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PLANT TISSUE AND THE COLOR INFRARED RECORD

by

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FOREWORD

A number of explanations have been proposed for the photographic reaction of infrared films to the near-infrared reflection from plants. Some have held that chlorophyll is endowed with a high reflectance in these wavelengths. Another has attributed it to water included within plant tissue. Dr. Pease has combined his background in botany with an intimate knowledge of color infrared film to analyze the nature of infrared reflectance of plant tissue, particularly as it applies to the red record of color infrared film. Conclusions have been checked with spectroradiometric measurements of both living plant tissues and tissue that has been subjected to a variety of purposely controlled laboratory modifications.

Color infrared film is one of the most useful tools for remotely sensed land use analysis, particularly for crops and natural vegetation. An understanding of the physiological causes of the record it yields should be of use in the analysis.

Leonard W. Bowden
Principal Investigator

ABSTRACT

Green plant tissue should not be considered as having a uniquely high near-infrared reflectance but rather a low visual reflectance. Leaf tissue without chloroplasts appears to reflect well both visual and near infrared wavelengths. The sensitometry of color infrared film is such that a spectral imbalance strongly favoring infrared reflection is necessary to yield a red record. It is the absorption of visual light by chlorophyll that creates the imbalance that makes the typical red record for plants possible.

Reflectance measurements of leaves that have been chemically blanched or which have gone into natural chlorotic decline strongly suggests that it is the rise in the visual reflectance that is most important in removing the imbalance and degrading the red CIR record. The role of water in leaves appears to be that of rendering epidermal membranes transluscent so that the underlying chlorophyll controls the reflection rather than the leaf surface itself.

PLANT TISSUE AND THE COLOR INFRARED RECORD

A number of explanations have been proposed for the "high" infrared reflectance of plants. A high infrared reflectance, however, is not a property solely confined to chlorophyll-filled tissue. A piece of white paper will reflect more near-infrared radiation than the most verdant plant foliage. Plant tissue appears red on color infrared (CIR) film, not because of the high infrared reflectance per se, but because of the difference between the infrared reflection and that in the visual wavelengths. When a piece of green tissue dies, dries, and turns straw colored, its near-infrared reflectance may drop but little. Rather, the visual reflectance will rise to reduce the spectral contrast that is necessary to yield the red color that is associated with infrared reflection when this film is used.

A patch of growing alfalfa was photographed with color infrared (Kodak Ektachrome Infrared Aero Type 8443) using only the minus-blue filter of the film system. As would be expected, there was a strong red record. Dry but still green alfalfa hay showed some red from the greenest samples under the same photographic conditions. Older, straw-colored alfalfa hay required strong auxiliary enhancement (Pease and Bowden, 1969) to yield red. Quite clearly, the red record was not due to some mysterious high infrared reflecting property of the chlorophyll or to water in the spongy mesophyll, but simply to the reshaping of the daylight illumination by the reflectance properties of the plant material to create the necessary spectral imbalance in reflection and in the last case by the selective attenuation of the enhancing filter. The same patterns of reflectance change and their CIR record were observed with Yellow Pine needles.

Figure 1. shows the reflectance curves for the alfalfa and pine needles. Reflected energy was measured with an ISCO Spectroradiometer at each wavelength and divided by the incident illumination at the same wavelength to obtain reflectance. The suggestion from these not-too-untypical examples seems clear. It is the rise in visual reflectance between 550 and 750 nanometers that is the greatest change that takes place as plant tissue dries or chlorotic decline occurs. This is perhaps more important in reducing a CIR red than a decline in infrared reflectance. This is not surprising since chlorophyll, whether <u>in vivo</u> or dry, reduces visual reflectance in the waveband that is utilized in the process of photosynthesis.

To test this hypothesis, the reflectance of the underside of an hibiscus leaf was measured when freshly plucked from the plant. The leaf was then chemically blanched with sodium hypochlorite to remove all green chlorophyll color. When measurements were made of the still soft and spongy blanched parenchyma tissue (Fig. 2,a) the infrared reflectance was virtually the same as the fresh leaf but the visual reflectance had drastically risen. Drying the blanched leaf caused the overall reflectance to rise slightly.

To test the role of water in reflectance, another hibiscus leaf was measured fresh, then subjected to dehydration in hot methanol. The drying was carried to the point that intercellular water was removed but the chloroplasts in the cells remained relatively intact. When dry, the dehydrated epidermis tended to become white and somewhat opaque, masking the effect of the chlorophyll beneath to reflected light, although the leaf still appeared green to transmitted light. As Figure 2,b shows, this causes a moderate rise in visual reflectance and a dramatic rise in the infrared reflectance.

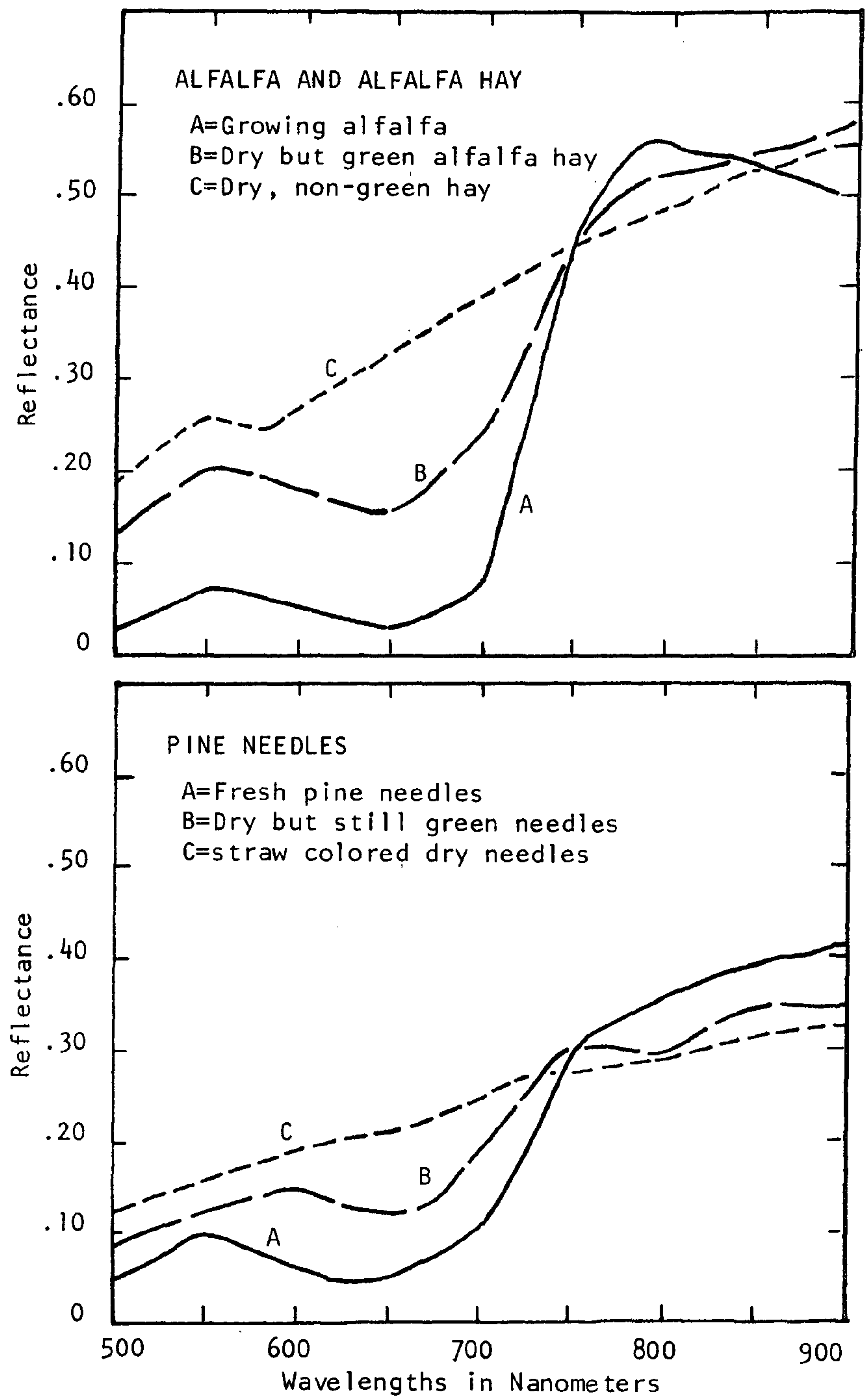


Figure 1. Reflectances of alfalfa and pine needles in sequential stages of dessication and chlorotic decline. Measurements were made with an ISCO Spectroradiometer using daylight illumination. Only the CIR spectrum is included.

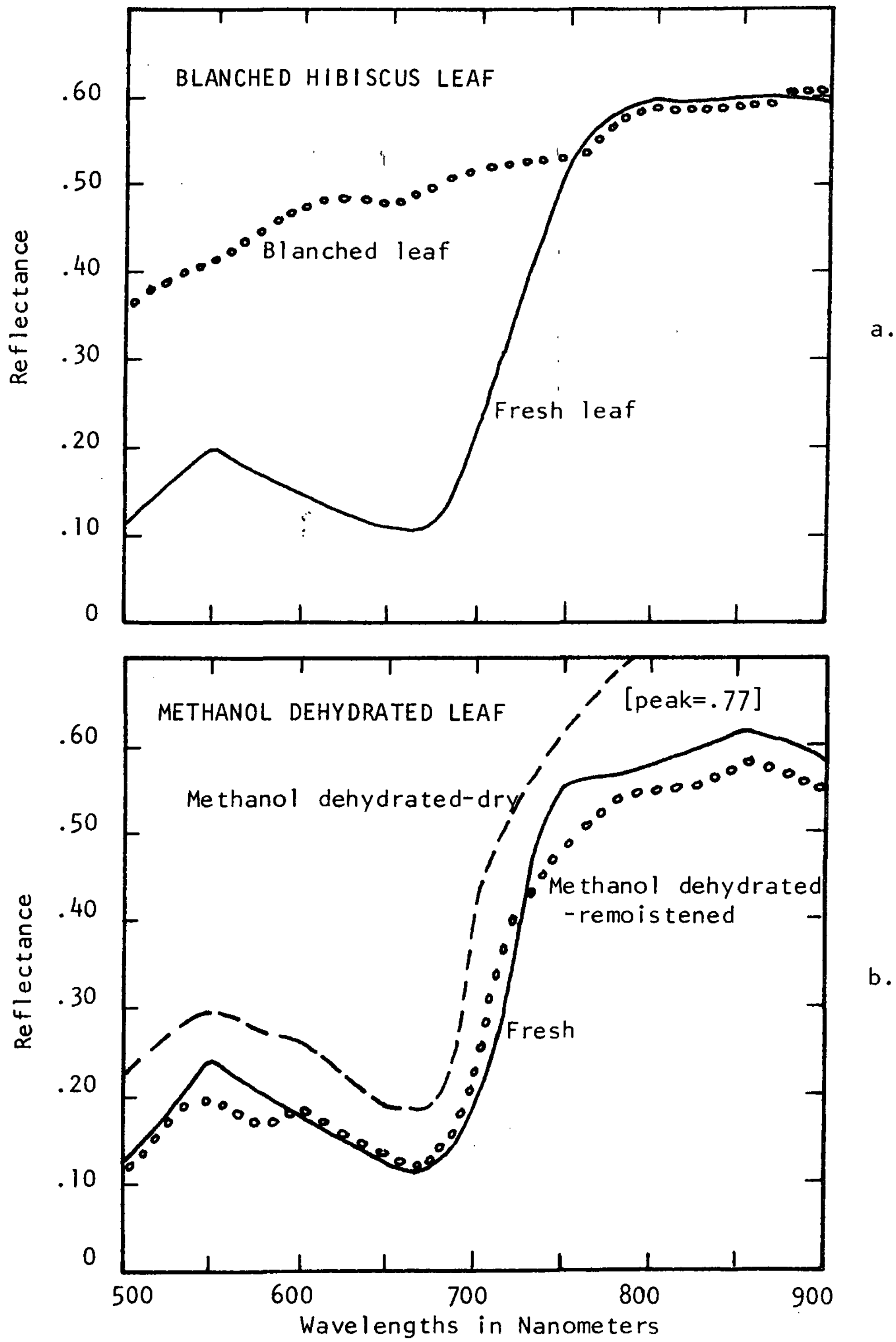


Figure 2. Reflectances from hibiscus leaves, (a) fresh versus blanched in sodium hypochlorite and (b) fresh versus methanol dehydrated. When dried, the transmittance of the epidermis is lowered with the tissue gaining in overall reflectivity. In the visual realm enough chlorophyll still shows to depress reflectance. In the near-infrared, where this effect is absent, the reflectance approaches that of white paper (.77). Remoistening the epidermis causes it to lose its reflective significance.

Resoaking of the leaf in warm water to replace intercellular moisture and re-moisten the epidermis restored the reflectance close to the curve of the fresh leaf. This suggests that the role of water in the intercellular spaces is to make the epidermis more translucent and thus permit the chlorophyll inclusions to affect the light that is reflected. As intercellular water declines, the epidermis begins to take over the role of the reflecting surface.

This does not mean to say that a drop in infrared reflectance will not occur after a leaf wilts or chlorotic decline occurs. A number of observations, however, suggests that such decline is due to the weathering of the dead plant material, and, with the exception of very fragile tissue, takes some time to occur. It may well be a secondary phenomenon following the death of the tissue, rather than decline in water content.

That a rise in visual reflectance can cause CIR film to lose its red infrared record, requires an understanding of the film's sensitometry. Under normal daylight illumination, the basic system will render a neutral target cyan instead of gray. This is due to the fact that the cyan dye-forming layer which controls the infrared record has less than half the sensitivity of either the yellow or magenta layers. Healthy plant targets, with their strong infrared reflections, are able to overcome this imbalance as is shown in Figure 3. In the lower graph, the reflection from the growing alfalfa is plotted as measured in curve A and then attenuated with the basic-minus-blue filter to give curve B, the spectral distribution of energy reaching the film. Energy is low in those wavelengths that expose the yellow and magenta dye forming layers but is high in the spectral domain that

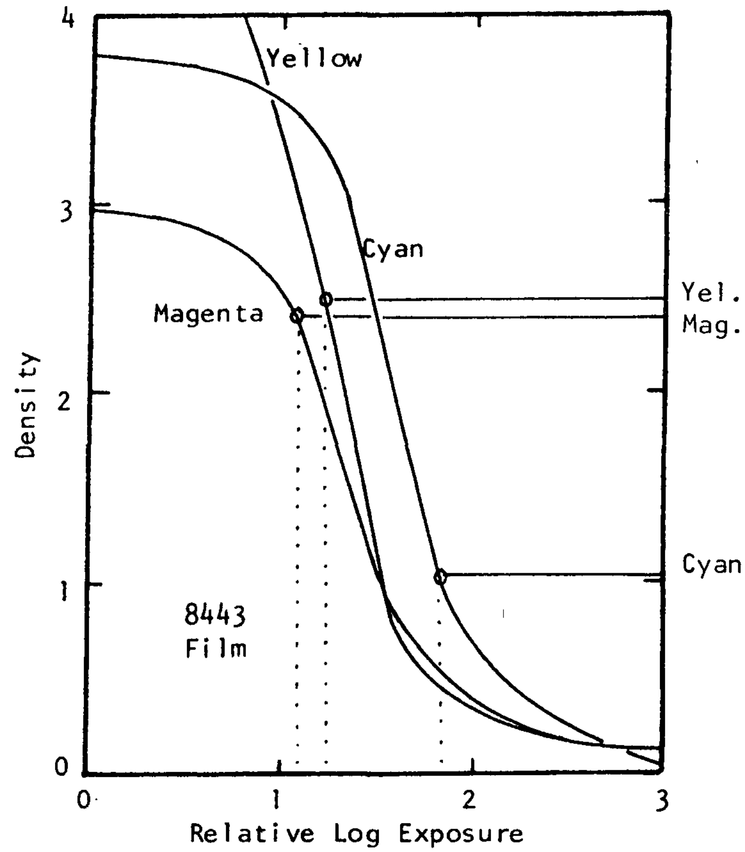
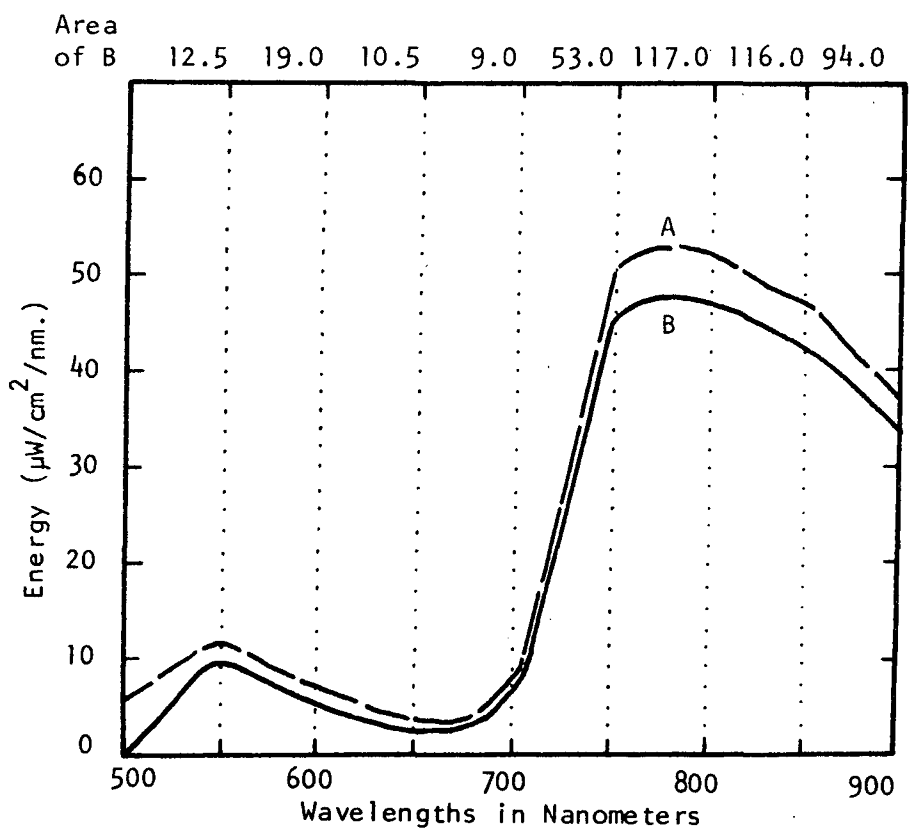


Figure 3. The spectral distribution of energy reaching the film (bottom) and resulting dye-layer densities on type 8443 film (top). Note that the imbalance of energies exposing the layers favors cyan exposure sufficiently to overcome the lower sensitivity of this layer.



A=Measured reflection from growing alfalfa.

B=Energy reaching film. (A attenuated by a 15 filter).

most affects the cyan layer. When dye-layer exposures are plotted on the accompanying D log E diagram for the film, the cyan layer is less dense than the other two despite its lower sensitivity. Since the lowest density, cyan in this case, sets the neutral tone of the transparency, the magenta and yellow dye-layers will show in concert as red. If on the other hand, the yellow and magenta dye-forming layers receive greater relative exposure due to a rise in visual reflectance, their densities are lowered below that of the cyan layer and no red will show (Fig. 4). The infrared reflection has changed but little. It has been the rise of the visual reflection that has masked the red.

The sensitivities of the three dye-layers do not quite fit the discrete spectral domains implied above. There is overlap with the magenta sensitivity extending into the yellow domain below 600 nm. and the cyan sensitivity extends across the whole CIR spectrum to some degree. As measured from the spectral sensitivity curves of Fritz (1967), the fraction of the total exposure of each layer falling in discrete 50 nm. wavebands is as follows (Pease, 1969). These assume the use of a minus-blue filter.

	Dye-forming layers		
Waveband	Yellow	Magenta	Cyan
500-550 nm.	.52	.04	.01
550-600	.48	.13	.03
600-650		.50	.08
650-700		.33	.14
700-750			.25
750-800			.21
800-850			.20
850-900			.08

By weighting the energy (area) within the spectral curve of energy reaching the film in each 50 nm. waveband for each dye-forming layer, then totaling

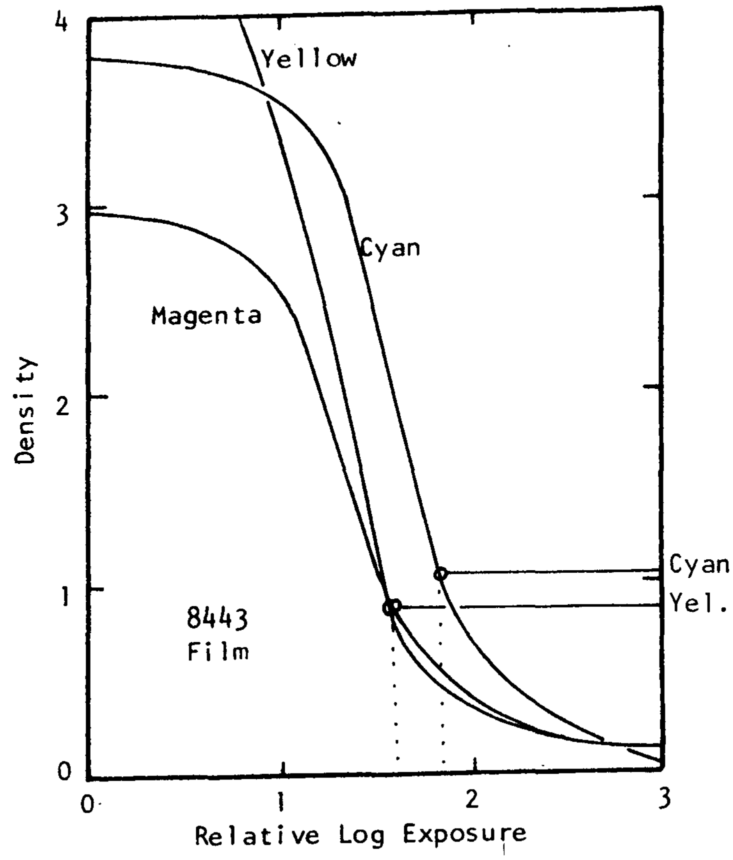
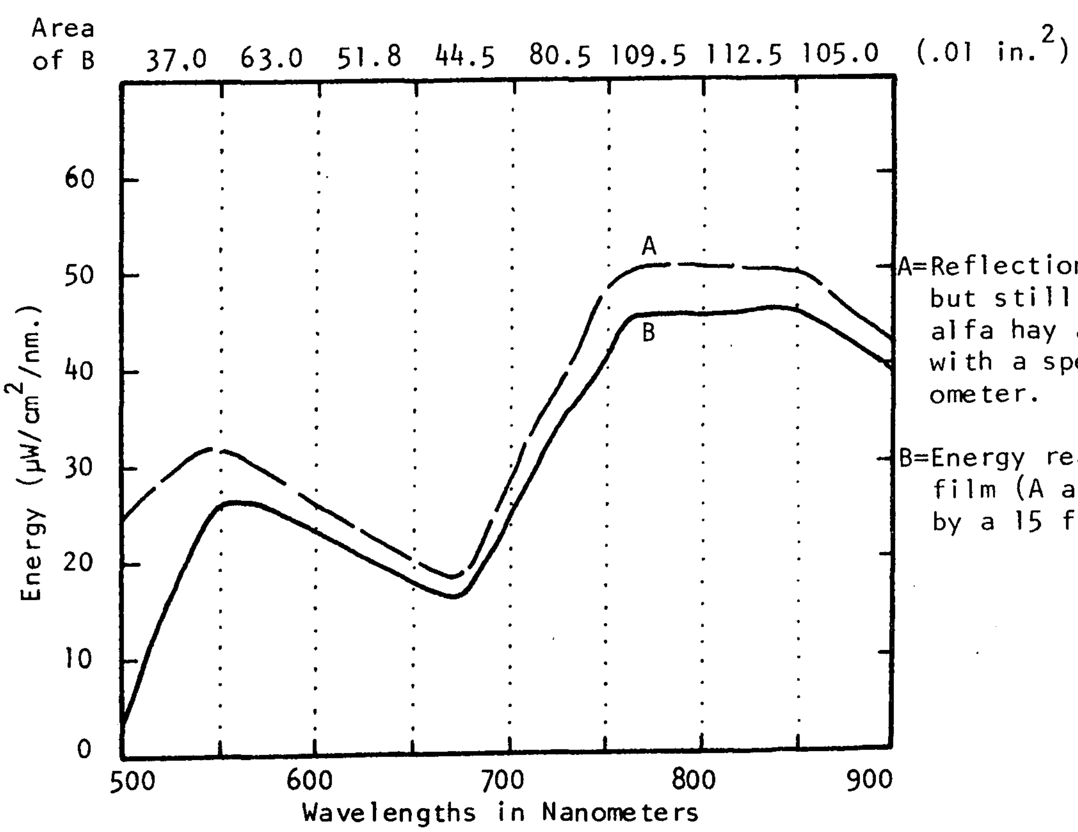


Figure 4. Dry but still green hay is marginal in yielding a red infrared record. The averaged reflection measurements plotted below will yield a cyan dominance. Greener than average samples will show some red. This illustrates how only a moderate rise in visual reflectance eliminates the red record when only the minus-blue CIR filter is used.



A=Reflection from dry but still green alfalfa hay as measured with a spectroradiometer.

B=Energy reaching the film (A attenuated by a 15 filter).

the resulting exposure for each layer, and finally placing the log of the exposure on the D log E diagram, the relative dye-layer densities can be predicted with considerable accuracy as has been done for figures 3 through 5. For this graphical prediction, the D log E diagram for type 8443 film has been modified to fit an illumination with constant spectral intensity rather than daylight (Pease, 1969).

The curve of energy reaching the film exhibits attenuation by all filters used with the CIR system. For this reason it can be reshaped by using certain filters as auxiliaries to the basic minus-blue which attenuate far more in the visual than in the near infrared (Pease and Bowden, 1969).[1] By reshaping the energy curve with a Wratten 78A filter, neutral targets are rendered neutral on the CIR image using normal daylight as illumination. When this is done, targets with only a slight rise in near-infrared reflectance begin to yield a red record. Figure 5 shows that a red record can be obtained for the non-green alfalfa hay when the filter reshapes the moderate infrared rise of this dry organic matter into a reflection curve that resembles that from the living plant.

These diagrams then make clear that it is not the infrared reflection alone that controls the red record, but rather the shape of the spectral curve of energy reaching the film. Reduction of spectral contrast wrought by the rise in visual reflectance as plant tissue dries, effectively removes the red. To be sure, as dead plant matter weathers it darkens and lowers all reflectance, but this only further equalizes the spectral intensities.

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[1]These filters must fit Wratten specifications particularly as regards near infrared transmittance. Certain vat-dyed glass filters tested had low transmittances in the near-infrared and were thus not usable. Wratten gelatine material performs well.

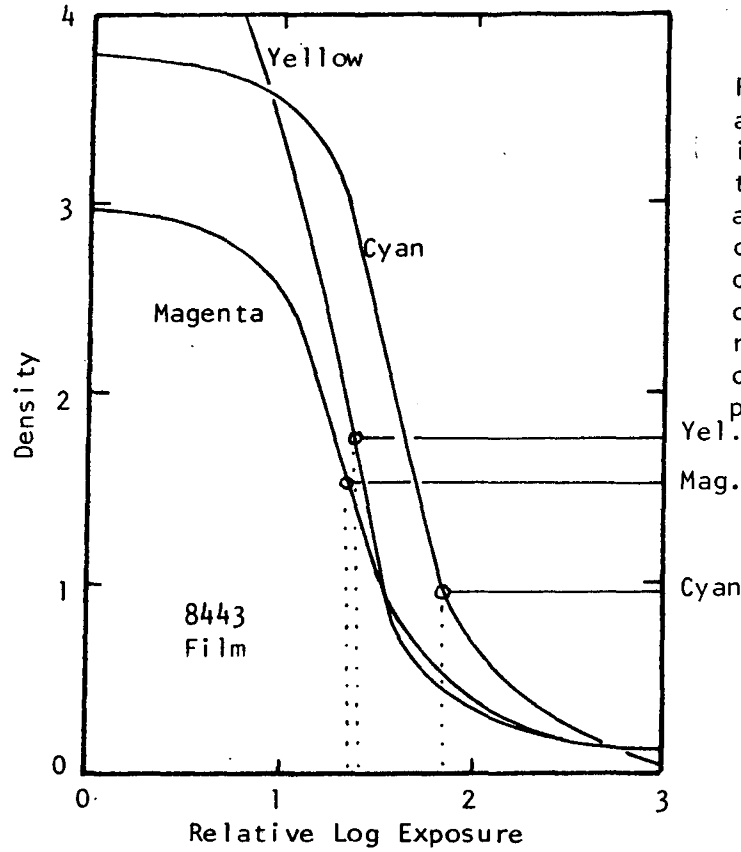
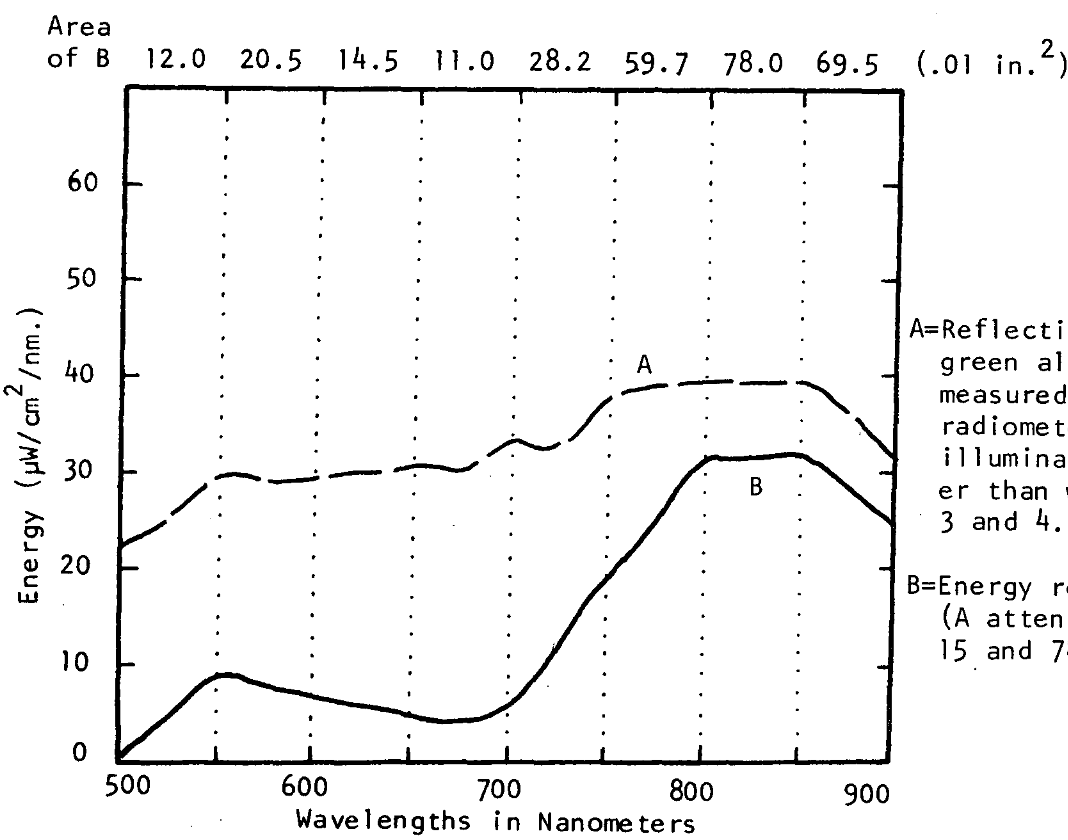


Figure 5. Dry (not green) alfalfa hay has a rising infrared reflectance, although this is not readily apparent when it reflects daylight. Reshaping the curve with a 78A filter creates a curve of energy reaching the film that is close to that of fresh plant material. Red then shows.



A=Reflection from non-green alfalfa hay, measured with spectroradiometer. Incident illumination was lower than with Figures 3 and 4.

B=Energy reaching film (A attenuated with 15 and 78A filters)

The reflectance curves (and under certain conditions the red record) of dry but still green plant tissue suggest that the light-absorbing properties of chlorophyll are significantly involved in selectively lowering the visual reflectance which makes the red record possible. As has been noted, this is compatible with the assimilation needs of plants and fits absorption studies that have been made of chlorophyll. Following this lead, a chromatogram was made from an extract of chlorophyll in the solvents methanol, acetone, and chloroform. When dry, the chlorophyll evaporite band had a near-infrared reflectance of .68 or nearly as high as the .80 of the unaffected chromatography paper[2]. In the visual waveband, however, the existence of the chlorophyll even though dry dropped the reflectance from the .80 of the paper to .15.

The role of chlorophyll as an absorber of visual light rather than a reflector of near-infrared light has been given black and white multispectral demonstration in Plate 1. The spectral bands imaged simulate the dye-layer sensitivities of color infrared film. The top photograph was made with illumination reaching the film in the spectral band between 700 and 900 nm. The illumination for the center photograph was restricted to 600-700 nm. while that for the bottom view reached the film between 500 and 600 nm. The targets include on the right a piece of the chromatogram with the unaffected paper above the chlorophyll evaporite bands and on the left a fresh geranium leaf above a dry geranium leaf from which the chlorophyll has been removed by solvents.

In the near-infrared photograph (top), reflectances are all close to that of the unaffected chromatography paper. The chlorophyll evaporite band

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[2]These reflectance measurements were made with a broadband spectroradiometer that measures a band in the near-infrared from 700-1000 nm. and in the visual a band from 400-700nm.

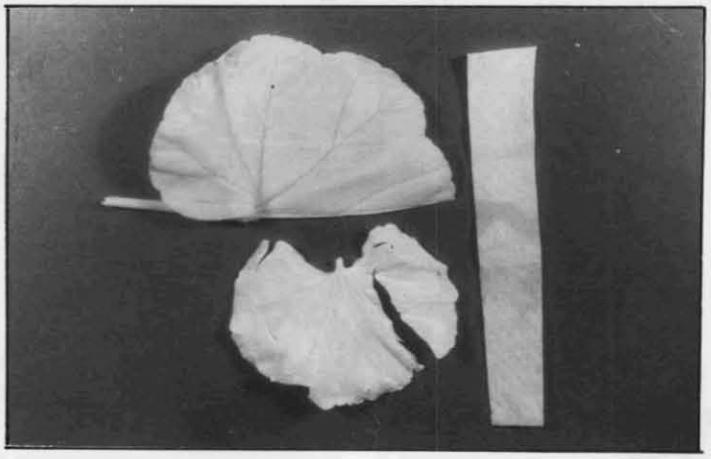
Plate 1. Multispectral analysis of chlorophyll reflectance. Targets in each view are as follows: the chromatogram is on the right with the unaffected paper at the top. Fresh geranium leaf is in the upper left with the dry geranium leaf below from which the chlorophyll has been extracted. The background for each view is a Kodak 18 percent neutral test card. Illumination is from a 3400°K source.

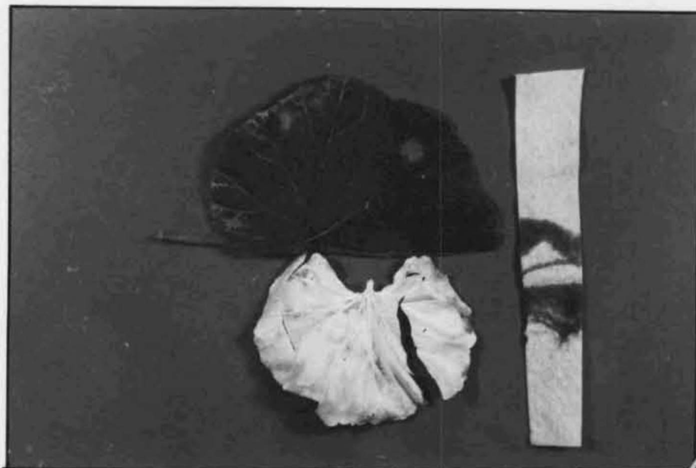
Upper: Made onto high speed black and white infrared sensitive film through a Wratten 89B filter, illumination falls into a spectral band between the 700 nm. cutoff of the filter and the 900 nm. cutoff of the film sensitivity. Note that all reflectances are close to the .80 of the chromatography paper and that the reflectances of the green leaf and dry chlorophyll extract are only slightly lower.

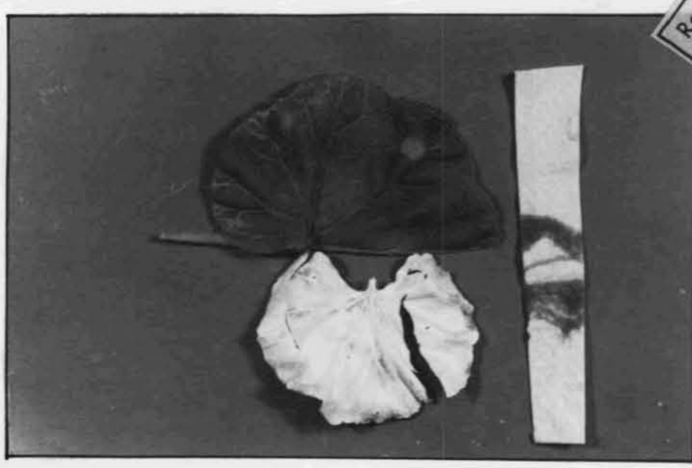
Center: Made onto Plus-X film through a Wratten 25 filter, illumination reaching the film is restricted to the spectral band between 600 and 700 nm. Reflectances of the dry chlorophyll extract and the fresh leaf are the lowest of the three photographs.

Lower: Made onto Plus-X film through a Wratten 58 filter, illumination reaching the film is restricted to the spectral band between 500 and 600 nm. As would be expected, chlorophyll reflectances are slightly higher than in the center view because this is the waveband of the "green peak."

Tissues of the leaf from which the chlorophyll has been removed have reflectances that are close to that of the white paper at all wavelengths in the CIR spectrum illustrated.







shows faintly as a darker band with a slightly lower reflectance that matches the tone of the fresh leaf. On the other hand, between 600 and 700 nm. (center) both the dry chlorophyll and the fresh tissue have reflectances below that of the .18 reflectance card that forms the background of each photograph. Between 500 and 600 nm. (lower), neither chlorophyll reflectance is as low as in the center photograph. The reflectance of the leaf from which the chlorophyll has been removed remains high in all wavebands shown. In translating the multispectral views to CIR dye-layer densities it must be kept in mind that the strong exposure in the near-infrared causes the cyan to be least apparent in the image. Its absence forms the window through which the high densities from the low exposure of the magenta (center) and yellow (lower) can show together as red.

A high reflectance in both the visual and near-infrared spectral bands appears to be an inherent property of cellulose plant tissue. It is the lowering of the visual reflectance by the addition of chlorophyll that causes a spectral imbalance that yields the typical red infrared record. Water is a good absorber of near-infrared light. Since absorbtivity is additive inverse to reflectivity, water <u>per se</u> cannot be considered a good reflector at these wavelengths, particularly when no specular reflection can occur. The role of water inclusions may be that of rendering the epidermal membranes more transluscent and thus permit the visual absorption of chlorophyll to control the reflection.

In this inquiry undersides of certain leaves were examined because this is where most intercellular water occurs and the upper epidermis may be rendered transluscent by other than water (oils, etc.). That the upper side, most frequently exposed to the aerial camera, may be less affected by dessication than the underside was indicated by the methanol-dried samples. Proximity of the chloroplast-rich palisade cells to the upper epidermis in a broadleaf may account for this side appearing visually darker yet yielding the brighter red infrared record. ISCO spectroradiometric reflectance measurements of the two sides of an hibiscus leaf bear this out.

It is acknowledged that this inquiry has involved but a few samples. Alfalfa, pine needles, hibiscus, and geraniums may or may not be typical of most green plant tissue. The conclusions presented must therefore be considered as only strongly suggestive pending further examination of leaf reflectance.

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