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Changes in Vegetation Spectra with Leaf Deterioration under Two Methods of Preservation

Spectral readings over three treatments—fresh, bottled, and bagged vegetation—were indistinguishable in bands TM3 and TM5 for up to four days after collection.

Introduction

Portable radiometers, which are used to measure reflectance from vegetation in the field, use 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 (Duggin, 1980; Richardson, 1981). The researcher should try to avoid confounding variation in reflectance associated with these environmental factors with variation related to the phenomena under study. From an experimental perspective, this can be achieved 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 constraints) of the experiments, 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

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 as detected by TM3 and TM5, respectively. 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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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 collected 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 temperature climates and will provide us with:

- the lower limit on the sensitivity of the experimental procedure to measure stress;
- 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; and
- a comparison of two single 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. To insure that leaves of approximately the same age were collected, sampling was restricted to the upper canopies of white oak trees growing in the same stand. 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 with 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 flew on Landsat in 1982—TM3 (0.63 to 0.69 μm), TM4 (0.76 to 0.90 μm), and TM5 (1.55 to 1.75 μm). 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 sample were taken, each containing approximately 25 to 30 leaves. The samples were randomly divided into two groups:

- Leaves from 24 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.
- Leaves were left on the branches of the other 24 samples. To enhance water absorption, about 3 to 5 inches or bark were 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 A.M. and 3:30 P.M. Eastern Daylight Time (10 A.M. to 2:30 P.M. Solar Time). All the measurements were taken in the principal plane, with the sensor positioned about 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 sampled, 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 on 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, ventral surface up, in a monolayer on a 1-foot square (929 cm^2), 1/8″ (3-mm) 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 about 20 cm (8 inches) above the plate. Because the field of view of the sensor was 12.5 degrees, energy from an area of about 15.5 cm^2 was measured. Readings were taken alternately on the leaves and the barium sulfate panel. A pair of readings took about 5 to 10 sec, and several pairs of readings were made in an attempt to minimize instrument variation. The procedure was repeated for all six samples (two of each mode of preservation).

The length of the experiment was nine days, from 3 September to 11 September 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

In order to examine the variation in reflectance measurements as a function of date after clipping

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 (x 10^{-3})	DEGREES OF FREEDOM	MEAN SQUARE (x 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 (x 10^{-3})	DEGREES OF FREEDOM	MEAN SQUARE (x 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 (x 10^{-3})	DEGREES OF FREEDOM	MEAN SQUARE (x 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		

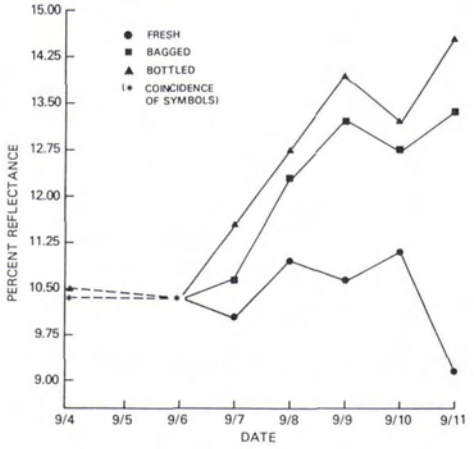

FIG. 1. Means of replicated reflectance measurements from TM3 over time by preservation type.

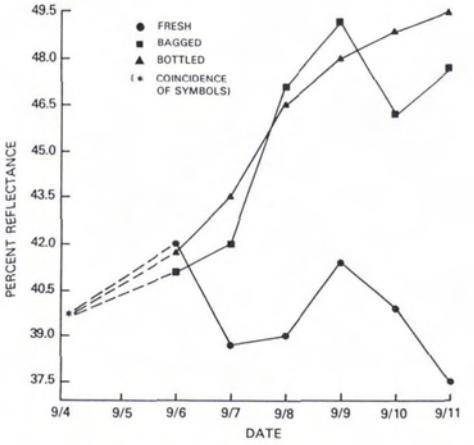

FIG. 3. Means of replicated reflectance measurements from TM5 over time by preservation type.

and type of sample preservation, we used an analysis of variance (ANOVA), (Fisher, 1970). The two main effects in our experiment were day after clipping (date) and type of preservation (type). The date factor had seven levels and the type factor had three levels—fresh (fr), bagged (bg), and bottled (bt). For each combination of date and type, there are two replications (reps). Thus, on any day of the experiment six leaf samples and three levels of type times two repetitions were collected. Measurements of the reflected energy in bands TM3, TM4, and TM5 were made on each sample. 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 nonspecified effects (the error).

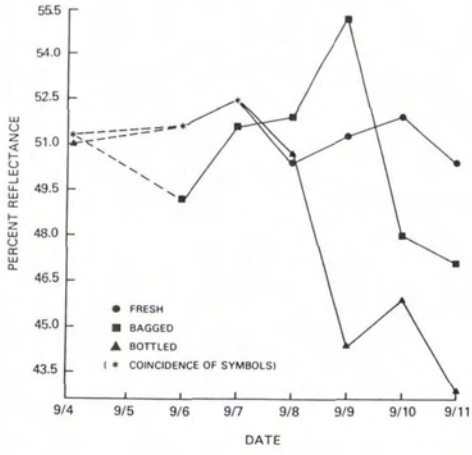

FIG. 2. Means of replicated reflectance measurements from TM4 over time by preservation type.

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 α_i, β_j, and $\alpha\beta_{ij}$ respectively. The important points to note are

- All three sources of variation are significant at any conventional α level for TM3 and TM5, and
- 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 because 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 the date for each type of preservation (Figures 1 to 3), 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 length of time since clipping increased, the differences in the reflectance between fresh versus bagged and bottled samples increased. This is the pattern of variation being captured in the type and date-type interaction terms of the analyses of variance. TM4 (Figure 2) 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

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		

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 measure-

TABLE 3. ANOVA TABLE FOR TWO FACTOR-MIXED EFFECTS-FACTORIAL DESIGN, FIRST THREE EXPERIMENT DAYS.

TM 3

SOURCE	DF	SUM OF SQ ($\times 10^{-3}$)	MEAN SQ ($\times 10^{-3}$)	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 ($\times 10^{-3}$)	MEAN SQ ($\times 10^{-3}$)	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 ($\times 10^{-3}$)	MEAN SQ ($\times 10^{-3}$)	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		

ment procedure over the experimental period. Because 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 (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 proceeded 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 4 to 6. Because we were performing six 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

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		

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

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 5); this was in agreement with the results of Tables 2 and 4.

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 2, 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.

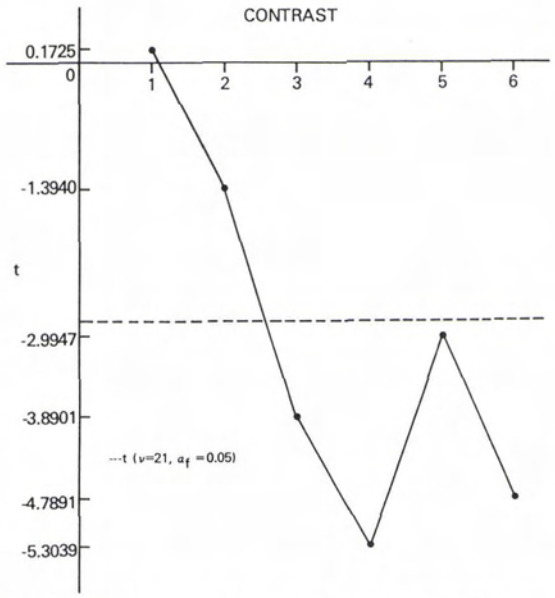

FIG. 4. Values of t statistics for the orthogonal difference contrasts from combined means of bagged and bottled samples for TM3.

We may interpret these results using Figure 7, 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 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

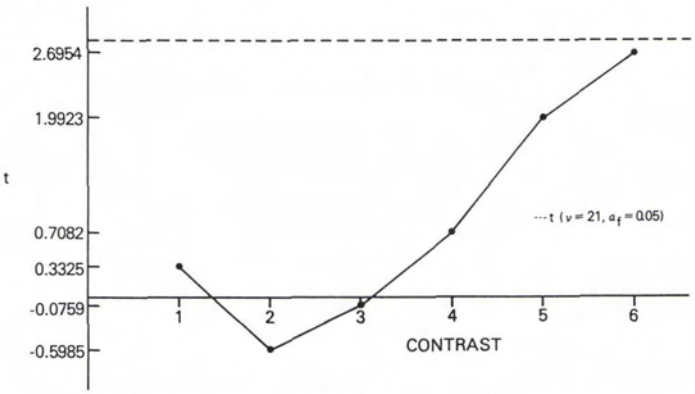

FIG. 5. Values of t statistics for the orthogonal difference contrasts from combined means of bagged and bottled samples for TM4.

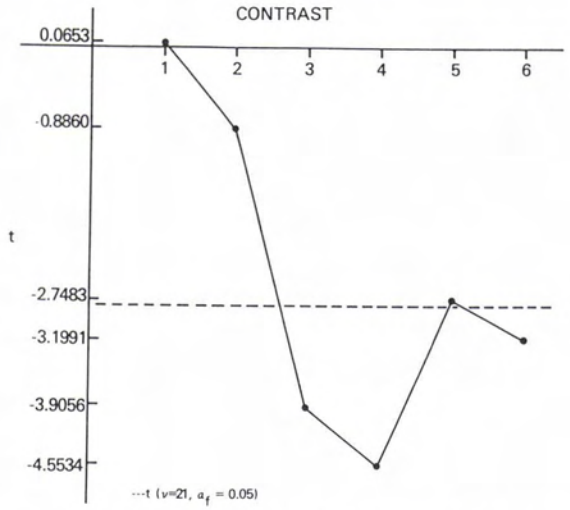

FIG. 6. Values of t statistics for the orthogonal difference contrasts from combined means of bagged and bottled samples for TM5.

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 to 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 between air filled cavities and 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 and cell contents shrink away from cell walls, creating more reflecting surfaces (Knipling, 1967) as cell walls become oriented parallel to the leaf's surface (Sinclair, 1971).

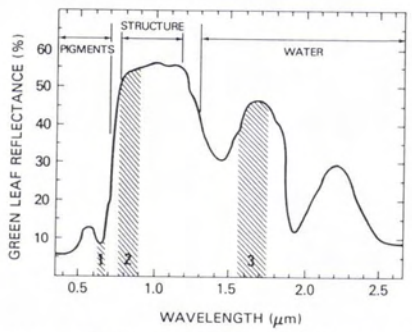

FIG. 7. Spectral reflectance of green leaf vegetation over 0.35 to 2.50 μm (modified after Tucker, 1978) showing bands used and dominating phenomena.

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 to 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:

- 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.
- 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.
- 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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