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Changes in Vegetation Spectra
with Deterioration of Leaves
Under Two Methods of Preservation

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OF LEAVES UNDER TWO METHODS OF PRESERVATION

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ABSTRACT

Motivated by the needs of a field project to measure spectral manifestations of mineralization in vegetation, the authors set up an experiment to measure changes in leaf spectra under differing methods of preservation over time. The spectral measurements were made using a three band hand-held portable radiometer which simulated three Thematic Mapper (TM) bands — TM3, TM4 and TM5.

Using a procedure identical to that used in an ongoing geobotanical field project, daily spectral measurements of white oak (*Quercus alba*) leaves under three preservation treatments were made. The spectral readings over three treatments — fresh, bottled and bagged vegetation — were indistinguishable in bands TM3 and TM5 for up to four days after collection. After that time bagged and bottled samples showed significant increases in reflected energy. This was interpreted as being related to loss of chlorophyll from and dehydration of the vegetation. There was no significant variation in the reflectance values from TM4 over preservation type for the experimental period. This was interpreted as indicating the persistence of the air space-cellular interface.

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CHANGES IN VEGETATION SPECTRA WITH DETERIORATION OF LEAVES UNDER TWO METHODS OF PRESERVATION

INTRODUCTION

Portable radiometers which are used to measure reflectance from vegetation in the field employ the sun as a light source. Measurement of this reflected solar radiation even in topographically simple areas is complicated by solar zenith angle, haze and cloud cover. The researcher should try to avoid confounding variation in reflectance associated with these environmental factors within variation related to the phenomena under study. From an experimental perspective this can be achieved either by randomizing the conditions under which measurements are made or by restricting ourselves to one set of conditions, i.e., "keeping other things constant." While the first approach, if successfully applied, leads to more general results, it is an untenable methodology in field experiments as the weather is unlikely to change in a fashion to meet other constraints (particularly time contrasts) of the experiment, and the resulting error variance may be too large to permit us to discern other patterns in the data. We alternatively may restrict our measurements to periods of high zenith angle and relatively little cloud cover. This too can be a serious problem in remotely located field experiments where observations must be made at regular intervals during the growing season. However, on occasions when the conditions prove unsuitable, vegetation may be transported to another location, stored for a period of time and measured when weather conditions are satisfactory. It must be stressed that removing vegetation from the field has the implicit assumption that its removal and storage will not significantly change the spectral properties of the vegetation.

The objectives then of this study were two-fold: 1) to determine how long leaf reflectance remained unchanged after leaves are collected; and 2) to assess the effects of leaf storage methods on leaf reflectance. In this experiment two storage methods were evaluated: 1) storing the leaves still attached to branches which were placed in distilled water; and 2) refrigerating leaves in paper bags.

The present study was designed to provide information to be used in establishing vegetation collection procedures for a project studying the reflectance of white oaks (*Quercus alba*) growing on soils in a mineralized region (metal sulfides). Of primary importance to this research is the sensitivity with which we may detect stress in vegetation.

While the samples used in this study were drawn from five white oak trees growing along the edge of a wooded lot in Greenbelt, Maryland, the results of this study are considered applicable to other hardwood species growing in temperate climates and will provide us with:

- (1) the lower limit on the sensitivity of the experimental procedure to measure stress;
- (2) an estimate of the time period for which one can preserve vegetation, specifically leaves, and still relate spectral readings from the vegetation to readings made in the field;
- (3) a comparison of two simple methods of vegetation preservation.

METHODOLOGY

Daily reflectance measurements of vegetation were made in three spectral bands to determine what relationship, if any, existed between spectral reflectance and the method of preservation or the length of storage time. Reflectance measurements were noted for vegetation preserved using two methods: 1) refrigerating the leaves in paper bags and 2) placing branches with leaves intact in distilled water. The reflectance values of the preserved leaves were compared to freshly clipped vegetation for a period of eight days. Reflectance measurements were made using a three band hand-held radiometer (Tucker et. al., 1981).

Hand-Held Radiometer

The radiometer used possesses two silicon detectors which have a sensitivity range of approximately 0.4 to 1.1 μm and one lead sulfide detector with a sensitivity range of approximately 1.1 to 3.0 μm . The spectral range of the device was determined by mounting interference filters in front of the detectors. The radiometer was configured to mimic three bands of the Thematic Mapper (TM) sensor which will fly on Landsat in 1982 -- TM3 (0.63-0.69 μm), TM4 (0.76-0.90 μm)

and TM5 (1.55-1.75 μm). The transmission curves for the filters used in this research are given in Figure 1. These bands are used because of their relation to the chlorophyll content, mesophyll structure and H_2O content, respectively, of vegetation (Tucker, 1978).

Modes of Preservation

The population of leaves used in the experiment was restricted to those from white oaks, the same species used in our geobotanical study. For reasons of logistics, trees within the confines of the Goddard Space Flight Center were used. All the cuttings (samples) were second year or older twigs from five randomly selected trees. The samples were selected randomly with respect to orientation (azimuth) and height in the canopy. In all, 48 samples were taken, each containing approximately 25 to 30 leaves. The samples were randomly divided into two groups:

(1) Leaves from twenty-four of the samples were removed from their branches and placed in an orderly manner in paper bags, which were then sealed with tape and carefully (to avoid damaging the leaves) stored in a refrigerator.

(2) Leaves were left on the branches of the other twenty-four samples. To enhance water absorption, approximately 3 to 5 inches of bark was stripped off of the bottom part of each branch. The branches were then set in clean bottles filled with distilled water. Distilled water had to be added to each bottle daily to replace water lost due to evapotranspiration.

In addition to the bagged and bottled samples, fresh samples, cut daily only minutes before reflectivity readings were to be made on them, acted as a control on the other two modes of preservation.

Procedures of Daily Measurements

Daily reflectivity measurements proceeded in the following manner. Readings were taken only under clear weather conditions in direct sunlight. To reduce differences related to sun angle, all spectral measurements were taken between 11 AM and 3:30 PM Eastern Daylight Time (10 AM-2:30 PM Solar Time). All the measurements were taken in the principal plane with the sensor

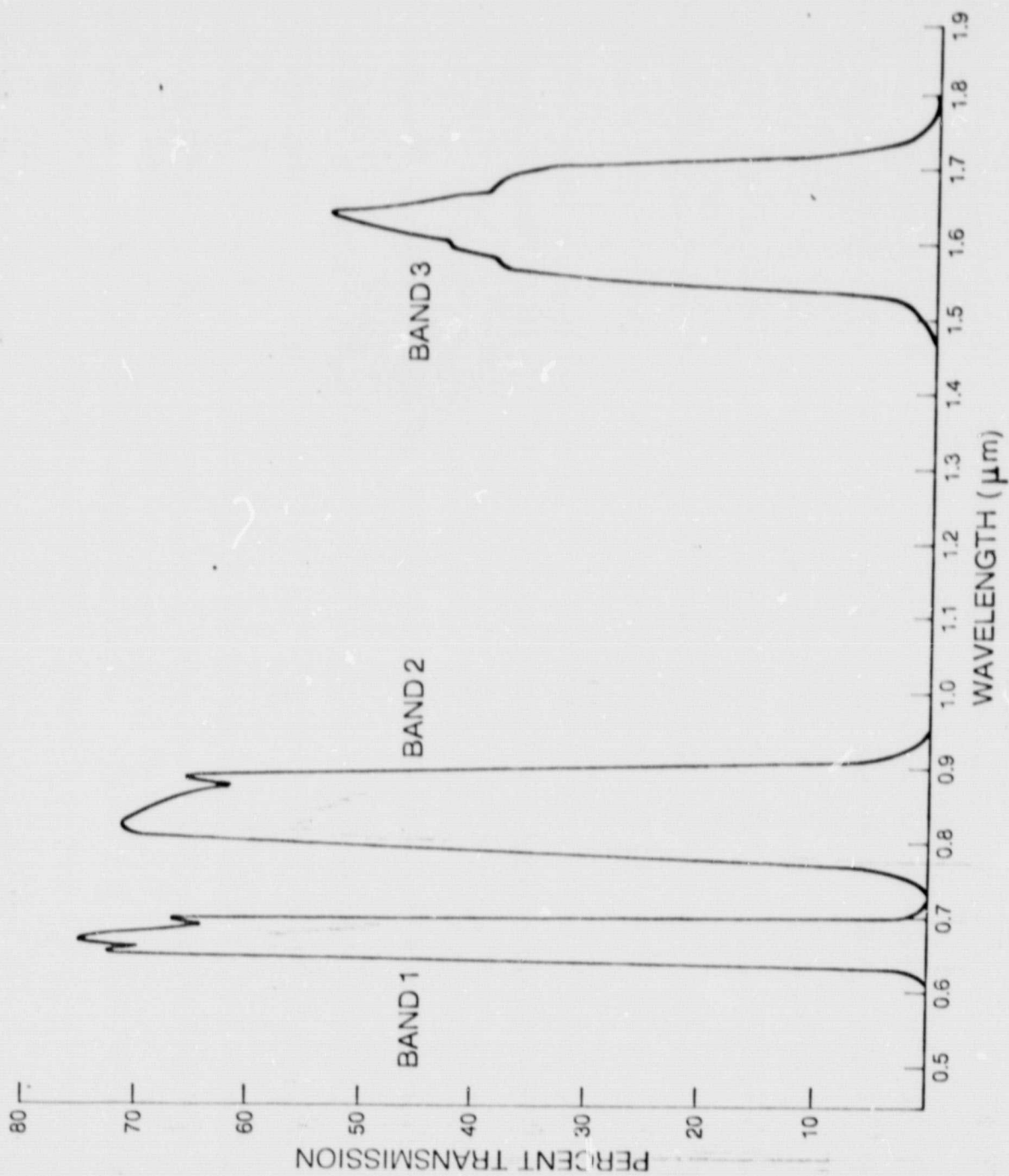


Figure 1. Transmission curves of three interference filters used in this study (from Tucker, et. al, 1981).

positioned approximately 10° off nadir to prevent casting shadows across the sample. Every day two bottled and two bagged samples were selected at random for measurement. The reflectivity measurements were made in a clearing near the trees which had provided all of the samples, in order to insure fresh cuttings for the control readings. Sample measurements were randomized with respect to the mode of preservation (bagged, bottled, or fresh) to control for potential biases accompanying the start up of daily experimental procedure and the time of day measurements were taken.

The leaves were separated (those in the bottles were removed from their branches) and placed, adaxial surface up, in a monolayer on a 1 foot square, 1/8" thick aluminum plate, so that they covered the entire plate. The plate's surface was painted with a potassium-silica based flat black paint possessing a very low reflectance over the spectral region which includes the bands examined here. A second plate surfaced with barium sulfate was used as a standard (J. Schutt, personal communication, 1980).

Once the sample was prepared on the black plate, a photograph was taken showing the vegetation annotated with the date, type of preservation, and the replicate number of the sample. A dark reading, achieved by covering the radiometer's sensor, was also taken so that drift could be noted and the lead sulfide detector zeroed. The measurements were made at a height of approximately 20 centimeters (8 inches) above the plate. Since the field of view of the sensor was 12.5 degrees, energy from an area of approximately 15.5 square centimeters was measured. Readings were taken alternately on the leaves and the barium sulfate panel. A pair of readings took approximately five to ten seconds and several pairs of readings were made in an attempt to minimize instrument variation. The procedure was repeated for all 6 samples (2 of each mode of preservation).

The actual length of the experiment was 9 days, from September 3 to September 11, 1980. The samples to be bottled and bagged were all cut on the 3rd, and the reflectivity measurements

were taken from the 4th to the 11th, inclusively. No readings were taken on the 5th of September due to a heavy cloud cover.

RESULTS

Experimental Design

The procedure described in the previous section was set up so that the specific questions outlined in the Introduction could be answered. To answer these questions we set up a statistical analogue of the experimental situation. The experimental design is that analogue and its algebraic operations are performed using a procedure known as analysis of variance (ANOVA) – Fisher, 1970. Within the ANOVA procedure the variation in a measurement is partitioned among sources of variation, these are equivalent to the subjects of the questions (e.g. date since clipping of vegetation). The question itself is constructed as two hypotheses (the null and alternative hypotheses) which are structured to include all possible answers to the question. A decision as to which hypothesis is more likely correct (which in turn can be translated into an answer to the original question) is based upon whether or not the source of variation “explains” significant amounts of the total variation. In this section we formally describe the experimental design.

In this experiment we have explicitly taken account of two factors (also known as main effects) – day after clipping (date) and type of preservation (type). Since the experiment was performed on seven days, we say that the date factor has seven levels. Similarly, the type factor has three levels – fresh (fr), bagged (bg) and bottled (bt). For each combination of date and type level, there are two leaf samples or replications (reps). Measurements of the reflected energy in the bands TM3, TM4 and TM5 were made on each rep. These measurements are the dependent variables for this experiment. Because each level of the date factor occurs in the experiment in combination with each level of the type factor, the design is known as a factorial design. The construction of a factorial design permits us to examine the interaction between the main effects. The meaning of the interaction effect will be discussed below within the context of specific data analyses.

The problem is then to examine variation in reflectance measurements and assign it to the date factor, the type factor, the interaction between date and type or some collection of non-specified effects (the error). The structural form of the relationship between reflectance and the sources of variation is given by

$$Y_{ijk}^c = \mu^c + a_i^c + \beta_j^c + a\beta_{ij}^c + \epsilon_{ijk}^c \quad (1)$$

where:

Y_{ijk}^c is the percent or proportion of reflectance in band c ($c = 1, 2, 3$) for the k^{th} rep ($k = 1, 2$) subjected to the i^{th} type of preservation ($i = 1, 2, 3$) and measured on the j^{th} day ($j = 1, 2, \dots, 7$);

μ^c is the overall mean reflectance from band c ;

a_i^c are constants subject to the restriction $\sum_{i=1}^3 a_i^c = 0$ and represent the variation contributed by the i^{th} method of preservation to the reflectance in band c ;

β_j^c are independently and normally distributed random variables with mean equal to zero and variance equal to $\sigma_{\beta(c)}^2$ [NID $(0, \sigma_{\beta(c)}^2)$], and represent the variation contributed by the j^{th} date to the reflectance in band c ;

$a\beta_{ij}^c$ are NID $(0, (2/3) \sigma_{a\beta(c)}^2)$ random variables subject to the restriction $\sum_i a\beta_{ij}^c = 0$ for all j , and represents the variation contributed by the interaction of the i^{th} type of preservation and the j^{th} date to the reflectance in band c ;

ϵ_{ijk}^c are NID $(0, \sigma_c^2)$ random variables representing all other unspecified sources of variation, commonly called the error;

β_j , $a\beta_{ij}$, and ϵ_{ijk} are independent.

If we take the expectation over reps for each treatment and subtract the overall mean (μ), equation (1) becomes

$$E[Y_{ijk}^c - \mu^c] = E[\tilde{Y}_{ijk}] = a_i^c \quad (2)$$

and the variance of Y_{ijk} is given by

$$\text{Var}(Y_{ijk}) = \text{Var}(\tilde{Y}_{ijk}) = \sigma_{\beta(c)}^2 + (2/3) \sigma_{a\beta(c)}^2 + \sigma_c^2 \quad (3)$$

The problem is now clearly to determine which of the terms on the right hand sides of equations (2) and (3) are likely to be non-zero. This determination is accomplished by partitioning the total variation among the above terms and comparing sum of squares.

In summary:

- (1) the experimental design is a two-factor factorial design;
- (2) the date factor has seven levels and is a random effect;
- (3) the type factor has three levels and is a fixed effect;
- (4) the presence of a non-zero term among the a_i^c 's will imply that not all preservation types have the same effect on reflectance;
- (5) the presence of a non-zero variance among the β_j^c 's will imply that the reflectance changes with date;
- (6) the presence of a non-zero variance among the $a\beta_{ij}^c$'s will imply that changes in reflectance over date are not the same for each preservation type.

Data Analysis

Table 1 gives the results for the two factor-mixed effects-factorial design described in the previous section. Each sub-table is an analysis of variance for one of the three TM Bands. The analyses were accomplished using the BMDP3V program (Jennrich and Sampson, 1979). Computation for these and all other analyses in this paper were performed on an IBM 370/3033 computer located at the Pennsylvania State University. In each sub-table, the first three sources of variation correspond to a_i , β_j and $a\beta_{ij}$ respectively. The important points to note are:

Table 1

Analysis of variance (ANOVA) table for two factor-mixed effects-factorial design, all treatments included.

TM3 (0.63 - 0.69 μ m)

SOURCE	SUM OF SQUARES ($\times 10^{-3}$)	DEGREES OF FREEDOM	MEAN SQUARE ($\times 10^{-3}$)	F*	P (F > F*)
DATE	3.50	6	0.58	8.87	0.000
TYPE OF PRESERVATION	3.24	2	1.62	8.53	0.005
DATE X TYPE	2.33	12	0.19	2.95	0.014
ERROR	1.38	21	0.07		

TM4 (0.76 - 0.90 μ m)

SOURCE	SUM OF SQUARES ($\times 10^{-3}$)	DEGREES OF FREEDOM	MEAN SQUARE ($\times 10^{-3}$)	F*	P (F > F*)
DATE	11.90	6	1.98	2.85	0.034
TYPE OF PRESERVATION	6.11	2	3.05	2.28	0.145
DATE X TYPE	16.14	12	1.34	1.93	0.090
ERROR	14.60	21	0.70		

TM5 (1.55 - 1.75 μ m)

SOURCE	SUM OF SQUARES ($\times 10^{-3}$)	DEGREES OF FREEDOM	MEAN SQUARE ($\times 10^{-3}$)	F*	P (F > F*)
DATE	15.85	6	2.64	6.39	0.001
TYPE OF PRESERVATION	29.18	2	14.59	11.14	0.002
DATE X TYPE	15.68	12	1.31	3.16	0.010
ERROR	8.69	21	0.41		

- (1) All three sources of variation are significant at any conventional α level for TM3 and TM5.
- (2) There is no evidence to indicate that the type of preservation or interactions are significant contributors to reflectance in TM4. Conclusions about the date effect are less certain, and since we are using an $\alpha = 0.02$ level of significance for individual tests in Table 1 we conclude that date is not significant.

If we examine the plots of the treatment means over date for each type of preservation (see Figures 2 to 4), we can develop tentative hypotheses for the results of Table 1. In the figures associated with TM3 and TM5, it appeared that the type of preservation was not an important factor for the first few days after clipping. However, as the time since clipping got longer, the differences in the reflectance between fresh versus bagged and bottled samples increase. This is the pattern of variation being captured in the type and date-type interaction terms of the analyses of variance. TM4 (Figure 3) is considerably more difficult to interpret. Clearly the date effect is significant and the figure shows that there is an increase in reflectance to the middle of the experiment with the reflectances of bottle and bagged samples decreasing towards the end of experimental period. This trend would likely have yielded a significant interaction had the experiment lasted longer. This pattern will be dealt with further in the Discussion section.

Patterns of variation were examined further by looking at subsets of the data. We first wished to rule out that the variation noted was due to environmental effects such as changes in atmospheric conditions or systematic changes in the measurement procedure over the experimental period. Since the fresh samples were our standard for comparison and were not subjected to a period of deterioration prior to measurement, a significant date effect among these samples would be attributed to external effects. Table 2 demonstrates that there was no significant change in the reflectance of fresh samples over date for any of the spectral bands. Next, to examine the conjecture that there was no difference in the reflectance over the three preservation types for a finite but undetermined period of time, we examined data for the first three experiment days

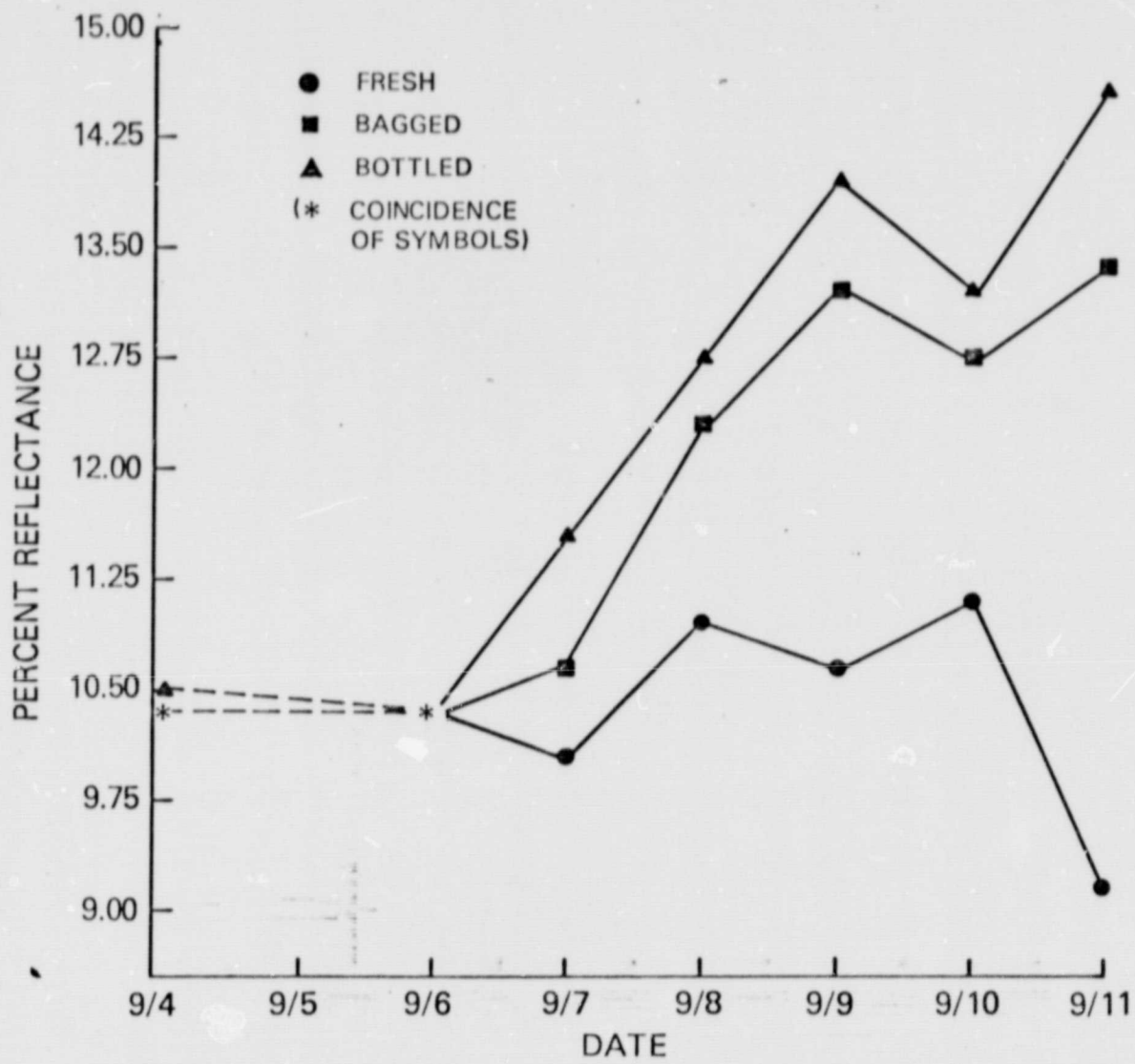


Figure 2. Means of replicated reflectance measurements from TM3 over time by preservation type.

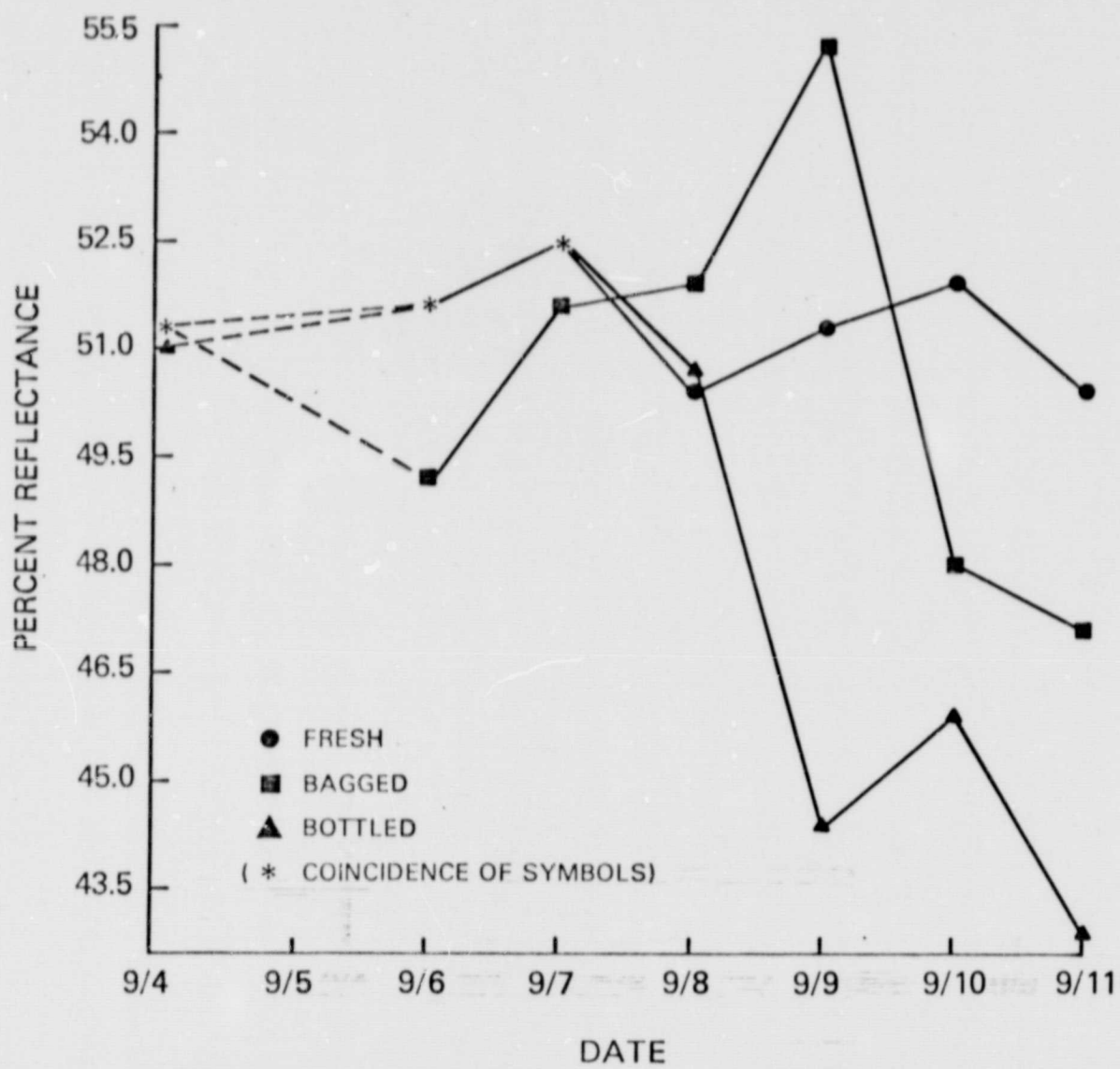


Figure 3. Means of replicated reflectance measurements from TM4 over time by preservation type.

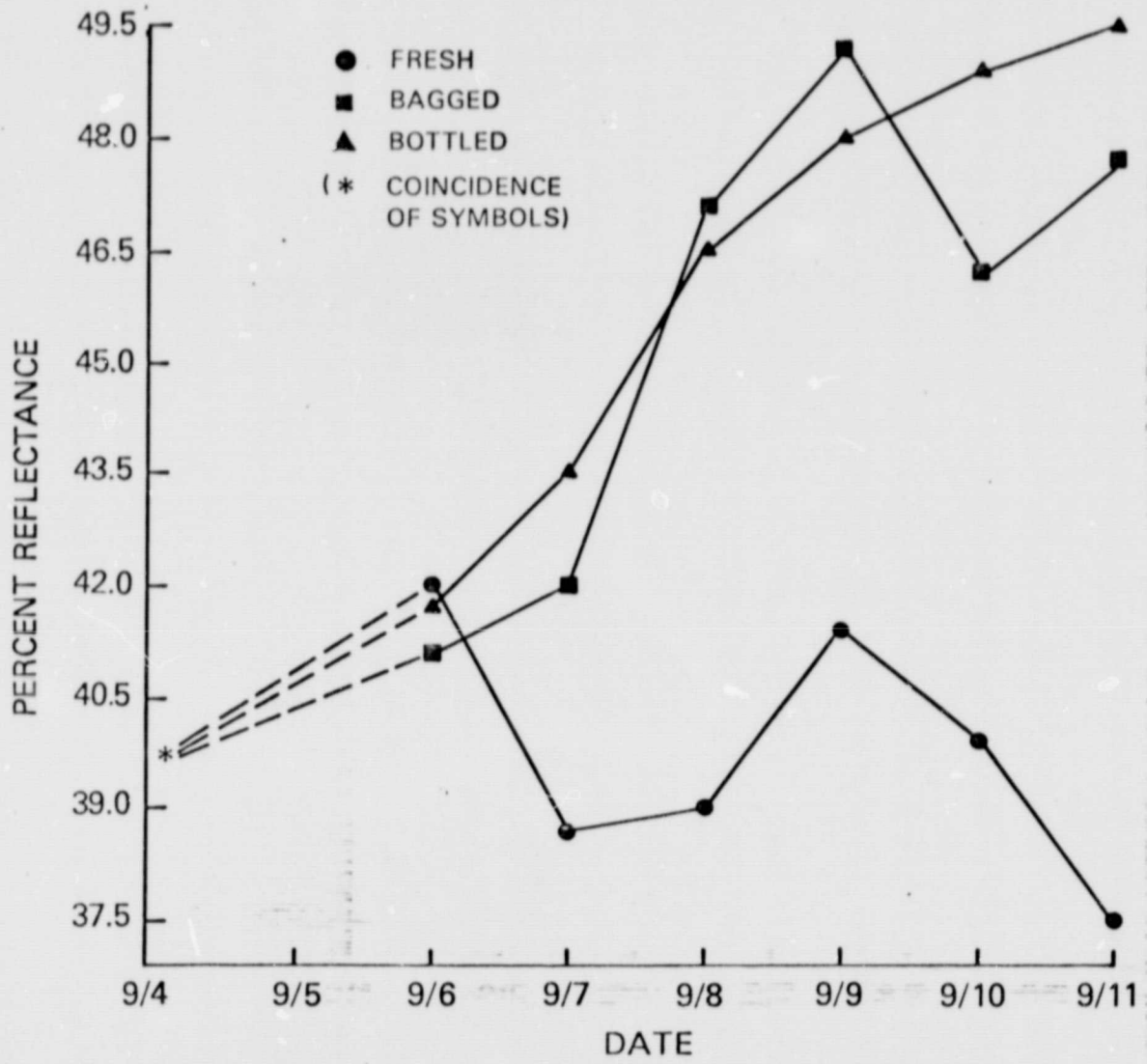


Figure 4. Means of replicated reflectance measurements from TMS over time by preservation type.

Table 2
 One-way ANOVA testing variation in reflectance from fresh vegetation over time.

TM3

SOURCE	DF	SUM OF SQ ($\times 10^{-3}$)	MEAN SQ ($\times 10^{-3}$)	F*	P(F > F*)
DATE	6	0.5	0.1	2.19	0.164
ERROR	7	0.3	0.0		

TM4

SOURCE	DF	SUM OF SQ ($\times 10^{-3}$)	MEAN SQ ($\times 10^{-3}$)	F*	P(F > F*)
DATE	6	0.8	0.1	0.538	0.766
ERROR	7	1.7	0.2		

TM5

SOURCE	DF	SUM OF SQ ($\times 10^{-3}$)	MEAN SQ ($\times 10^{-3}$)	F*	P(F > F*)
DATE	6	3.0	0.5	2.002	0.1924
ERROR	7	1.8	0.3		

Table 3
ANOVA table for two factor-mixed effects-factorial design, first three experiment days.

TM 3					
SOURCE	DF	SUM OF SQ (x 10 ⁻³)	MEAN SQ (x 10 ⁻³)	F*	P(F > F*)
DATE	2	0.05	0.02	0.83	0.469
TYPE OF PRESERVATION	2	0.11	0.06	1.50	0.326
DATE X TYPE	4	0.15	0.04	1.32	0.334
ERROR	9	0.25	0.03		

TM 4					
SOURCE	DF	SUM OF SQ (x 10 ⁻³)	MEAN SQ (x 10 ⁻³)	F*	P(F > F*)
DATE	2	0.64	0.32	1.27	0.326
TYPE OF PRESERVATION	2	0.45	0.22	1.83	0.273
DATE X TYPE	4	0.47	0.12	0.47	0.758
ERROR	9	2.26	0.25		

TM 5					
SOURCE	DF	SUM OF SQ (x 10 ⁻³)	MEAN SQ (x 10 ⁻³)	F*	P(F > F*)
DATE	2	0.15	0.07	0.31	0.739
TYPE OF PRESERVATION	2	1.36	0.68	0.92	0.469
DATE X TYPE	4	2.95	0.74	3.11	0.073
ERROR	9	2.14	0.24		

Table 4
 One-way ANOVA testing variation in reflectance from bagged
 and bottled vegetation over time.

TM3					
SOURCE	DF	SUM OF SQ ($\times 10^{-3}$)	MEAN SQ ($\times 10^{-3}$)	F*	P(F > F*)
DATE	6	5.2	0.9	12.86	0.000
ERROR	21	1.4	0.1		

TM4					
SOURCE	DF	SUM OF SQ ($\times 10^{-3}$)	MEAN SQ ($\times 10^{-3}$)	F*	P(F > F*)
DATE	6	16.0	2.7	2.035	0.105
ERROR	21	27.4	1.3		

TM5					
SOURCE	DF	SUM OF SQ ($\times 10^{-3}$)	MEAN SQ ($\times 10^{-3}$)	F*	P(F > F*)
DATE	6	25.6	4.3	9.094	0.000
ERROR	21	9.8	0.5		

(1, 3, and 4 days after clipping). The results are given in Table 3 and we noted that there were no significant effects in any of the three spectral bands. However, when we examined variation over the entire experimental period solely in the bagged and bottled samples, (Table 4) we saw that the variation in TM3 and TM5 was significantly different for at least one of the experiment days.

The previous three results lead us to the logical conclusion that the significant variation found in the date effect is due to the changes in the reflectance measurements of the bagged and bottled samples from the early to late portion of the experiment. It is clear from the previous results that the preserved sample reflectance does not differ significantly from fresh cutting responses in the early stages. The point in time of the change can be determined by setting up a series of orthogonal difference contrasts (Dayton, 1970). These contrasts are based upon the combined means of the bagged and bottled samples over date. The coefficients associated with the difference contrasts are given in Table 5. Note that there are six contrasts which correspond to the six degrees of freedom associated with the date factor. The logic behind these contrasts is that we step through the experiment days, starting with days 1 and 3, comparing the mean of all the previous days with the current one. For example, we first compared the combined mean of bagged and bottled samples for day 1 versus those of day 3, then we combined the means of days 1 and 3 and compared this new mean to the mean for day 4, and proceed in this fashion until we were comparing the combined means of day 1 through 7 against day 8. The coefficients given in Table 5 are the weights by which we multiplied the means for any given contrast. Use of the contrasts allows us to orthogonally decompose the sums of squares, which upon suitable manipulation can be transformed into a t statistic with 21 degrees of freedom for each contrast. The results of these analyses are given in Figures 5 to 7. Since we were performing 6 sequence tests for each band, we have used a Bonferroni adjustment (Fisher, 1970) to adjust the family confidence limit (for each band) to 0.05. This means that the α level for each t test is set at $0.05/6 = 0.0083$, which is achieved by using $t(\nu = 21, \alpha = 0.0083) \cong 2.84$.

A dashed line corresponding to this value is present in each figure. We noted that for TM3 and TM5 the first significant t value occurs at contrast three giving a change point between the fourth and fifth day. Further, there was no significant contrast for TM4 (Figure 6); this was in agreement with the results of Tables 2 and 4.

Table 5
Orthogonal Difference Contrast Coefficients used in Determining First Change Date.

Contrast	Coefficients						
	$\bar{Y}_{B1.}$	$\bar{Y}_{B3.}$	$\bar{Y}_{B4.}$	$\bar{Y}_{B5.}$	$\bar{Y}_{B6.}$	$\bar{Y}_{B7.}$	$\bar{Y}_{B8.}$
1	1	-1	0	0	0	0	0
2	1	1	-2	0	0	0	0
3	1	1	1	-3	0	0	0
4	1	1	1	1	-4	0	0
5	1	1	1	1	1	-5	0
6	1	1	1	1	1	1	-6

Discussion

In this experiment we have reported that no significant change occurred in the reflectance in any of the three spectral bands for clipped leaves with either method of preservation for at least four days after clipping. Beyond four days, reflectance in bands 1 and 3 from bt and bg leaves increased to the end of the experiment. No statistically significant changes occurred in band 2 reflectance from the three treatments over the experimental period. However, examination of the band 2 plot, Figure 3, hints at an initial increase in reflectance, followed by a decline in the reflectance values for the bt and bg samples. Further, the results suggest that leaves placed in paper bags have reflectances closer to those of freshly clipped leaves than leaves left on the branches and placed in distilled water. However, the differences in reflectance were not statistically significant. These results are similar to the conclusions of Keegan et al., 1955a, 1955b. In these reports, personnel at the National Bureau of Standards studied reflectance changes of white oaks under natural drying conditions or stored in metal containers.

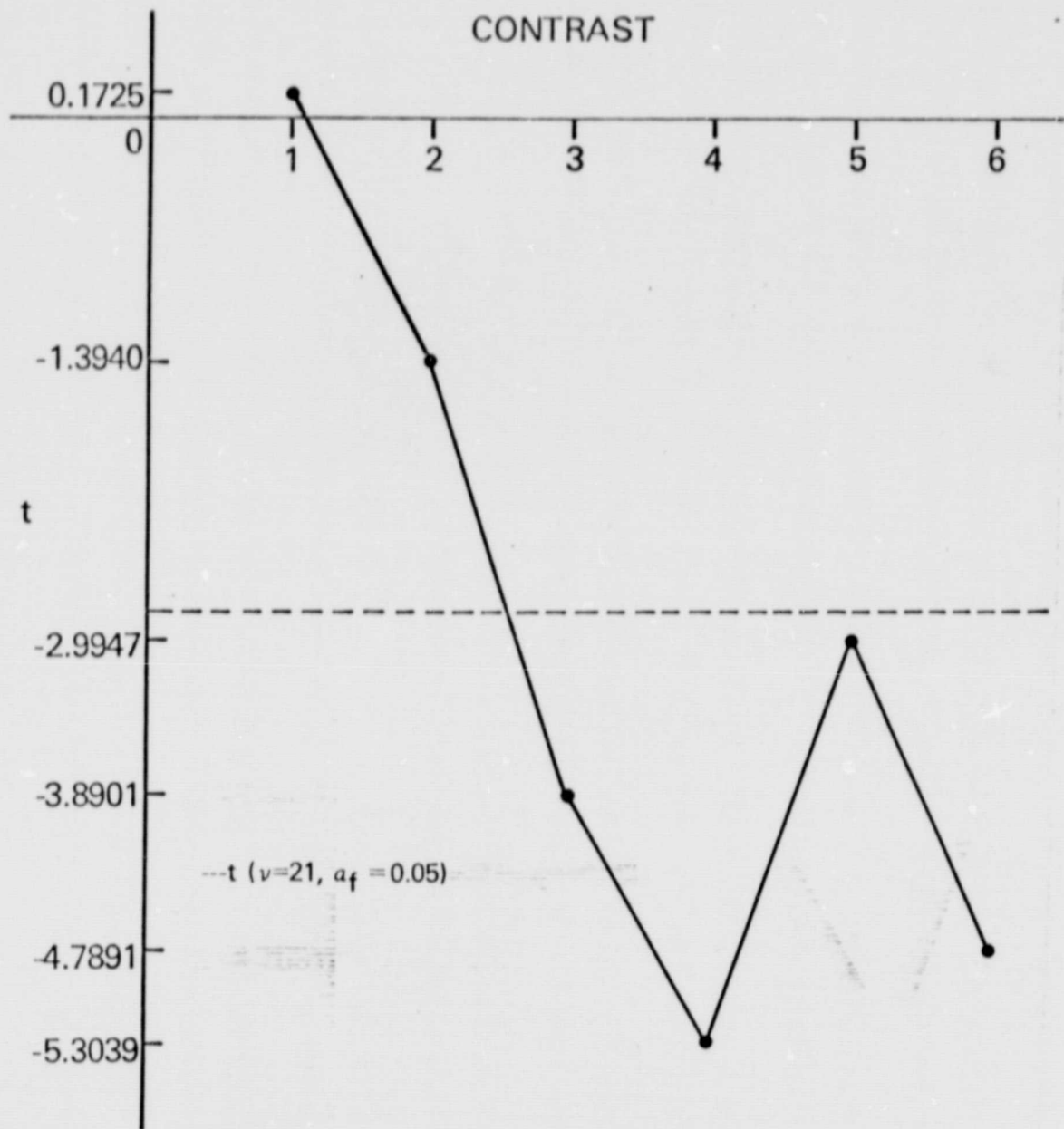


Figure 5. Values of t statistics for the orthogonal difference contrasts from combined means of bagged and bottled samples for TM3.

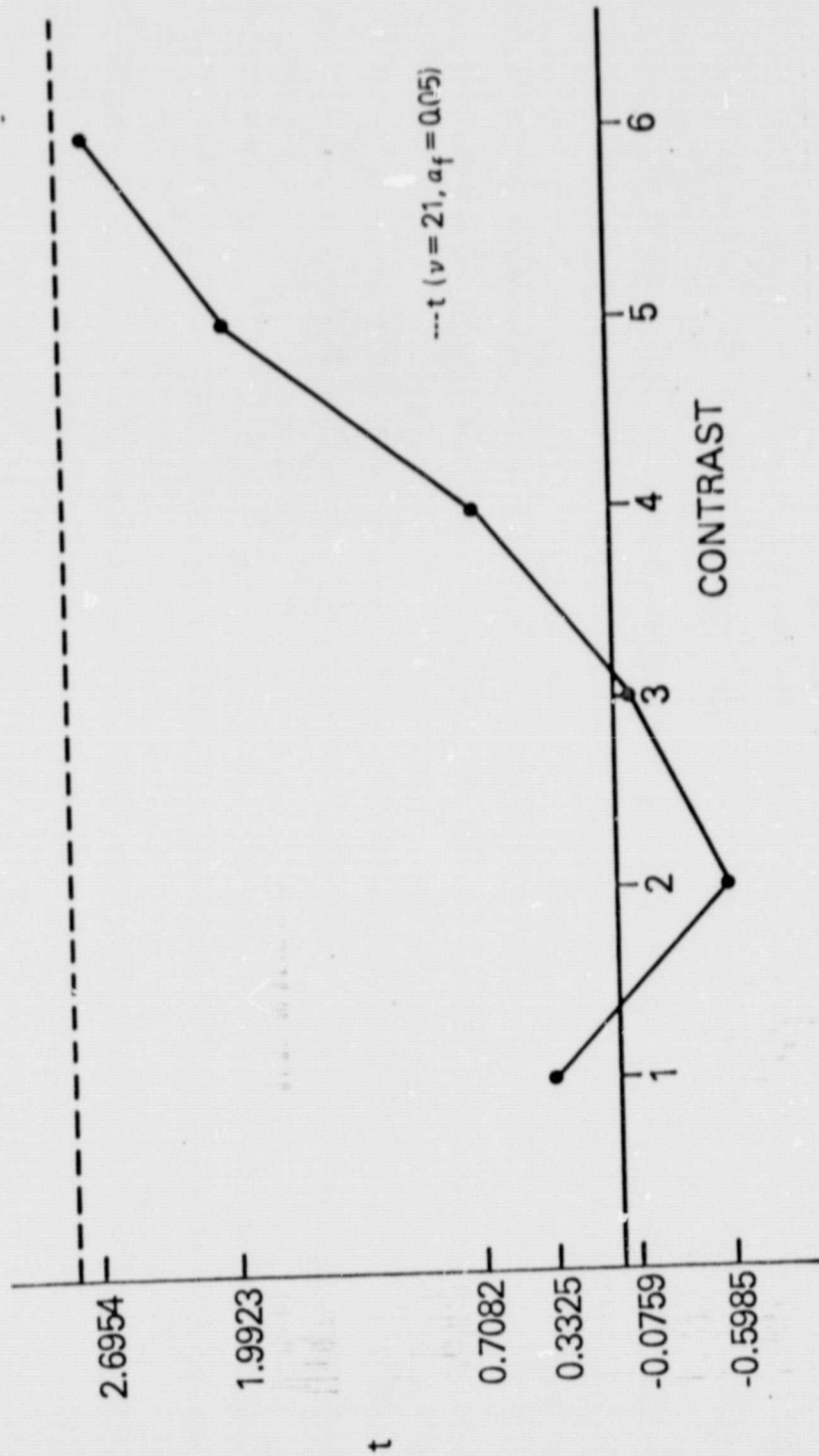


Figure 6. Values of t statistics for the orthogonal difference contrasts from combined means of bagged and bottled samples for TM4.

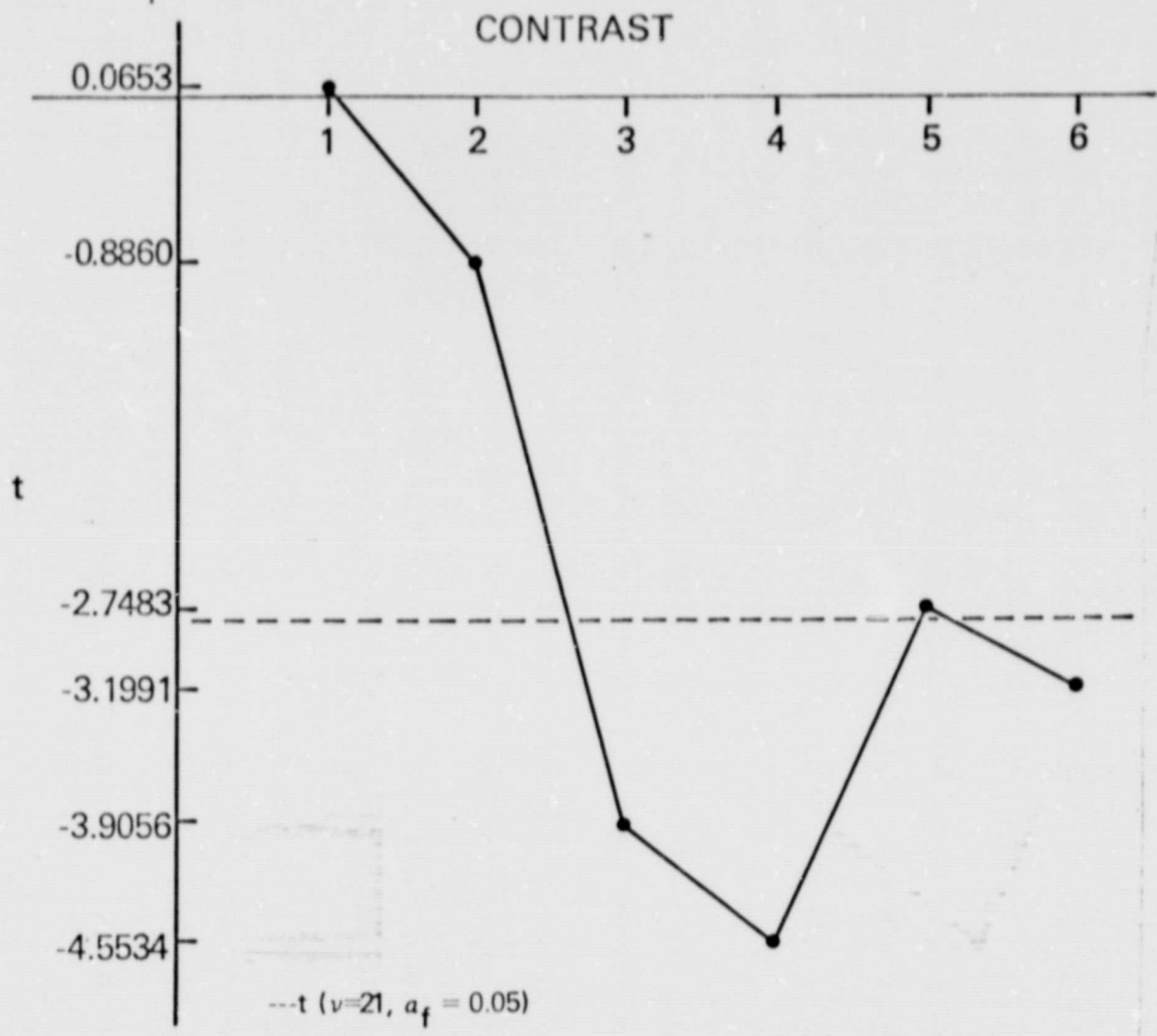


Figure 7. Values of t statistics for the orthogonal difference contrasts from combined means of bagged and bottled samples for TMS.

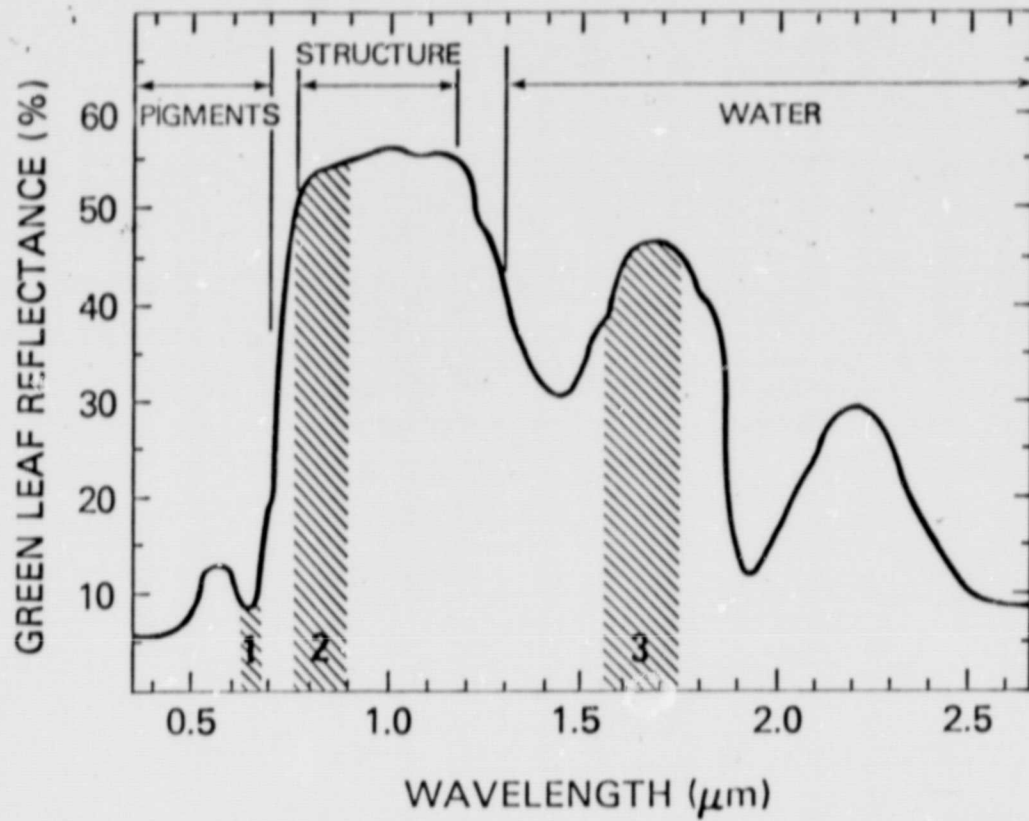


Figure 8. Spectral reflectance of green leaf vegetation over 0.35-2.50 μm (modified after Tucker, 1978) showing bands used and dominating phenomena.

We may interpret these results using Figure 8 modified from Tucker (1978) illustrating the location of the three hand held radiometer bands and the phenomena which dominate variation in regions of the green leaf reflectance spectrum. The first band TM3, (0.63 to 0.69 μm) is centered on a chlorophyll absorption maximum. From the onset of leaf senescence, about day 5, to the end of the experiment the reflectance of clipped samples increased dramatically in TM3. An increase in reflectance in the visible region is well documented for diseased, stressed and senescing leaves and has been attributed to a breakdown of the chlorophyll pigments in a leaf (Gates et al., 1965; Knipling, 1970; Rabideau et al., 1946). The second band, TM4, (0.76-0.90 μm) falls within a region of the spectrum in which leaf structure governs changes in the infrared reflectance (Gates et al., 1965; Knipling, 1969; Gausman et al., 1970; Sinclair, 1971). Infrared reflectance in the leaf is due to scattering in the mesophyll layers. In the palisade and spongy mesophyll the cell walls act both as reflecting surfaces and boundaries where refraction occurs as the light passes from air filled cavities into the hydrated cellulose of the cell walls. During the early stage of senescence an increase in reflectance may occur as adjacent cell walls are torn apart creating more reflecting surfaces (Knipling, 1967). In late stages of senescence, reflectance decreases as cell walls disintegrate (Knipling, 1969). This would suggest a pattern of reflectance similar to the one we witnessed, i.e. a slight increase in reflectance prior to the decrease in reflectance over time. Band 3, TM5, of the hand held radiometer (1.55-1.75 μm) measures infrared reflectance between two strong water absorption maxima at 1.45 μm and 1.95 μm . Above about 1.3 μm leaf senescence is manifested by an increase in reflectance which is due to the loss of water by the leaf (Myers and Allen, 1968; Thomas et al., 1971). This too conforms to the pattern of variation we see in Figure 4.

In summary, then, referring back to the questions set out in the Introduction:

- (1) The measurement procedure used is sensitive enough to discern documented patterns of variation in reflectance measurements which have been associated with changes in the leaf pigments (particularly chlorophyll) and the water content of drying (stressed) or senescing vegetation.

- (2) We may use reflectance measurements from clipped vegetation for up to four days and be able to directly relate the results to freshly picked vegetation.
- (3) Neither of the two methods of preservation was statistically superior. However, the bagged samples were easier to handle and appeared to yield reflectances which remained closer to the fresh samples.

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Figure Captions

Figure 1. Spectral sensitivity of three interference filters used in this study.

Figure 2. Means of replicated reflectance measurements from TM3 over time by preservation type.

Figure 3. Means of replicated reflectance measurements from TM4 over time by preservation type.

Figure 4. Means of replicated reflectance measurements from TM5 over time by preservation type.

Figure 5. Values of t statistics for the orthogonal difference contrasts from combined means of bagged and bottled samples for TM3.

Figure 6. Values of t statistics for the orthogonal difference contrasts from combined means of bagged and bottled samples for TM4.

Figure 7. Values of t statistics for the orthogonal difference contrasts from combined means of bagged and bottled samples for TM5.

Figure 8. Spectral reflectance of green leaf vegetation over 0.35-2.50 μm (modified after Tucker, 1978) showing bands used and dominating phenomena.