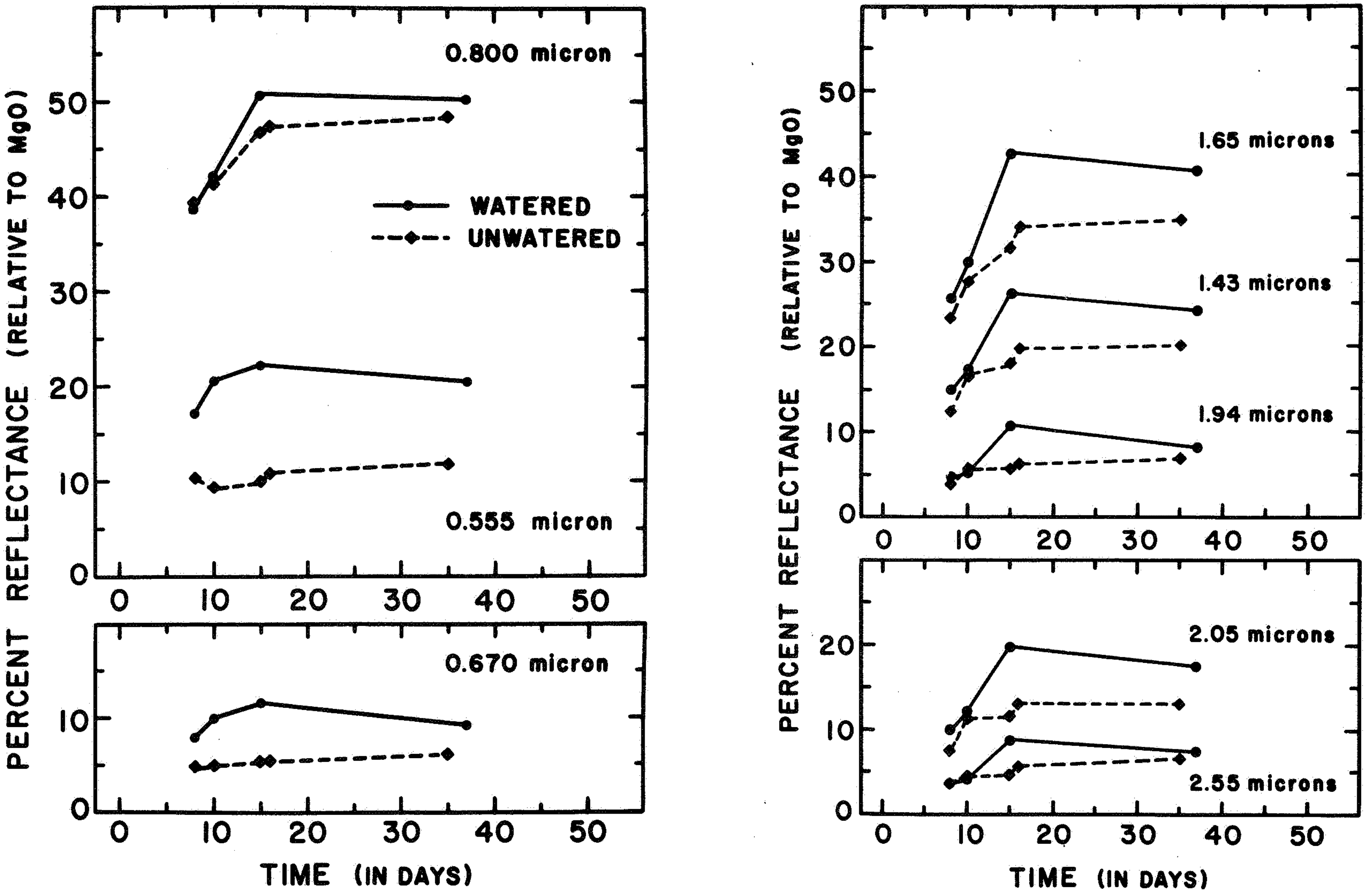


Figure 16. --Change in reflectance at eight wavelengths as a function of time for the upper surfaces of single immature leaves attached to watered (2W) and unwatered (2NW) yellow poplar seedlings. March 28, 1967, is presented as day one.

Immature leaves selected on March 28 on the first group of plants represent a condition intermediate between the low stress under which the "mature" leaves developed and the high stress associated with the second immature leaves. Typical data for one pair of immature leaves selected on March 28 are shown in Figure 17. These data represent the longest series of measurements obtained from single leaves during the study. No data are available for the unwatered plant after May 2 because leaf abscission occurred shortly after the unwatered plants of the first group were watered heavily on the evening of May 7. Yellowing of all mature and most immature leaves occurred promptly on all four of the previously unwatered plants and by May 14 all of mature and all but two of the marked immature leaves had fallen.

One consistent difference in the shape of the reflectance curves of mature and immature leaves was observed. This occurred in the band of high reflectance between 0.75 and 1.35 microns. Reflectance was nearly constant across this band for mature leaves, creating a plateau that sloped gently downward toward longer wavelengths. For immature leaves the slope in the plateau was much more pronounced but decreased as the leaf matured (Figure 18). This change in slope of the high reflectance plateau in the near-infrared region was observed for immature leaves on plants at all levels of moisture stress.

The more extreme slope of the near-infrared plateau typical of very young leaves, and the sharply reduced reflectance from leaves developing under high water stress, are well shown in Figure 19. These

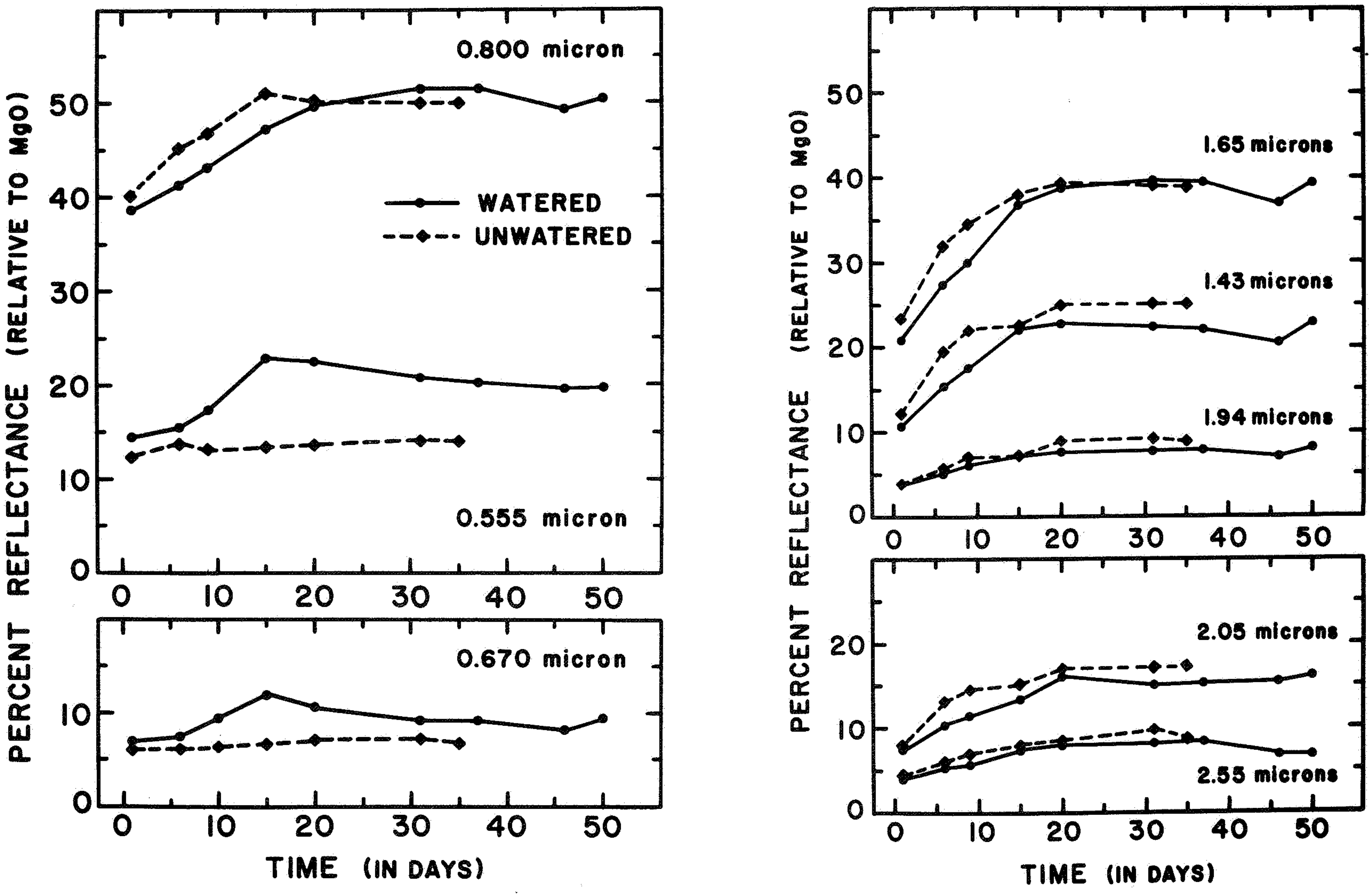


Figure 17. --Change in reflectance at eight wavelengths as a function of time for the upper surfaces of single immature leaves attached to watered (1W) and unwatered (1NW) yellow poplar seedlings. March 28, 1967, is represented as day one.

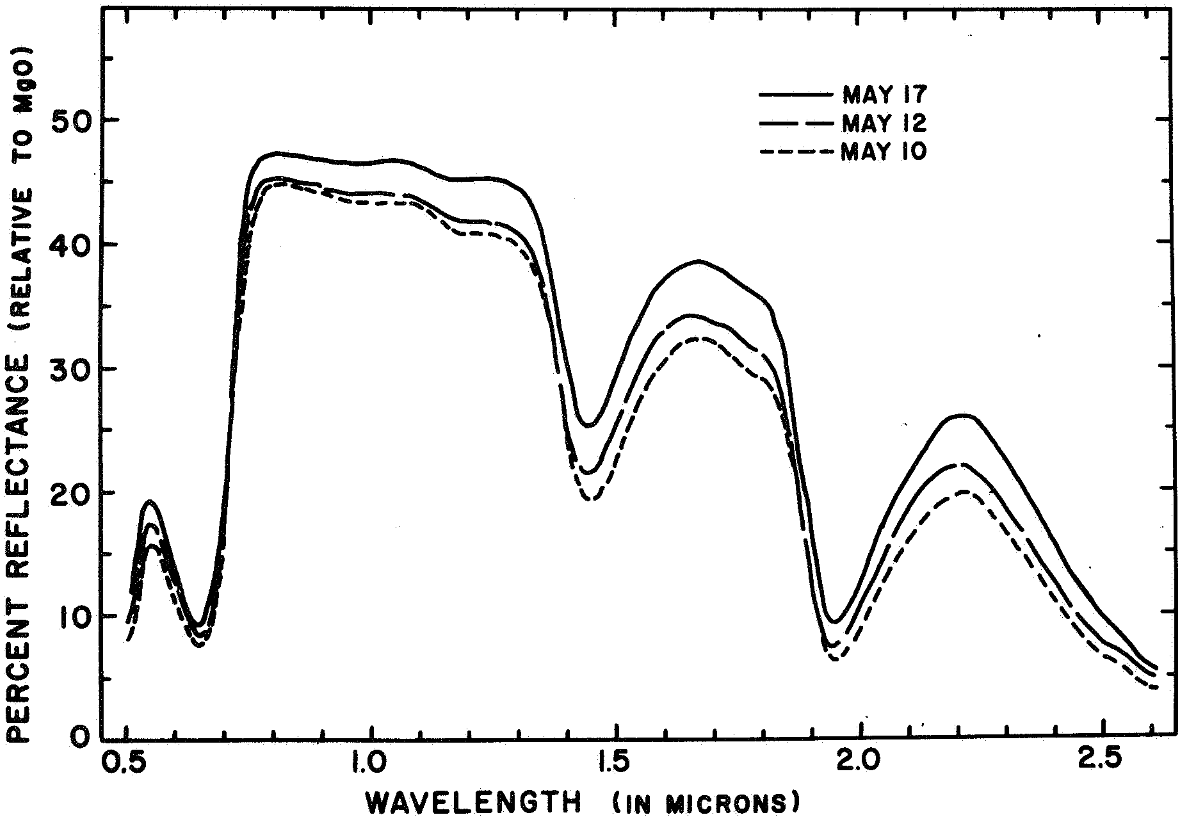


Figure 18. --Change in spectral reflectance from the upper surface of a single immature leaf developing on a watered yellow poplar seedling (leaf number $45W_1$) as a function of time.

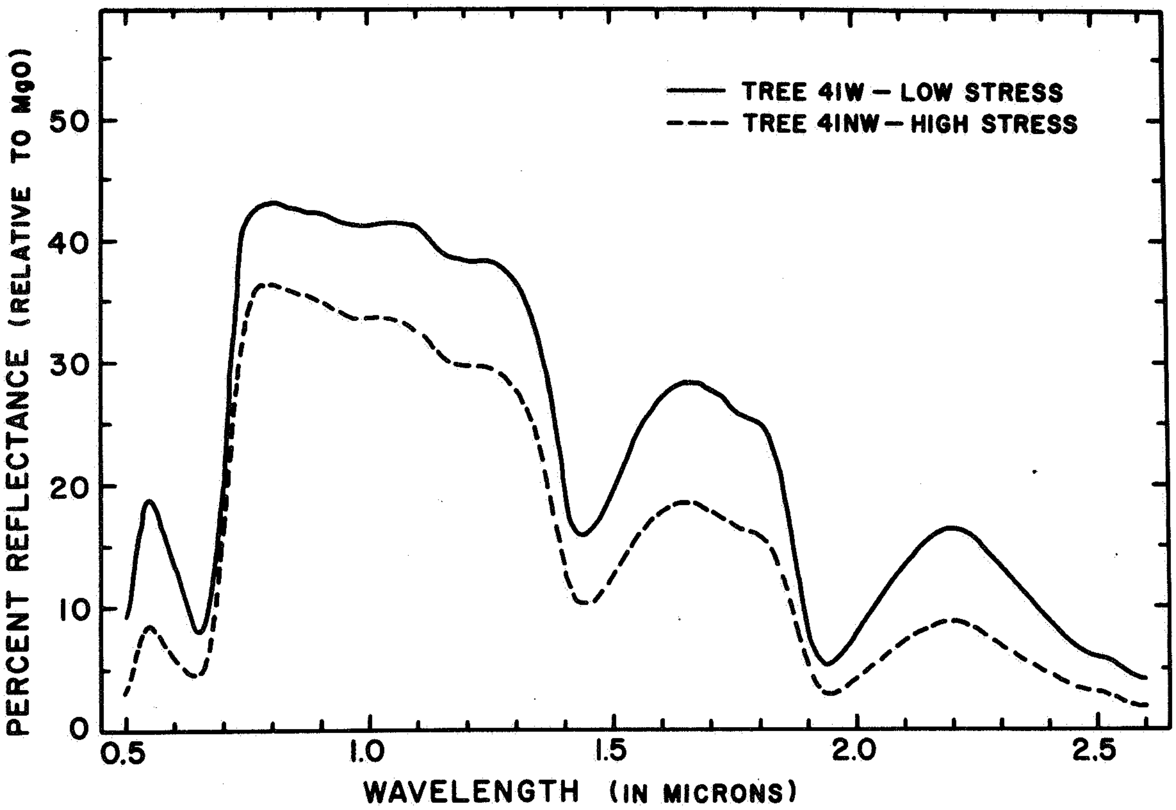


Figure 19. --Spectral reflectance from the upper surfaces of recently unfolded leaves of yellow poplar seedlings growing under low (41W) and high (41NW) water stress regimes.

data came from two of the youngest and smallest leaves used during the study. The leaf from tree 41NW had emerged and unfolded less than 24 hours before the measurements were obtained, and the leaf from tree 41W had unfolded less than 48 hours before. Leaf areas at the time the reflectance data were obtained were 10.1 sq. cm. and 20.0 sq. cm. for the leaves from trees 41NW and 41W, respectively.

Many factors made it impossible to obtain periodic radiometric data from the yellow poplar seedlings. However, one set of measurements in the 8.1-13.2 micron band was obtained in mid-May, and these measurements are summarized in Table 3.

Leaf Moisture Tension

Efforts to maintain low levels of moisture stress in the watered trees were not completely successful, even when the trees were watered daily. Non-uniformity in rate of water application, uneven development of distribution of roots, differences in amount of transpiring leaf surface, and mechanical injury to roots resulting from frequenty handling of the plants, may all have contributed to the differences in moisture tension observed between plants and, for any one plant, over time. Moisture tension data obtained with the Scholander pressure cell are shown in Table 4 and indicate the magnitude of the differences in water stress that developed during the study.

Differences in the rate of growth and in ultimate leaf size were observed between leaves on different plants. Representative data showing change in leaf size over time are included in Table 5.

Table 3. Summary of radiometric data obtained from three year old yellow poplar seedlings under different degrees of moisture stress.

Tree No.	1W 3/	1NW 3/	41W	42NW	43NW	44NW
Leaf Moisture Tension (bars) 1/	-8.2	-8.2	-5.4	-15.7	-13.6	-13.6
Apparent Temperature (C°) 2/	19.0	19.5	18.3	25.5	26.4	24.2

1/ Measured in a Scholander pressure cell between 1408 and 1421 hours on May 19, 1967.

2/ Measured with a Stoll-Hardy radiometer filtered to measure energy in the 8.1-13.2 micron region between 1320 and 1400 hours on May 19, 1967. Each figure shown is an average of three or more replications.

3/ W - watered seedling; NW - unwatered seedling

Table 4. Water stress measurements made with a Scholander pressure cell for watered (W) and unwatered (NW) yellow poplar seedlings growing in a greenhouse.

Date	4-5-67	4-19-67	4-28-67	5-1-67	5-4-67	5-7-67	5-10-67	5-12-67	5-14-67	5-14-67	5-17-67	9-19-67
Time Measured	1610 to 1650	1330 to 1415	0819 to 0908	0704 to 0838	0731 to 0855	1748 to 1825	0640 to 0754	0653 to 0803	0615 to 0654	1926 to 1939	0631 to 0733	1437 to 1500
Tree No.	Water Stress in Pounds per Square Inch (14.7 psi = -1 bar of moisture tension)											
1W	109(1)	143	113		88	65	73	40			40	
2W	97	162			91	38	67	65			54	
3W	110	157			66	48	100	81			68	
4W	85	160			65	68	71	74			95	
1NW(2)	123	219			280	310	58	56	65		73	161
2NW(2)	128	223			308	350+	148	134			162	
3NW(2)	87	178	230		245	275	50	58			96	302
4NW(2)	129	227		265	300	305	62	66			101	283
41W			120	54	92		68	83	62		87	100
42W			119	60	107		74	64	57		68	
43W			108	57	92		75	59	64		54	
44W			123	62	95		65	77	77	85	54	
45W					96		75	81	50	57	55	
41NW			108		153		175	211	167		223	310+
42NW			165		194		253	248	158		175	
43NW			115	93	125		162	174	166		194	315+
44NW			115	77	136		161	175	173	178	190	
45NW				95	121		142	143	136	145	163	

(1) This measurement made at 1330 hours.

(2) Tree watered heavily at 1930 hours on May 7 and watered daily through May 17, 1967.

Table 5. Summary of leaf area measurements for selected leaves from watered (W) and unwatered (NW) yellow poplar seedlings growing in a greenhouse.

	Mature Leaves						Immature Leaves					
Leaf No.	1W	1NW	2W	2NW	3W	3NW	$1W_1$	$1NW_1$	$2W_2$	$2NW_2$	$45W_1$	$45NW_1$
Date	Leaf Area in Square Centimeters											
3-28-67							10.1	37.9				
4-3-67							41.5	132.0				
4-5-67	168.5	238.8	254.5	146.7	158.3	172.9			26.2	12.4		
4-6-67							60.9	175.8				
4-7-67									41.2	16.9		
4-12-67							92.2	174.9	73.4	24.7		
4-17-67	168.7	236.0	253.1	142.4	159.1	171.5	105.0	174.5				
4-18-67										25.4		
4-28-67	168.3											
4-29-67		233.9					105.0	171.0				
5-2-67		234.8		143.0		170.8		169.3		24.3		
5-4-67	168.6		253.3		157.1		105.5		91.4			
5-7-67		231.3										
5-10-67											29.1	36.0
5-12-67											48.6	36.3
5-13-67	170.1						106.2					
5-17-67	169.0						106.4				98.9	37.0

Despite variations in moisture tension and leaf size, relationships between foliar reflectance and water stress were essentially the same in September as for either group of seedlings in March, April and May. In all cases, the level of water stress at the time of leaf formation and development appeared to exert a greater influence on foliar reflectance than did the level of water stress at the time the reflectance measurements were made.

At least three distinctly different leaf conditions were encountered during the study: (1) leaves that were never subjected to high water stress, (2) leaves that developed under little water stress and were then subjected to increasingly severe moisture tensions, and (3) leaves which developed under high water stress and were kept at high stress throughout. It proved impossible to work with leaves which had developed under high water stress and then had this stress sharply reduced; leaves that developed under high stress always yellowed and fell off shortly after the stressed plants were watered.

Microscopic examinations of leaf sections were not made for any yellow poplar leaves. However, previous work with other species suggests what the internal differences probably were between the three types of leaves described above. Kramer and Kozlowski (1960) stated: "The overall effect of internal water deficits is to reduce vegetative growth, -- water deficits not only reduce the quantity of growth, but also change the quality, largely by increasing the amount of lignification and thickness of cell walls." (p.359).

Based on a study with tomato plants, Gates (1955) reported that leaves of different ages were differentially sensitive to moisture stress, with growth of the younger leaves reduced more than growth of older leaves. Although growth was reduced less, older leaves did not recover from brief periodsof water shortage as rapidly as did the younger leaves.

During the study several of the seedlings went through two complete cycles of increasing water stress, terminated by abrupt reduction in that stress. In every case for which data are available, leaves that formed and unfolded during periods of high moisture stress possessed the reflectance characteristics of leaves from stressed plants. Leaves which formed during periods of low moisture stress (i.e. - those formed between the two periods of stress) possessed the reflectance characteristics of leaves from the watered, or low stress, plants. Thus, the continuous flushing and leaf formation typical of yellow poplar provided a continuing series of new, immature leaves whose reflectance characteristics changed as the plants went from high, to low, to high, and back to low water tensions. These changes were consistent, both between seedlings and over time, indicating a distinct causative relationship between water stress and leaf reflectance. The lower reflectance of young leaves forming on plants under high moisture stress, created a visibly darker leaf and this visible sign of water stress persisted even after the leaf had matured (Figure 20).

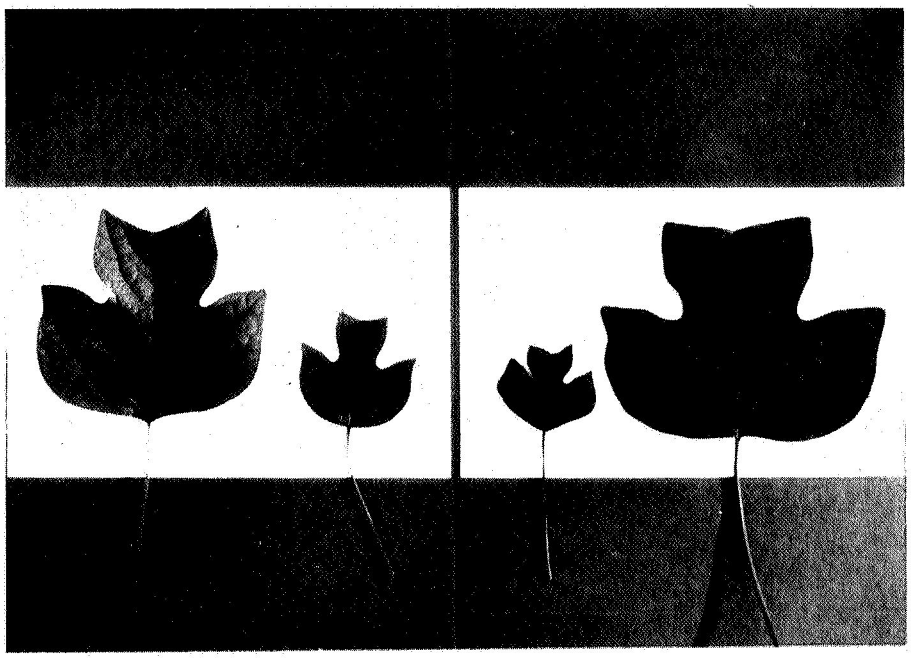


Figure 20. --Visual appearance of mature and immature leaves that developed on yellow poplar seedlings growing under low (45W) and high (45NW) water stress regimes. The dark color of leaves developing on trees under high water stress was a consistent symptom of that stress.

When the data relating reflectance with moisture content is compared with that relating reflectance and water stress, both similarities and differences are apparent. As previously indicated, 218 percent of its overdry weight was the lowest moisture content observed for any green leaf taken from one of the yellow poplar seedlings subjected to high moisture stress. If all reflectance data for mature leaves from both watered and unwatered plants are compared with reflectance data for picked leaves having moisture contents over 200 percent, (dry weight basis) the increasing reflectance of mature leaves as moisture stress increases is quite analagous to the changes observed as picked leaves dried. The same relationship does not hold for those leaves that developed under higher moisture stresses. Therefore, moisture stress at the time of leaf formation produces basically different changes in foliar reflectance properties than does moisture stress that develops after the foliage matures. This observation is in keeping with the results of Gates cited earlier, but is not meant to suggest that both effects can not interact. Such interactions may present a significant cause of uncertainty in remote sensing of tree vigor or foliar moisture content in deciduous species if the sensors are confined to reflected energy at the end of the growing season.

Although radiometric data are limited, data available suggest that emitted energy in the 8 to 14 micron band may provide more consistent indications of moisture stress than reflected energy of shorter wavelength. The data for pine indicate that emitted energy in the 4.5-5.5

micron band may, in addition to the 8 to 14 micron band, also give more meaningful data which can be related to tree vigor than any band of solely reflected solar radiation. However, multispectral comparison of reflected and emitted energy from tree foliage appears to offer the most promising method for detecting differences in moisture stress between trees.

LITERATURE CITED

1. Colwell, R. N. 1956. Determining the prevalence of cereal crop disease by aerial photography. Hilgardia, 26: 223-286.
2. Dixon, H. H. 1914. Transpiration and the ascent of sap in plants. Macmillan, London.
3. Esau, Katherine. 1965. Plant anatomy. John Wiley and Sons, New York. 767 pp.
4. Gates, C. T. 1955. The response of the young tomato plant to a brief period of water shortage. Australian Jour. Biol.. Sci., 8:215-230.
5. Gates, D. M. 1965. Characteristics of soil and vegetated surfaces to reflected and emitted radiation. Proc. 3rd. Symp. Remote Sensing of Environment, Univ. of Mich., IST Report No. 4894-9-x, pp. 573-600.
6. Heller, R. C., R. C Aldrich, and W. F. Bailey, 1959. An evaluation of aerial photography for detecting southern pine beetle damage. Photogram. Eng., 25:595-606.
7. Heller, R. C., W. F. McCambridge, R. C. Aldrich, and F. P. Weber. 1966. The use of multispectral sensing techniques to detect ponderosa pine trees under stress from insect or pathogenic organisms. Ann. Prog. Report, For. Remote Sensing Lab. for Nat. Resource Prog., NASA, by the Pacific Southwest Forest & Range Exp. Sta., 60 pp.
8. Heller, R. C., R. C. Aldrich, W. F. McCambridge, and F. P. Weber. 1967. The use of multispectral sensing techniques to detect ponderosa

pine trees under stress from insect of pathogenic organisms. Ann. Prog. Report For. Remote Sensing Lab. for Nat. Resource Prog., NASA, by the Pacific Southwest Forest & Range Exp. Sta., 65 pp.

9. Hoffer, R. M., R. A. Holmes, and J. R. Shay. 1966. Vegetable, soil, and photographic factors affecting tone in agricultural remote multispectral sensing. Proc. 4th Symp. Remote Sensing of Environment, Univ. of Mich., IST Report No. 4864-11-X, pp. 115-134.

10. Hoffer, R. M and L. D. Miller. 1965. Potential application of remote multispectral sensing in agriculture. Presented at 30th Semi-Annual Convention of the Am. Soc. Photogram., Dayton, Ohio, Sept. 24, 1965.

11. Kramer, P. J. and T. T. Kozlowski. 1960. Physiology of trees. McGraw-Hill, 642 pp.

12. L. A. R. S. 1967. Remote multispectral sensing in agriculture. Ann. Prog. Report, Lab. for Agric. Remote Sensing for Nat. Resource Prog., NASA Purdue University Agric. Exp. Sta. Res. Bull. No. 832.

13. Marshall, D. C. 1958. Measurement of Sap flow in conifers by heat transport. Plant Phys., 33:385-396.

14. Meyer, M. M. and D. W. French. 1967. Detection of diseased trees. Photogram. Eng., 33:1035-1040.

15. Olson, C. W., Jr. 1964. Light reflectance measurements compared with panchromatic and infrared aerial photographs. Univ. of Mich., IST Report No. 4864-8-T, 21 pp.

16. Olson, C. E., Jr. 1967. Optical remote sensing of the moisture content in fine forest fuels. Univ. of Mich., IST Report No. 8036-1-F, 21 pp.

17. Olson, C. E., Jr. R. E. Good, C. A. Budelsky, R. L. Liston, and D. D. Munter. 1964. An analysis of measurements of light reflectance from tree foliage made during 1960 and 1961. Univ. of Ill., Agric. Exp. Sta. Report on ONR Proj. No. NR-387-025, Contract 1834 (31), 222 pp.

18. Scholander, P. F., H. T. Hammel, D. Bradstreet, and E. A. Hemmingsen. 1965. Sap pressure in vascular plants, Science (AAAS), N. Y., 148:339-346.

19. Slatyer, R. O. 1967. Plant-water relationships. Academic Press, New York, 366 pp.

20. Swanson, R. H. 1965. Seasonal course of transpiration of lodgepole pine and engelmann spruce. Proc. International Symp. on For. Hydrology, Penn. State Univ., Aug. 29 to Sept. 10, 1965. pp. 217-432.

21. Thomas, J. R., V. I. Myers, M. D. Heilman, and C. L. Weigand. 1966. Factors affecting light reflectance of cotton. Proc. 4th Symp. Remote Sensing of Environment, Univ. of Mich., IST Report No. 4864-11-X, pp. 305-312.

22. Weber, F. P. 1965. Exploration of changes in reflected and emitted radiation properties for early remote detection of tree vigor decline. Unpuplished M. F. thesis, University of Michigan, Ann Arbor, 101 pp.

23. Weber, F. P. 1966. Multi-spectral imagery for species identification. Final report, For. Remote Sensing Lab. for Nat. Res. Program, NASA, by the Pacific Southwest Forest and Range Exp. Sta., 37 pp.

24. West, D. F. 1956. Aerial surveys for better crops. Crops and Soils, 11:17-18.

APPENDIX

The following is a list of University of Michigan and Forest Service, U. S. Department of Agriculture, personnel who have made contributions to this research study and represent a major salary contribution to it:

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