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Abstract

Effects of an imposed (excised) leaf orientation, differing species and differing venation patterns on reflectance measurements in the Landsat-4 Thematic Mapper (TM) channels TM3 (0.63-0.69 μm), TM4 (0.76-0.90 μm), and TM5 (1.55-1.75 μm) were investigated. Orientation of leaves (random vs. systematic placement) was found to affect measurements in the TM4 channel, but not the TM3 and TM5 measurements. Venation caused no significant changes for any band. Azimuth of incident radiation was not a significant main effect, but in conjunction with changes in orientation, angle did have a significant effect on reflectance values in TM3, TM4 and TM5. Specific differences were highly significant ($P>F\leq 0.006$) in all but one borderline ($P>F\leq 0.0222$) case for TM5. For spectral examination of excised leaves, the sampling arrangement of the leaves should as closely approximate in situ positioning as possible (with respect to remote sensing instrumentation). This dictates a random rather than aligned arrangement.

Introduction

A considerable literature has arisen in geobotanical detection of soil-mineral anomalies over the past decade mainly due, we believe, to a realization of the potential for use of remote sensing technology in delineating such anomalies (Collins et al., 1980; Horler et al., 1980; Labovitz et al., 1983; Lyon, 1975; Yost, 1975). While these references cover a variety of techniques, the present paper focuses on one of the many approaches to the problem of anomaly detection from a remote platform. Labovitz et al. (1983) concentrated on accumulating extensive ground truth for modeling purposes with the ultimate goal being the use of a remote (satellite) platform for anomaly detection. As the available satellite data were to be from the Landsat-4 Thematic Mapper (TM), a hand-held instrument simulating three of the satellite bands (Tucker et al., 1980) was used in their ground work (a red, TM3; near infrared (IR), TM4; and a middle-IR, TM5). TM3 (0.63-0.69μm) and TM5 (1.55-1.75μm), when utilized in these geobotanical anomaly detection studies, proved to be sensitive to metal related changes in leaf reflectance. TM4 (0.76-0.90μm) yielded no significant results (Labovitz et al., 1983). This was unexpected, as TM4 should be sensitive to differences in leaf structure, as a result of changing refraction between wet cellulose walls (IR≃1.50), water (IR=1.33) and air. (Gates, 1970; Knipling, 1969; Woolley, 1971). For this reason it was felt that any morphological changes associated with mineralization would be captured in this 0.76-0.90μm channel. As we saw no variation, it was hypothesized that information contained in the TM4 band was lost or destroyed in the sampling and/or measuring process used.

In our method leaves were excised and placed on a black plate, adaxial surface up, in a monolayer with midveins parallel to each other, but normal to the radiation source. Labovitz, et al. (1983) offer a more detailed description of sampling technique. As most leaves in a temperate deciduous forest are oriented adaxial surface up (although at various angles), it was not felt that the experimental face-up orientation of excised leaves was causing a loss of information per se. However, it is known that variation in the angle of incidence (angle of illumination) causes variation in reflectance (Howard et al., 1971), and that even venation can change the reflectance pattern (Woolley, 1971).

Howard et al. (1971) found differences in reflectance between background and anomalous samples of <u>Pinus ponderosa</u> in the red and near infrared for field measurements. These differences were not found in laboratory measurements. They suggested therefore, that vegetation is primarily influenced by soil mineralization via foliage density and foliage pattern on the tree. The parallel midvein orientation previously utilized was theorized as being abnormal with respect to in situ azimuth orientation. If there were to exist a difference in reflectance due to any particular orientation, then the information monitored by TM4 would be destroyed during sampling.

Also of interest was whether a change in azimuth of the monolayer with respect to the light source would effect a significant change in reflectance values. As the leaves when oriented for sampling were not flat, but were curled up on the edges to various degrees, the question arose whether or not this curling would change reflectance values with varying azimuth. The lower surface of a leaf is more highly reflective in the visible, and less reflective in the near-IR than the adaxial face (Woolley, 1971). This is a readily observed phenomenon on many leaves, where the abaxial face appears a more yellow-green. This study was thus conducted to assess the impact of an imposed measurement procedure upon excised leaf reflectance values.

Materials and Methods

Several aspects of the measurement procedure were examined. Specifically, these were whether or not angular rotation of the leaves would effect changes in the reflectance, and whether such changes could be attributed to external, or internal, morphological differences. The authors also tested whether leaf orientation was important, i.e. did reflectance change with changes in leaf monolayer orientation. Finally, we investigated whether venation patterns affected reflectance patterns in the three measured channels.

Three venation types were used; arcuate (pinnate), palmate and reticulate, represented by six species. The species used were two per venation type, respectively: Carya tomentosa Nutt. (hickory), Nyssa sylvatica Marsh. (blackgum), Liquidambar styraciflua L. (sweet gum), Acer rubrum L. (red maple), Quercus alba L. (white oak), and Q. coccinea M. (scarlet oak) (Figure 1). Species chosen were the dominants found in a reconnaissance survey of two 20m by 20m plots of all woody dicotyledonous vegetation $\geq$6cm diameter breast height at Goddard Space Flight Center in Greenbelt, MD. Samples were collected in August, 1981 in the morning. Leaves were excised, bagged in plastic and refrigerated within thirty minutes of sampling. Measurements were made on the leaves within two days of sampling.

The investigation consisted of two concurrent experiments utilizing the same design and samples. First, each measurement series was made on a subject (plate of leaves) in an unflattened condition, duplicating the technique previously used as described by Labovitz et al. (1983) (Figure 2). Two orientations, random and arranged, were used (Figure 3). Second, a thin glass plate was used to flatten the sample, and the measurement series was repeated. The glass reading allowed the authors to suggest the relative importance of leaf curling (external) and/or leaf internal morphology in testing whether the azimuth angle of the irradiance was effecting significant changes in reflectance (Figure 4). All measurements were made using the NASA/GSFC Portable Illumination Source (PIS) unit in conjunction with a three band hand-held radiometer (Tucker, et al., 1980) (Figure 5). A Halon[1] plate was used as a standard for the first series, and a Halon plate with a superposed glass plate was utilized as a standard for the latter. The data were then transformed using 2*arcsin $(\sqrt{Xi})$, where Xi is the ith reflectance value. This transformation was used as the data were percentages (Dayton, 1970). Results are presented separately for glass covered and non-glass reflectances.

The model used for the experiment was

$$Y_{mlkji} = \mu + \alpha_m + \beta_l + \gamma_k + \delta_{j(k)} + \pi_{i(j(k)kl)} + \beta\delta_{lj(k)} + \alpha\delta_{mj(k)} + \alpha\beta_{ml} + \alpha\gamma_{mk} + \alpha\beta\gamma_{mlk} + \alpha\beta\delta_{mlj(k)} + \beta\gamma_{lk} + \alpha\pi_{mi(j(k)kl)} + \varepsilon_{mlkji}$$

where: Y_{mlkji} = reflectance of ith subject at the mth, lth, kth, and jth levels of angle, orientation, venation and species respectively, and

α_m = mth level of angle (azimuth) m = 1, 4;

β_l = lth level of orientation (arrangement) l = 1, 2;

γ_k = kth level of venation; k = 1, 3

$\delta_{j(k)}$ = jth level of species (nested within venation); j = 1, 6

$\pi_{i(j(k)kl)}$ = ith subject (nested within species, venation and orientation); i = 1, 11

and ε_{mlkji} = random error associated with each measurement.

The model used is a nested 4 x 2 x 3 factorial with repeated measures on the angle level. All effects are random.

Due to the repeated measures along one treatment in the design, a Levene's test for equality of variances was used to test for certain assumptions in the design. The program used to perform this analysis was the P7D program of the BMD system (Dixon, 1981). In a repeated measures design, the homoscedasticity requirement pertains to subjects at each level of the design in which the

[1]Highly reflective coating on aluminum base

subjects are nested (Dayton, 1970). Results indicated the assumption of homoscedasticity to be invalid, and thus, Box's conservative degrees of freedom were used in testing the null hypotheses for the within subjects sources of variation (Table 1) (Dayton, 1970). Three sets of ANOVAS had to be run on the data, as initially, for some effects there were no appropriate denominators for testing. A Bonferonni adjustment was made and the alpha level for each test was set at 0.0167 (Fisher, 1971) to produce an overall alpha level of 0.05.

Results and Discussion

All but two of the within-subject effects are significant regardless of whether or not the conservative degrees of freedom are utilized (Table 2 and 3). (Significance is here defined as $P>F\leq 0.017$.) The two tests at odds are the angle*orientation effect of TM5 glass and the angle*orientation*species interaction for TM4 non-glass. These interactions, while interesting, were not the main concern of this experiment, and no further analyses were conducted which might remove this ambiguity.*

The main effects, angle, venation, species and orientation, were tested without ambiguity. In no case was angle effecting significant changes in reflectance values. This is also true for the venation effect. Species differences, however, were highly significant for all but one test conducted. The TM5 species effect changed from significant to non-significant upon flattening (Table 4). Orientation (leaf arrangement on the black plate) was significant for only two of the six test situations (TM3, TM4, TM5; glass, no glass), but both of the significances were in the near IR (TM4) channel, for both the flattened (with glass) and unflattened samples.

*Ambiguity refers to an F ratio that is significant with normal degrees of freedom, but is not significant with the conservative degrees of freedom.

Of all effects examined, orientation is the most intriguing. For TM3 and TM5, the pigment and water monitoring bands, respectively, there is no discernable effect of changing orientation on the reflectance values, either flattened or unflattened. For the near-IR band, TM4 (which monitors tissue structure), there are no changes in the significance pattern upon flattening. It does, however, make a difference how the samples are oriented. Orientation significantly affects reflectance in both the flattened and unflattened conditions. This implies that it is internal morphology which TM4 is monitoring, rather than the curling (external), or non-planar orientation of the leaves. Apparently, how the tissue is arranged with respect to incoming radiation controls the dominant reflectance pattern for excised leaves. This is particularly interesting in light of the fact that our previous investigations in geobotanical anomaly detection did not find the TM4 band useful in separating mineralized from non-mineralized excised leaf samples (Labovitz, et al., 1983).

As these earlier investigations utilized an arranged orientation, and orientation definitely has an effect on reflectance values, perhaps the information contained in the leaves was altered by the very process of orientation. Leaves in situ are arranged to optimize solar energy interaction. This arrangement, in plane view, more approximates a random, rather than an oriented distribution of leaves, for any particular leaf in the canopy of a temperate deciduous tree. It is possible that any potential information in TM4 was destroyed by the systematic placement of leaves for sampling. Thus, random arrangement will be examined in future geobotanical ground study work with hand held instrumentation to see if TM4 is useable in geobotanical investigations of mineralization.

The change from significance to a non-significant status upon flattening with glass for the TM5-species effect appears to be random, as in all other cases species is highly significant ($P>F\leq 0.007$). Even for the one non-significant test, it is just barely not significant ($P<F\leq 0.023$). (Recall that the critical level is 0.0167.) It is thus difficult to imagine this main effect, approximating a total leaf water effect as measured in TM5, being changed by flattening with a glass plate. Intuitively, the leaf water would remain the same under both conformations. As there is no significant difference between reflectance samples at the level of venation, the physical spacing or ordering of the major vascular tissues in the leaves apparently does not significantly affect reflectance readings. The species within these major venation patterns are, however, highly different from one another, indicating that it is the pigmentation, numbers of cell layers, leaf shape and/or general tissue organizaton that are effecting reflectance changes. In other words, generic/specific differences are being discerned with the three channels utilized in this study.

A Duncan's Multiple Range Test was used to separate species' means (Sall, 1979). As the two oak species' reflectance means are never significantly different for any channel, flattened or not, the level of differentiation is probably genus (Table 5). At least one subject differed from the other ten (eleven subjects per species) in all cases tested, as in each test, the subjects effect is highly significant. The angle of rotation effects no change in the reflectance value for either flattened or unflattened samples. Due to significant interactions involving the angle factor, however, one must assume that angle, in conjunction with sampling orientation, does indeed affect reflectance values.

Of the second and third order results, there were five changes (or reversals) between the flattened and unflattened analyses. For the angle*orientation interaction of TM3, the non-glass went from significant to non-significant with flattening (Table 4). TM5 went from non-glass non-significant to flattened ambiguous status.

The angle*orientation significance under TM3 non-glass indicates that while there is no significant portion of reflectance variability ascribable to either angle or orientation alone for TM3, the effects are synergistic. That the significance is removed when the sample is flattened reveals an association between this second order effect and leaf morphology. Specifically, the change in significance suggests a correspondence with curved perimeters of the leaves sampled. Angle*orientation significance can be explained when the sampling physiognomy is recalled. If leaves are oriented in a specifc direction, the curled edges will also be preferentially oriented. This would cause a relatively large change in the angle of incidence upon upturned surfaces with different azimuths. The angle effect would not interact to this degree if leaves are randomly oriented. However, this same effect for TM5 shows the reverse pattern, where unflattened the effect is not significant. Glass plate readings for TM5 angle*orientation are ambiguous, but most likely significant. This judgment is made due to the same pattern shown by TM5 angle*orientation*species, which combines the second order interaction in question with the species term. This third order interaction, while non-significant in an unflattened condition is unquestionably significant ($P>F\leq 0.002$) with glass. The change from non-significance in an unflattened condition to significance with flattening has no immediately obvious explanation. For the former physiognomy, variation in reflectance was perhaps high enough to mask or defeat any significant interaction. Once this variation is removed and the confidence limits around the true mean are sufficiently narrowed, the effect is seen to be significant. Admittedly, it is difficult to assign a biological explanation to a significant difference for the flattened conditions.

This same third order effect has a reversed pattern for TM4, where the reflectance change becomes non-significant upon flattening the samples. As before, the lack of significance indicates that there were changes in reflectance associated with the degree of edge curling of the samples. There is a possibility that there was no change in this effect with flattening as the unflattened significance is ambiguous. The probability of the calculated F ratio exceeding the tabular value however, is very low ($P>F\leq 0.006$).

Conclusions

When excised leaves are to be examined spectrally using the technique of Labovitz et al. (1983) the arrangement of the leaves on the plate does affect reflectance, and thus should be as close to in situ positioning as possible (Table 4). This dictates that random, rather than aligned midribs should be used when the goal is to detect soil anomaly effects. Thus, the TM4 band should be re-examined for usefulness to geobotanical investigations in experiments where the arrangement of excised leaves to be sampled approximates a random distribution (if not in situ, then at least using a closer approximation in measurement procedure).

Variations in venation do not cause significant variation in reflectance values, nor do interactions involving the venation effect. Finally, the angle (azimuth) of incident radiation does not have an effect by itself, but causes variation in reflectance readings in conjunction with orientation.

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Table 1

Results of Levene's homogeneity of variance tests for TM3, TM4 and TM5

$\alpha = 0.05$

data transformed

without glass:

	TM3	TM4	TM5
vein	S	S	S
orientation	NS	NS	NS
species	S	S	NS
angle	NS	NS	NS

with glass:

	TM3	TM4	TM5
vein	NS*	S	S
orientation	NS	NS	NS
species	S	S	S
angle	NS	NS	NS

* the only value that went from significant to not significant with transformation

TABLE 2
ANOVA RESULTS FOR TM3, TM4, AND TM5-UNFLATTENED SAMPLES

	TM3			TM4			TM5		
EFFECT	DF NUM, DENOM	F RATIO	[a]P>F	DF NUM, DENOM	F RATIO	P>F	DF NUM, DENOM	F RATIO	P>F
ANGLE	1,1*	0.1858	0.7401	1,3*	1.56	0.2185	1,11*	1.36	0.2706
ORIENTATION	1,3*	0.4933	0.5331	‡1,3	15.75	0.0107	1,5	1.07	0.3493
VENATION	2,3	1.60	0.3366	2,3	0.24	0.8009	2,3	0.65	0.5839
SPECIES	‡5,22	15.76	0.0001	‡3,5	31.33	0.0011	‡3,5	16.81	0.0048
ANGLE*ORIENTATION	‡1,2*	23.71	0.0010	1,2*	1.88	0.2339	1,2*	0.98	0.4609
ANGLE*VENATION	2,2*	1.93	0.2222	2,2*	1.02	0.4922	2,2*	0.09	0.9945
ANGLE*SPECIES	3,3*	3.58	0.0355	3,3*	0.67	0.7217	3,3*	0.31	0.9542
ORIENTATION* SPECIES	5,120	0.23	0.9488	5,120	0.377	0.8637	5,120	0.689	0.6321
ORIENTATION* VENATION	2,3	0.02	0.9756	2,3	1.91	0.2915	2,3	1.27	0.3976
ANGLE*ORIENTATION* VENATION	2,3*	0.73	0.6379	2,3*	0.31	0.9149	2,3*	2.89	0.0746
ANGLE*ORIENTATION* SPECIES	3,120*	0.43	0.9202	‡3,120*γ	2.66	0.0054	3,120*	1.31	0.2267
SUBJECT	‡120,120*	27.21	0.0001	‡120,120*	320.79	0.0001	‡120,120*	211.85	0.0001

a - BASED UPON CONVENTIONAL DF

* - CONSERVATIVE DF USED

‡ - FACTOR SIGNIFICANT AT ADJUSTED $\alpha = 0.016$

γ - RESULTS OF TEST AMBIGUOUS DUE TO USE OF CONSERVATIVE DF

TABLE 31[1]
ANOVA RESULTS FOR TM3, TM4, AND TM5-FLATTENED SAMPLES

	TM3			TM4			TM5		
EFFECT	DF NUM, DENOM	F RATIO	aP>F	DF NUM, DENOM	F RATIO	P>F	DF NUM, DENOM	F RATIO	P>F
ANGLE	1,1*	2.40	0.2457	1,11*	0.22	0.8851	1,1*	0.03	0.9918
ORIENTATION	1,5	0.91	0.3851	‡1,5	14.11	0.0132	1,5	0.75	0.4270
VENATION	2,3	1.09	0.4405	2,3	0.19	0.8348	2,3	0.14	0.8716
SPECIES	‡3,3	40.83	0.0062	‡3,3	81.20	0.0023	3,3	16.83	0.0222
ANGLE*ORIENTATION	1,2*	4.97	0.0457	1,2*	0.11	0.9524	‡1,2*γ	7.92	0.0165
ANGLE*VENATION	2,2*	3.50	0.0765	2,2*	0.18	0.9713	2,2*	1.32	0.3718
ANGLE*SPECIES	3,3*	0.44	0.8795	3,3*	0.67	0.7200	3,3*	0.43	0.8885
ORIENTATION* SPECIES	3,120	0.560	0.6425	3,120	0.128	0.9433	3,120	0.439	0.7255
ORIENTATION* VENATION	2,3	0.34	0.7360	2,3	2.33	0.2450	2,3	1.26	0.4013
ANGLE*ORIENTATION* VENATION	2,3*	0.08	0.9966	2,3*	1.56	0.2631	2,3	0.15	0.9832
ANGLE*ORIENTATION* SPECIES	3,120*	2.24	0.0194	3,120*	2.03	0.0356	‡3,120*	3.01	0.0018
SUBJECT	‡120,120*	7.57	0.0001	‡120,120*	163.08	0.0001	‡120,120*	129.53	0.0001

[1] SYMBOLS AS PER TABLE 2

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OF POOR QUALITY

Table 4

Factors affecting reflectance values when using our leaf sampling technique (Laboyitz, et al., 1983)

Factor	No glass TM3	No glass TM4	No glass TM5	Glass TM3	Glass TM4	Glass TM5
angle	NS	NS	NS	NS	NS	NS
orientation	NS	S	NS	NS	S	NS
venation	NS	NS	NS	NS	NS	NS
species	S	S	S	S	S	NS
angle*subject	-	-	-	-	-	-
angle*orientation	S	NS	NS	NS	NS	S *
angle*venation	NS	NS	NS	NS	NS	NS
angle*species	NS	NS	NS	NS	NS	NS
orientation*venation	NS	NS	NS	NS	NS	NS
angle*orientation*venation	NS	NS	NS	NS	NS	NS
angle*orientation*species	NS	S*	NS	NS	NS	S
subject	S	S	S	S	S	S
orientation*species	NS	NS	NS	NS	NS	NS

NS - not significant
S - significant ($P>F\leq 0.0166$)
* - ambiguous results as conservative df do not agree with conventional df
\- - no appropriate test for significance
_ - change in status of significance

Table 5

Mean clustering with the Duncan's multiple range test

$\alpha = 0.05$

No glass			Species	Glass		
TM3	TM4	TM5		TM3	TM4	TM5
a*	c	b	*Acer rubrum* L.	a,b	c	b,c
b	b	a	*Carya tomentosa* Nutt.	c	b	a
b	a	c	*Liquidambar styraciflua* L.	d	a	b,c
c	b	b,c	*Nyssa sylvatica* Marsh	d	b	c
b	b	b	*Quercus alba* L.	b,c	b	a,b
b	b,c	b,c	*Q. coccinea* M.	a	b	b,c

* means with the same letter are not statistically different from one another

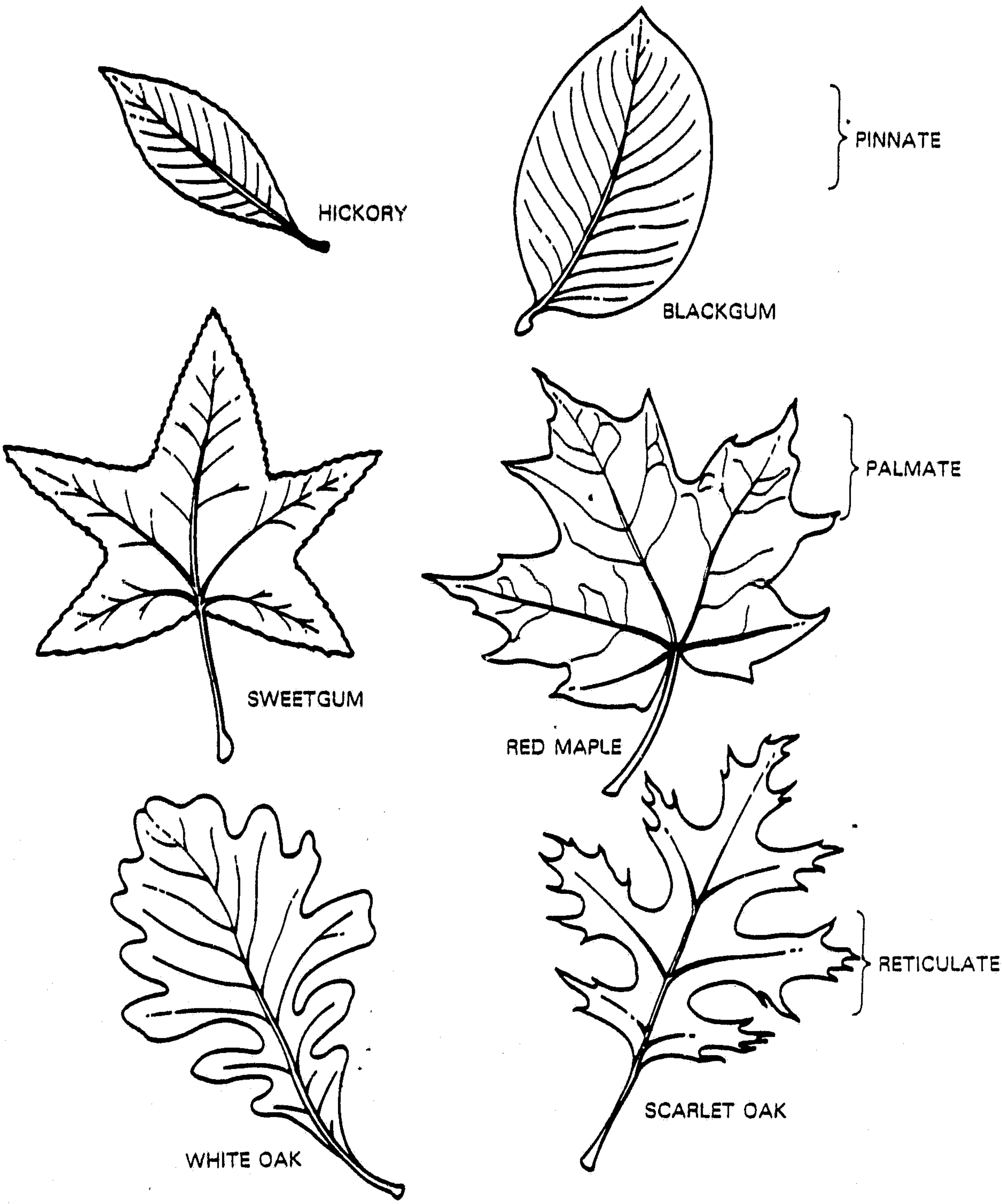


FIGURE 1
SPECIES AND VENATION PATTERNS

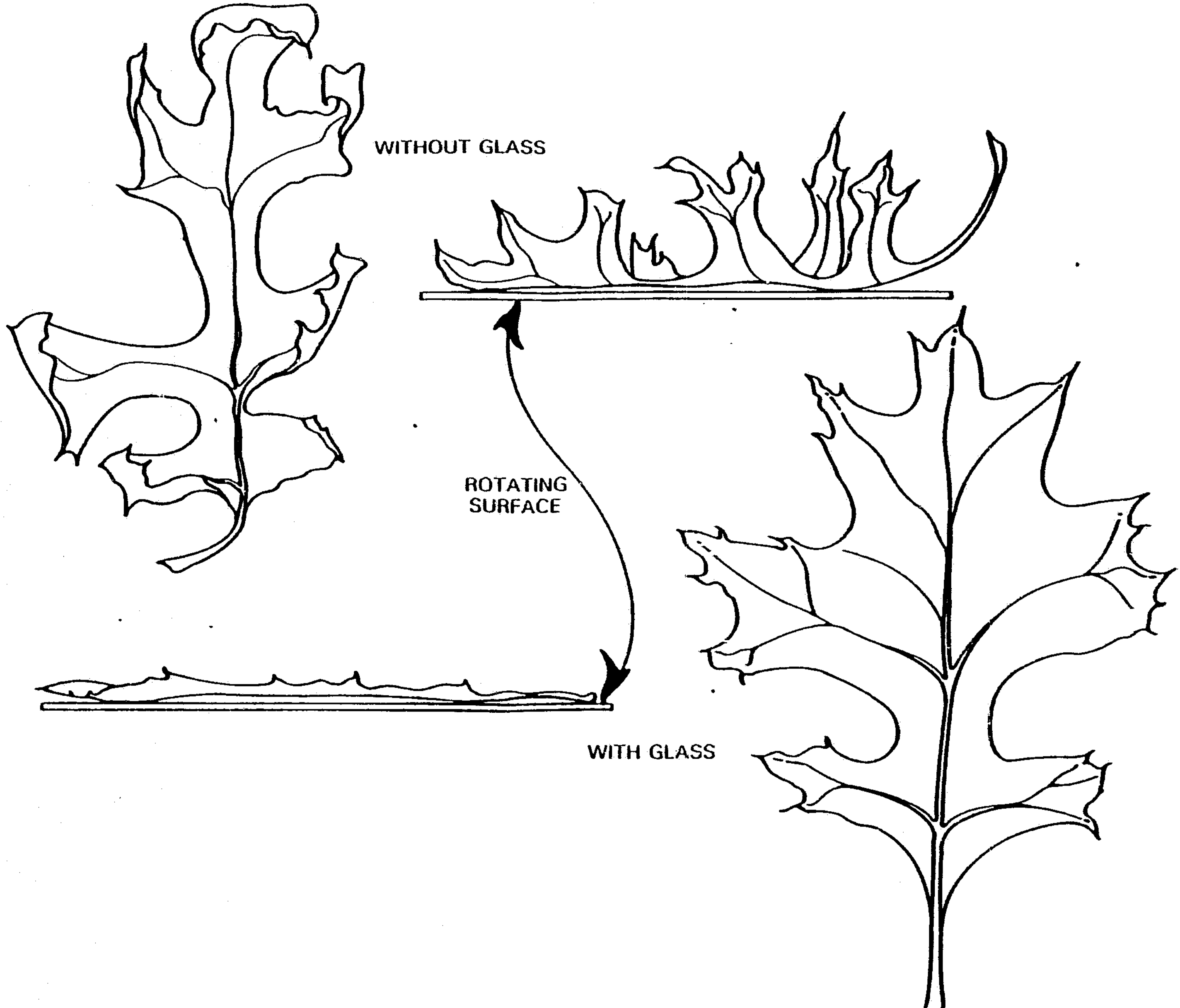
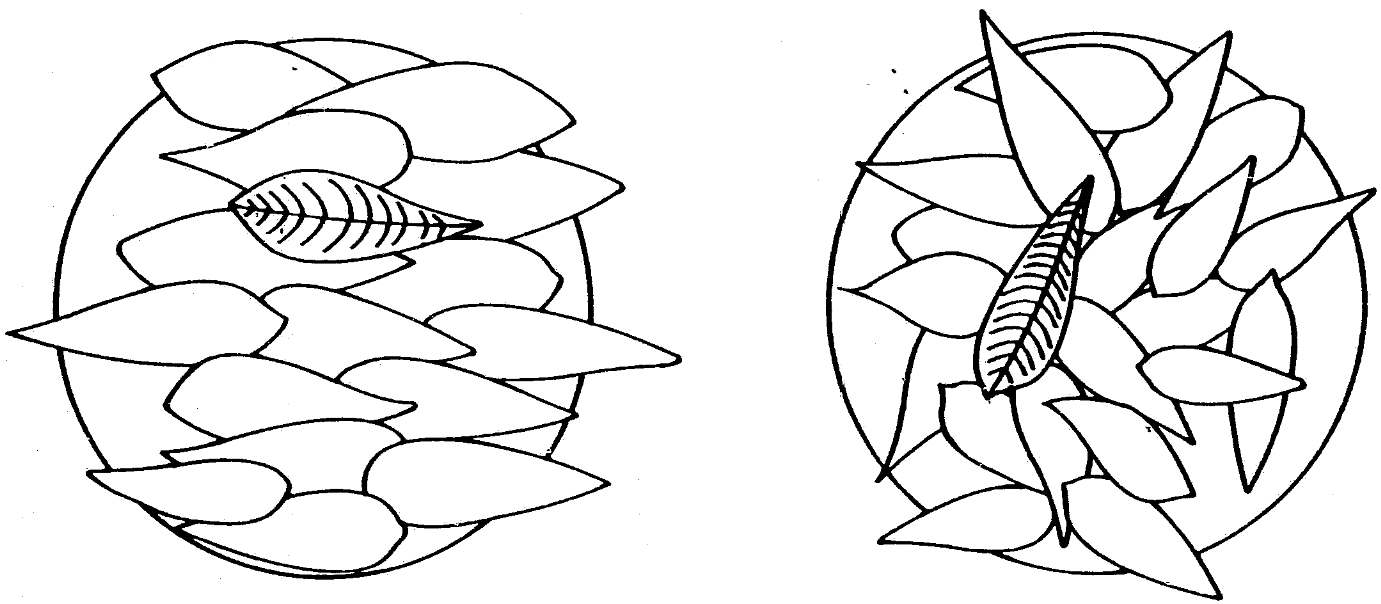


FIGURE 2
MORPHOLOGY (FLAT, CURVED)



ARRANGED RANDOM

FIGURE 3
ORIENTATION

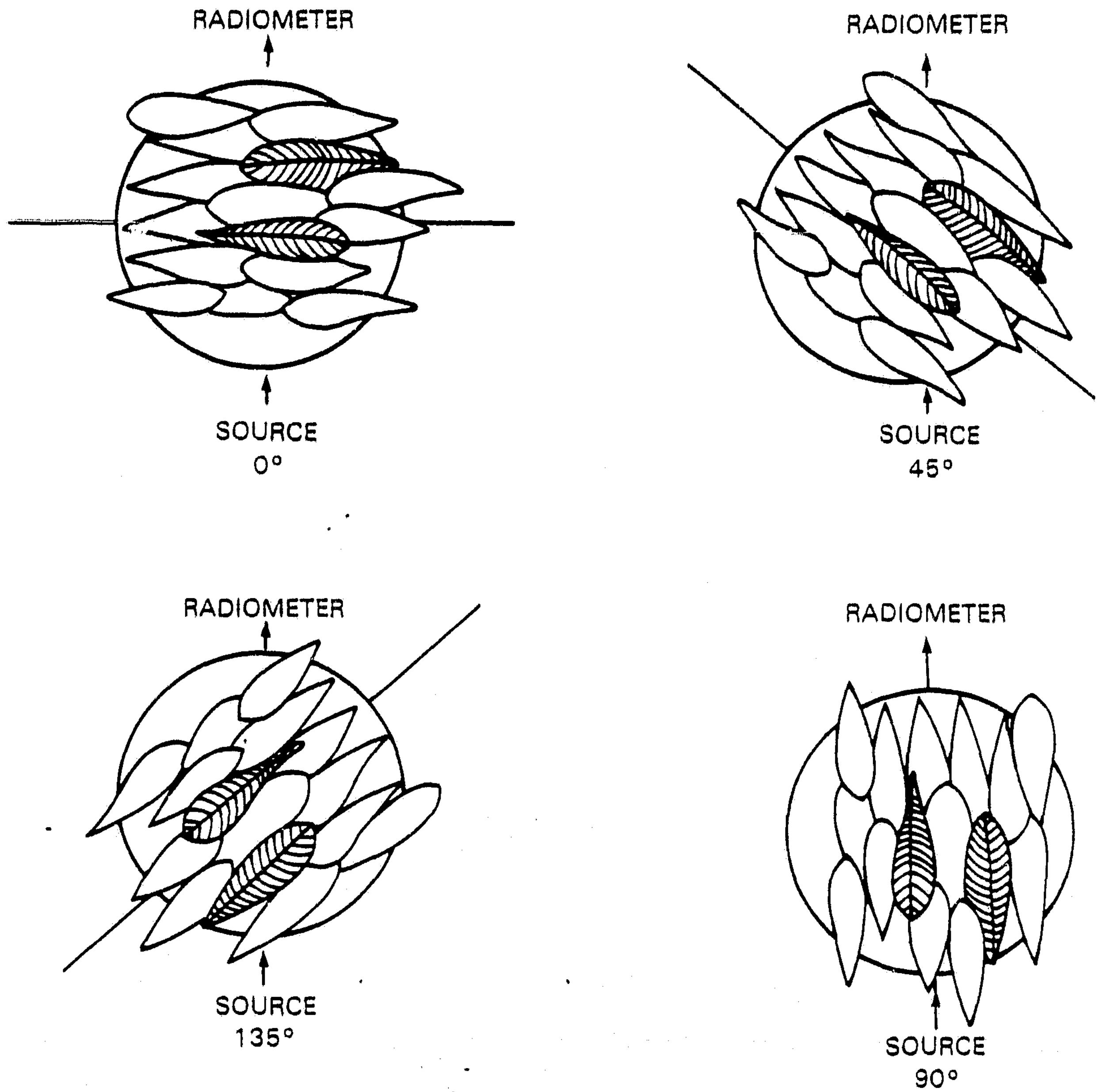


FIGURE 4
ANGLE (AZIMUTH)

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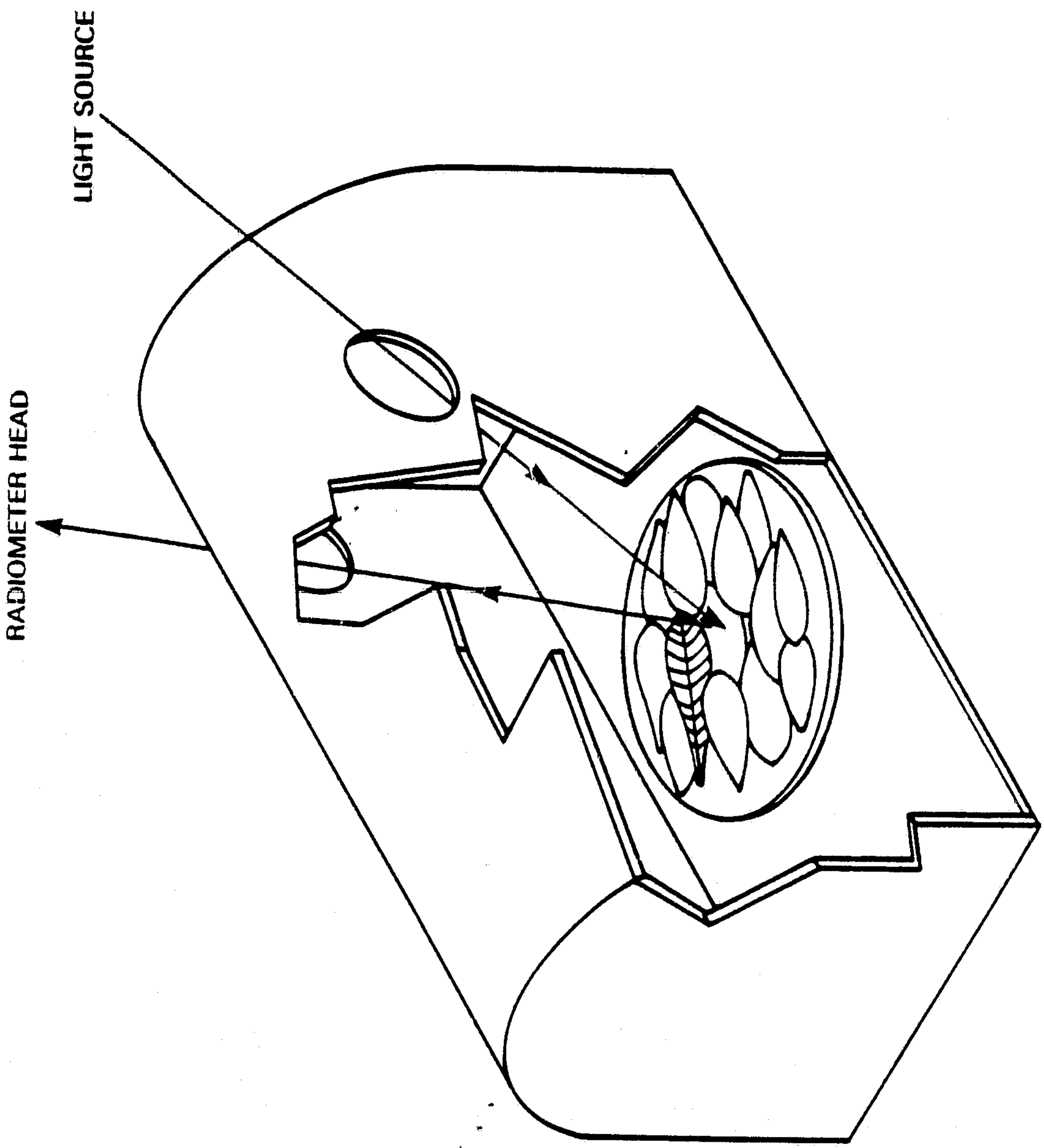

FIGURE 5
GEOMETRY OF APPARATUS