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Leaf Reflectance of Near-Infrared*

Inasmuch as the vigor and abundance of agricultural crops are being analyzed with aerial and space infrared imagery, a plant physiologist studies the causes of variations in reflectance.

(Abstract on page 191)

I shall discuss briefly and nonmathematically some research about the reflectance of near-infrared light from plant leaves and plant cellular constituents. My discussion will be mainly concerned with the near-infrared or 750- to 1350-nm wavelength interval. This region contains the photographic infrared area ranging from approximately 750 to 900 nm. I shall call near-infrared light *IR light*.

As Table 1 indicates, I show that *IR* light is scattered or reflected from leaves by refractive index discontinuities. The most important discontinuity is the cell-wall/air-space interface. If *IR* light travels at a critical angle from a hydrated cell wall with a refractive index of about 1.425 to an air space with a refractive index of 1.0, the *IR* light is scattered or reflected. Refractive index discontinuities among cellular constituents, however, are also of some importance in causing reflectance of *IR* light.

Table 1. IR Reflectance

Light scattered by Refractive Index Discontinuities:
1. Most important—Hydrated cell walls to intercellular air spaces.
2. Some importance—Discontinuities among cellular constituents, i.e., membranes *vs.* cytoplasm.

The function of wall-air interfaces in reflectance of *IR* light can be easily demonstrated by replacing air in leaves with a liquid. Figure 1 shows how vacuum infiltration of cotton (*Gossypium hirsutum* L.) leaves with water and castor oil, represented by the lower broken lines, reduced reflectance compared with normal, uninfiltrated leaves, represented by the upper solid line. Further work has shown that reflectance is minimal if the refractive index of the liquid presumably matches the refractive index of the cell wall.

The function of wall-air interfaces in reflecting *IR* light can also be shown by considering leaf maturity. Plate 1 shows transections of immature and mature citrus leaves. The upper transection represents a very young citrus leaf (fifth leaf from apex of new flush), and the lower transection represents a mature citrus (orange, *Citrus sinensis* (L) Osbeck) leaf (eighth leaf from apex of previous growth flush). The upper young leaf is compact with few air spaces in its mesophyll, whereas the lower old leaf is *spongy* or has many air spaces in its mesophyll. Figure 2 shows that the young compact leaf had lower light reflectance than the mature spongy leaf.

Figure 2 shows that the mature citrus leaf represented by the solid line, compared with the young citrus leaf represented by the

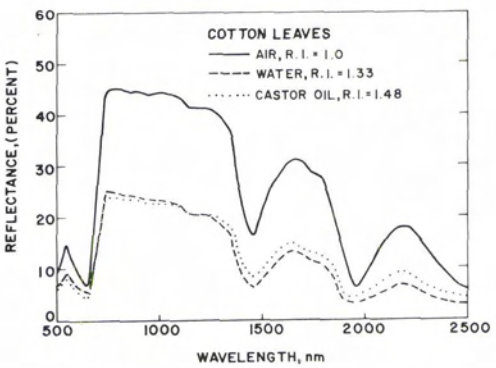

Fig. 1. Effect of replacing air in cotton leaves with liquid on the reflectance of *IR* light.

*Talk presented in a panel discussion on "What *IR* Films Measure in Plants" at the Fourth Biennial Workshop of Color Aerial Photography in the Plant Sciences, Univ. of Maine, July 1973.

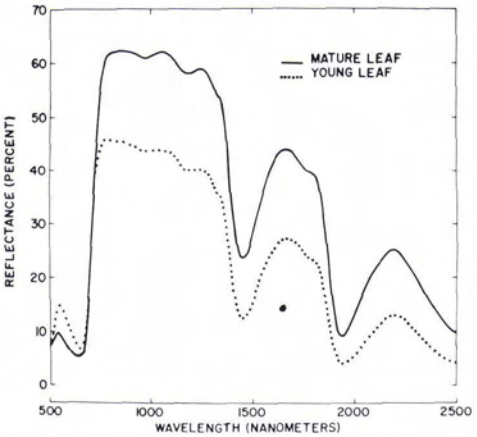

FIG. 2. Reflectance of young and mature citrus leaves.

broken line, had 15 percentage values of more *IR* reflectance over the 750- to 1350-nm wavelength interval.

It is extremely important to consider leaf maturity if spectrophotometric measurements are to be made comparing the reflectance of stressed with nonstressed leaves. For example, leaves were sampled fourth from the apex of salinity-stressed and nonstressed cotton plants. Spectrophotometric measurements showed that salinity-stressed leaves had higher reflectance than nonstressed leaves. However, nonstressed plants were growing more rapidly than stunted, salinity-stressed plants. Hence, leaves fourth from the apex of nonstressed plants were younger or more compact than more porous leaves fourth from the apex of stressed plants. If leaves were sampled at the same chronological age, stressed leaves had lower reflectance than nonstressed leaves because stressed leaves were stunted or more compact than the more porous, nonstressed leaves of the same chronological age. Thus, if leaf maturation was not considered, stressed leaves had higher reflectance than nonstressed leaves; if leaf maturation was considered, stressed leaves had lower reflectance than nonstressed leaves. Stressed leaves usually have lower reflectance than nonstressed leaves if stressed and nonstressed leaves are the same age.

Leaf mesophyll arrangements also affect reflectance of *IR* light. Plate 2 shows a transection of a corn (*Zea mays* L.) leaf. A corn leaf has a relatively compact mesophyll. Subsequently, it will have relatively low reflectance and high transmittance compared with a leaf with a more porous mesophyll. A leaf with a more porous mesophyll will be shown in Plate 3.

Plate 3 shows a transection of a maple (*Acer* spp.) leaf with a porous mesophyll, or a mesophyll with many cell wall-air space interfaces. The maple leaf will have higher *IR* reflectance and lower *IR* transmittance than the corn leaf that was shown in Plate 2.

Mesophyll arrangements vary considerably as Figure 3 shows. The three main types of leaf mesophylls can be represented by *A*, a compact corn leaf; *C*, an isolateral eucalyptus leaf (palisade cells both sides); and *H*, a dorsiventral ligustrum leaf with multiple palisade layers.

Figure 4 shows *IR* reflectance spectra for upper leaf surfaces of corn, eucalyptus, hyacinth, and oleander leaves. Compact corn leaves on a relative basis had lowest reflectance and dorsiventral oleander leaves with a very porous mesophyll had highest reflectance. Eucalyptus leaves with an isolateral mesophyll and dorsiventral water hyacinth leaves with large air chambers had inter-

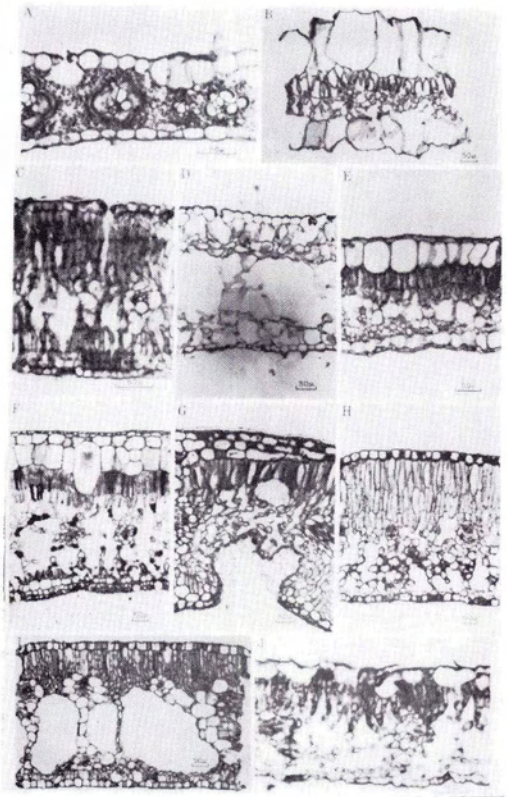

FIG. 3. Transections of leaves. *A (top left.)*, corn. *B (top right)*, begonia. *C (second row, left)*, eucalyptus. *D (second row, center)*, crinum. *E (second row, right)*, banana. *F (third row, left)*, ficus. *G (third row, center)*, oleander. *H (third row, right)*, ligustrum. *I (bottom left)*, hyacinth. *J. (bottom right)*, rose.

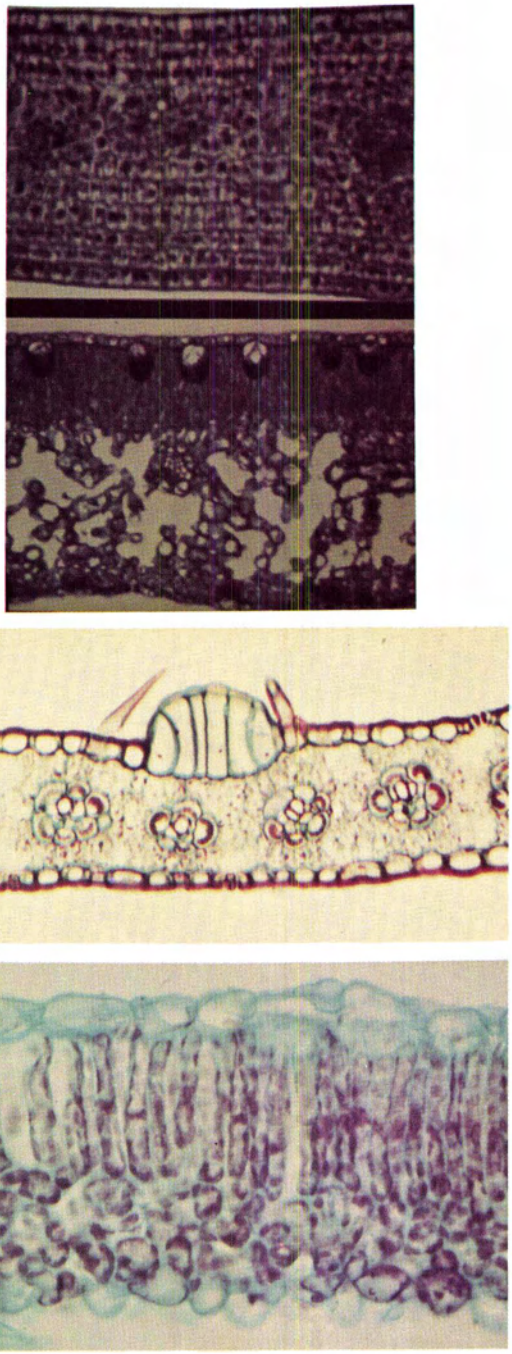

PLATE 1. *(top)* Transections of young (upper photo) and mature (lower photo) citrus leaves.

PLATE 2. *(center)* Transection of a corn leaf.

PLATE 3. *(bottom)* Transection of a maple leaf.

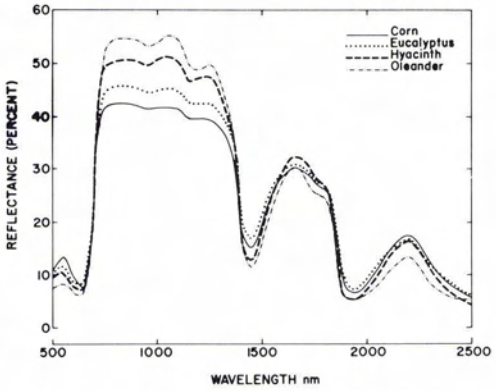

FIG. 4. Reflectance spectra for upper leaf surfaces of corn, eucalyptus, hyacinth, and oleander leaves.

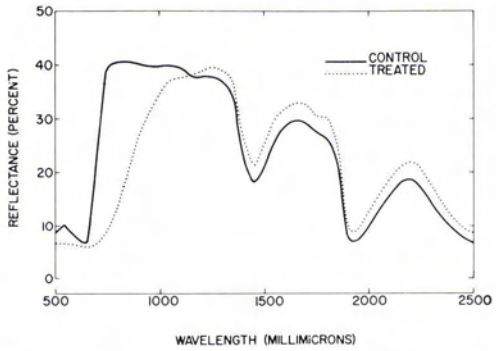

FIG. 5. Reflectance of upper surfaces of cotton leaf sections, control (untreated) and ammonia-treated.

mediate reflectances. Reflectances of leaves with porous compared with compact mesophylls were highest because light passed more often from hydrated cell walls to air spaces. This change in density or refractive index caused light scattering and subsequently reflectance was increased.

Internal discoloration of leaves or a black coating on their surface will cause a decrease in *IR* reflectance. In Figure 5 the solid line represents normal leaves and the broken line represents leaves infiltrated with ammonia that caused internal discoloration. The discoloration was caused by saponification of chlorophyll and oxidation and polymerization of polyphenol oxidase to a brown pigmentation. This change in reflectance is not caused by a change in internal air spaces, but absorptance of *IR* light is increased by internal discolorations or black coatings on leaf surfaces.

Often in literature in conjunction with remote sensing of plant diseases, spectrophotometric measurements show that diseased leaves have higher *IR* reflectance than normal leaves. Chances are that measurements were made on partially dehydrated tissue for diseased leaves. This tissue would collapse in such a manner that the number of air voids would increase. (Sinclair, T. R. 1968. "Pathway of solar radiation through leaves." M. S. Thesis, Purdue University Library, Lafayette, Indiana. 179 pp.) Subsequently *IR* reflectance would be higher for diseased leaves than for normal leaves. Figure 6 shows how dehydration affects *IR* light reflectance. The lower, solid line represents fresh leaves, and the upper broken line represents leaves that were air dried for seven days. As the leaves were progressively dried, *IR* reflectance increased because leaf structure was altered.

Subcellular particles interact with *IR* light as Figure 7 shows. Subcellular particles of

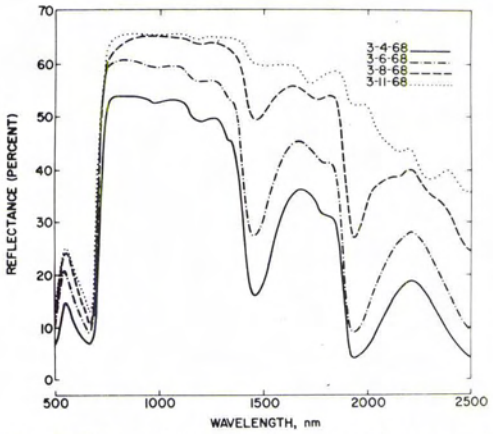

FIG. 6. Effect of dehydration on the reflectance of cotton leaves.

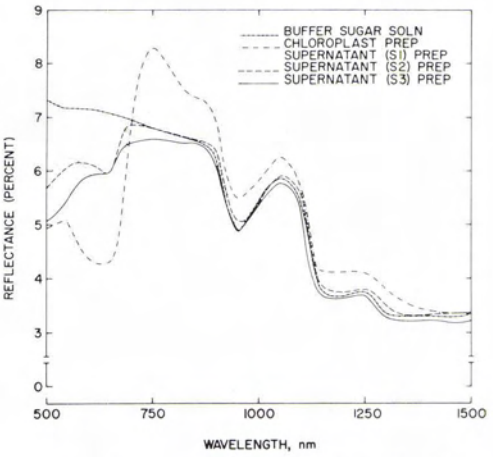

FIG. 7. Reflectance of resuspended subcellular particles of homogenized spinach leaves and the medium (buffered sucrose solution).

spinach leaves were differentially sedimented and resuspended in buffered sucrose solution. The chloroplast preparation, compared with buffered sucrose solution, increased *IR* reflectance. It is concluded that subcellular particles in intact leaves contribute to reflectance of *IR* light. However, the contribution of subcellular particles in leaves to reflectance of *IR* light is small compared with *IR* light reflectance caused by cell wall-air interfaces in leaf mesophylls.

We have conducted specific studies with a microspectrophotometer to test the premise that refractive index discontinuities other than wall-air interfaces cause reflectance of *IR* light from leaves.

Figure 8 shows a Leitz microspectrophotometer assembly equipped with an objective for reflected light that was used. Reflected light was recorded on high speed black-and-white infrared film or on a strip chart as a specimen was scanned over the 370- to 1100-nm wavelength interval.

The components are indicated as:

- *A* is the power source for a xenon lamp.
- *B* represents instrumentation for punching data on paper tape for computer input.
- *C* represents the housing of the xenon lamp, and it also depicts the monochromator.
- *D* is the microscope with attached photomultiplier tube and bayonet camera mount.
- *E* shows the power supply for the photomultiplier tube. An ammeter and strip-chart recorder are also shown.

Light from the monochromator shown at *C* goes through an illuminator on the microscope shown at *D* and impinges on the specimen. Light is reflected from the specimen upward to either the camera or the photomultiplier tube. The output of the photomultiplier tube is recorded by an ammeter or strip-chart recorder.

The sequence of phases from the glass slide on which the plant tissue was mounted to the objective was: plant tissue, tap water, No. 1 cover slip, immersion oil. A 17.5-percent reflectance standard was also used. The sequence of phases from the surface of the standard to the objective was: immersion oil, glass slide, tap water, No. 1 cover slip, immersion oil.

If wavelength scans were made on a specimen, reflecting power was calculated by dividing reflectance of the specimen by reflectance of the standard and multiplying the result by 17.5 percent.

Several plant genera were used to obtain desired cellular constituents. If specimens were scanned, the number of replications varied from 4 to 15. The diaphragm size that controlled the amount of light impinging on specimens averaged 2.5 X 21 micrometers. Black-and-white infrared photos were taken of some cellular constituents to demonstrate light reflectance.

Figure 9 shows infrared photos of light reflected at the 850-nm wavelength by *Lemna* L. and *Elodea* (*Anacharis canadensis* Planch.) leaves. Photos *A* and *C*, that represent centers of etiolated *Lemna* and green *Elodea* leaves with their upper epidermises in focus, respectively, demonstrate diffusion of incoming infrared light by epidermises. In photo *B*, the layer of cells below the upper epidermis was in focus, and artifacts resembling ball bearings are evident. The same type of artifact is apparent in photo *D* taken at the edge of an etiolated *Lemna* leaf. It is possible that the artifacts are tonoplasts—membrane separating cytoplasms from vacuoles.

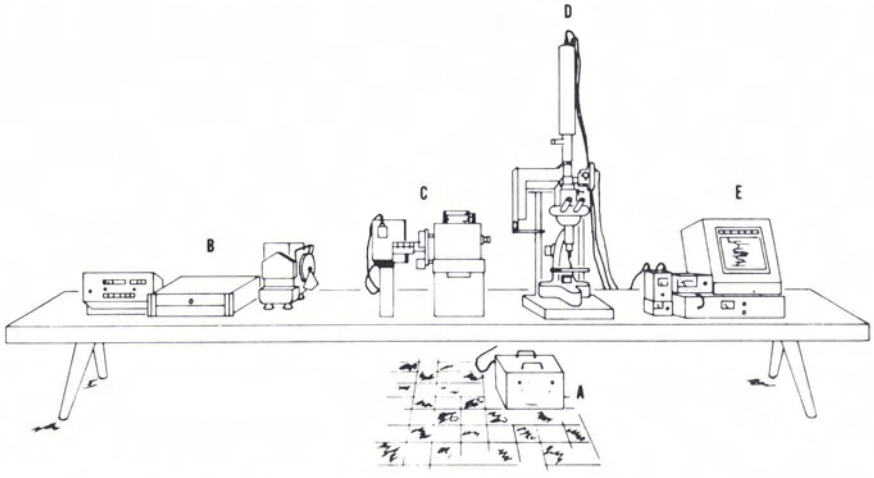

FIG. 8. The Leitz microspectrophotometer assembly.

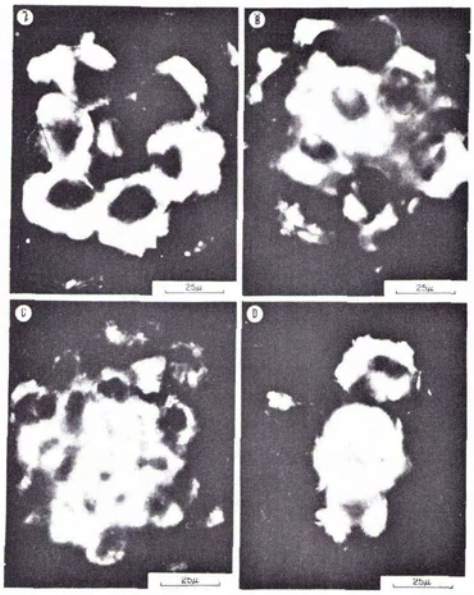

FIG. 9. Black-and-white infrared photos of light reflected at the 850-nm wavelength by: *A (upper left)*, center area of etiolated *Lemna* leaf with the upper epidermis in focus. *B (upper right)*, center area of etiolated *Lemna* leaf with the layer of cells below the upper epidermis in focus. *C (lower left)*, surface of *Elodea* leaf. *D (lower right)*, near perimeter of etiolated *Lemna* leaf.

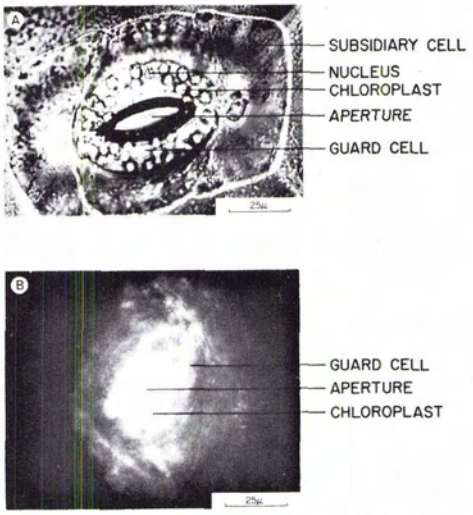

FIG. 10. Comparison of an ordinary black-and-white photo (*A, top)* taken with tungsten light with a black-and-white infrared photo (*B, bottom)* of light reflected at the 850-nm wavelength for *Rhoeo discolor* stomata.

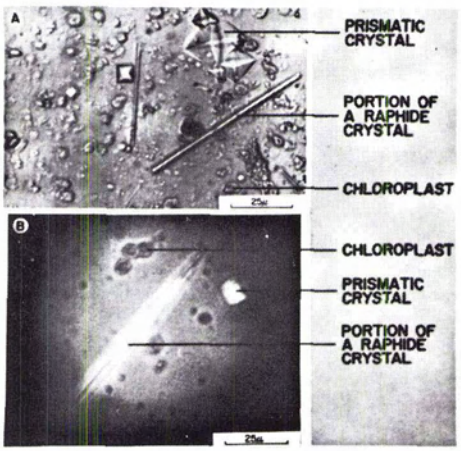

FIG. 11. Comparison of an ordinary black-and-white photo (*A, top)* taken with tungsten light with a black-and-white infrared photo (*B, bottom)* of light reflected at the 850-nm wavelength by crystals and chloroplasts in expressed cell sap of Wandering Jew.

Figure 10 compares an ordinary black-and-white photo designated *A*, taken with tungsten light, with a black-and-white infrared photo designated *B*. The infrared photo was taken of light reflected at the 850-nm wavelength. Both photos *A* and *B* show stomata on lower epidermises of *Rhoeo discolor* Hance. The lower infrared photo indicates that infrared light is strongly reflected from internal leaf surfaces through the stomatal aperture. The outlines of guard cells are also visible, and within guard cells, some chloroplasts appear to reflect infrared light.

Figure 11 compares an ordinary black-and-white photo designated *A*, taken with tungsten light, with a black-and-white infrared photo designated *B*. The infrared photo was taken of light reflected at the 850-nm wavelength. Both photos *A* and *B* show cellular particles and fragments in expressed cell sap of leaves of *Zebrina pendula* Schnizl. or Wandering Jew. The cells contain many prismatic and raphide crystals. Photo *B* demonstrates the ability of crystals to reflect infrared light. In addition, it appears that chloroplasts reflect some infrared light, since a *halo effect* surrounds their perimeters in photo *B*.

Figure 12 shows differences in reflectance of light over the 370- to 1100-nm wavelength interval between walls and internal areas of cells of *Heliconia humile* L. and *Agave americana* L. (Century Plant). For *Heliconia* (upper graph), there was no statistically significant difference between reflectance measurements made on cell walls and those made on internal cell areas. However, there was a highly significant difference for reflectance measurements made between the cell

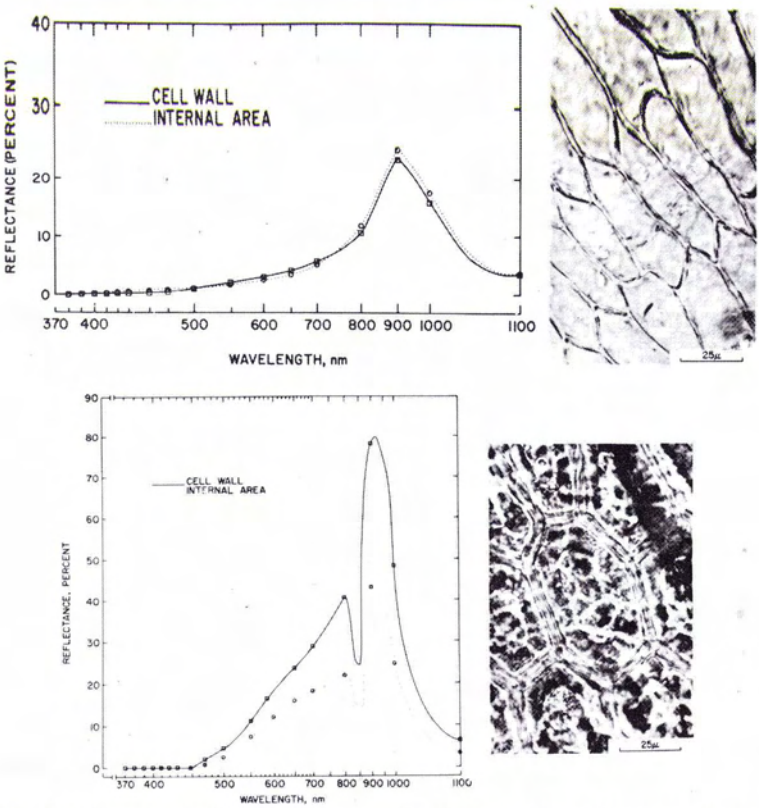

FIG. 12. Influence of cell walls and internal cell areas on the reflectance of light over the 370- to 1100-nm wavelength interval for *Heliconia humile* (upper left) and *Agave americana* (lower left). Photos depict cell walls of *H. humile* (upper right) and *A. americana* (lower right).

wall and internal cell area for the Century Plant (lower graph). The largest difference in reflectance (23 percent) between the cell wall and internal cell area for Century Plant occurred at the 900-nm wavelength. The marked decrease in reflectance that occurred for both the cell wall and internal cell area at the 850-nm wavelength was apparently caused by water absorption.

The different light reflectance results obtained between cell walls and internal cell areas for *Heliconia* and Century Plant can be explained by comparing photos of live, unstained epidermal cells (Figure 12). Cell walls of Century Plant (lower photo) are very complex compared with simpler cell walls of *Heliconia* (upper photo). Striated cell walls of Century Plant apparently induced more light scattering relative to internal cell area than simpler cell walls of *Heliconia*.

Figure 13 shows differences in reflectance of light over the 370- to 1100-nm wavelength interval between nuclei and adjacent areas of cells of broad bean (*Vicia faba* L.). According to an analysis of variance for 15 replications, mean differences between reflectances of nuclei and internal areas were significant at

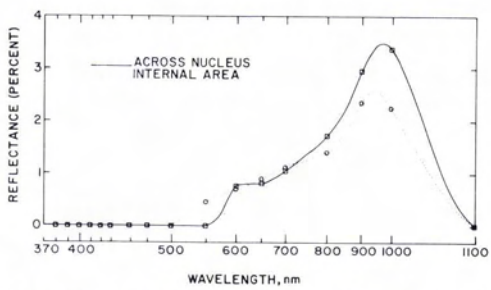

FIG. 13. Influence of nuclei and internal cell areas on the reflectance of light over the 370- to 1100-nm wavelength interval for broad bean.

the 10 percent probability level. Student's paired *t*-test at the 5 percent probability level indicated that nuclei had higher reflectance than internal areas at the peak of 950 nm. The higher reflectance for nuclei compared with adjacent cytoplasm can be attributed to refractive index discontinuities because the measuring diaphragm covered the nucleolus and nuclear membrane.

Figure 14 shows the influence of normal and plasmolyzed onion (*Allium cepa* L.) skin

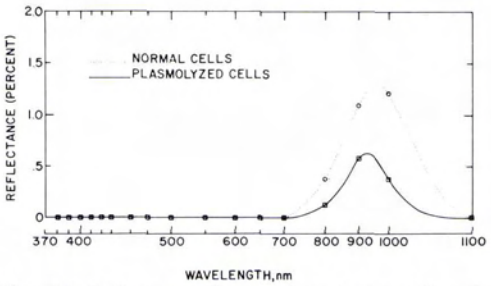

FIG. 14. Influence of normal and plasmolyzed onion skin cells on the reflectance of light over the 370- to 1100-nm wavelength interval.

cells on reflectance of light over the 370- to 1100-nm wavelength interval. To make measurements, the diaphragm was positioned across the cytoplasm of normal cells; and for plasmolyzed cells, the diaphragm was positioned mainly across cytoplasm, but it was allowed to extend across the cytoplasmic membrane and reach slightly across the hypotonic solution phase. The same onion epidermal strip was used for measurements on normal and plasmolyzed cells.

Cytoplasm of plasmolyzed cells had lower light reflectance over the 800- to 1000-nm wavelength interval than cytoplasm of normal cells. The mean difference of all wavelengths between normal and plasmolyzed cells was significant at the 5 percent probability level. Apparently, shrinkage of cell protoplasm reduced light reflectance by lengthening pathways of light, thereby increasing absorptance.

Figure 15 shows a photo of *IR* light reflected by a portion of a Wandering Jew leaf epidermal cell. The photo indicates that membranes reflected *IR* light more strongly

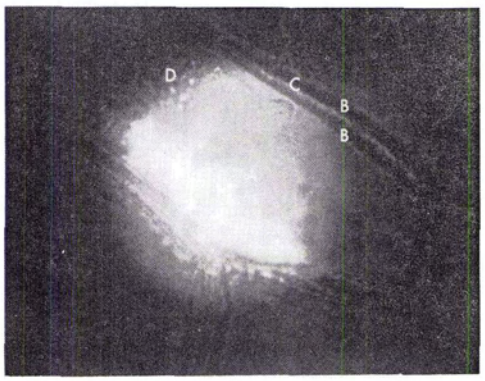

FIG. 15. Photomicrograph of a portion of the lower epidermal cell of a leaf of Wandering Jew. White and dark areas denote high and lower light reflectance at the 850-nm wavelength, respectively. Strongly reflected light is apparent for nuclear membrane *A*, plasmalemmas *B*, middle lamella *C*, and epidermal plastids *D* compared with adjacent darker areas.

than surrounding protoplasm. This is evident with the plasmalemma or cell membrane at *B*, and nuclear membrane at *A*. Epidermal plastids at *D* also appear to reflect light more strongly than surrounding protoplasm. In addition, the middle lamella at *C* reflected *IR* light more strongly than cell walls that are evident on each side of *C*.

Results with the microspectrophotometer show that refractive index discontinuities of cellular constituents contribute some to *IR* light reflectance by plant leaves. However, the amount of *IR* light reflected by cellular discontinuities is small compared with the amount of *IR* light reflected by cell wall-air space interfaces.

ABSTRACT: *The paper was presented at the Fourth Biennial Workshop of Color Photography during a panel discussion on "What IR Films Measure in Plants." IR reflectance is light scattered by refractive index discontinuities (1) at the interface of hydrated cell walls with intercellular air spaces and (2) among cellular constituents, i.e., membranes vs. cytoplasm. The author is a plant physiologist.*

1974 Convention in St. Louis, March 10-15

(See page 174)