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Citation: Curran, P. J., Dungan, J. & Gholz, H. (1990). Exploring the relationship between reflectance red edge and chlorophyll content in slash pine. Tree Physiology, 7(1-2-3-4), pp. 33-48. doi: 10.1093/treephys/7.1-2-3-4.33

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Tree Physiology 7, 33–48
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Exploring the relationship between reflectance red edge and chlorophyll content in slash pine

PAUL J. CURRAN,[1] JENNIFER L. DUNGAN[2] and HENRY L. GHOLZ[3]

[1] *Department of Geography, University College of Swansea, Singleton Park, Swansea SA2 8PP, UK*

[2] *TGS Technology Inc., NASA, Ames Research Center, Moffett Field, CA 94035, USA*

[3] *Department of Forestry, University of Florida, Gainesville, FL 32611, USA*

Summary

Chlorophyll is a key indicator of the physiological status of a forest canopy. However, its distribution may vary greatly in time and space, so that the estimation of chlorophyll content of canopies or branches by extrapolation from leaf values obtained by destructive sampling is labor intensive and potentially inaccurate.

Chlorophyll content is related positively to the point of maximum slope in vegetation reflectance spectra which occurs at wavelengths between 690–740 nm and is known as the "red edge." The red edge of needles on individual slash pine (*Pinus elliottii* Engelm.) branches and in whole forest canopies was measured with a spectroradiometer. Branches were measured on the ground against a spectrally flat reflectance target and canopies were measured from observation towers against a spectrally variable understory and forest floor. There was a linear relationship between red edge and chlorophyll content of branches ($R^2 = 0.91$). Measurements of the red edge and this relationship were used to estimate the chlorophyll content of other branches with an error that was lower than that associated with the colorimetric (laboratory) method. There was no relationship between the red edge and the chlorophyll content of whole canopies. This can be explained by the overriding influence of the understory and forest floor, an influence that was illustrated by spectral mixture modeling.

The results suggest that the red edge could be used to estimate the chlorophyll content in branches but it is unlikely to be of value for the estimation of chlorophyll content in canopies unless the canopy cover is high.

Introduction

The chlorophyll content of a forest canopy can be an indicator of such measures of physiological status as photosynthetic capacity, developmental stage, productivity, and stress (Vernon and Seely 1966, Whittaker and Marks 1975, Danks et al. 1983, Ustin et al. 1988). However, the distribution of chlorophyll within a forest canopy varies in time and three-dimensional space. As a consequence, estimating the chlorophyll content of branches and canopies by extrapolation from individual leaf chlorophyll measurements obtained by destructive sampling is labor intensive and potentially inaccurate. Ideally a method is required that is accurate, nondestructive, simple to use, and is at the spatial scale of the branch or canopy rather than the leaf. The measurement of radiation reflected in visible and near infrared wavelengths may be such a technique.

Chlorophyll absorbs red radiation strongly at a waveband around 680 nm, as a result of electron transitions centered around the magnesium component of the photoactive site of the chlorophyll molecule (Devlin and Witham 1983, Goetz et al.

1983, Schanda 1986). Red reflectance, especially when standardized by reflectance in a non-absorbing waveband, is highly correlated with chlorophyll content (Turrell et al. 1961, Everitt et al. 1985, Thomas and Gausman 1977). Therefore, red reflectance should be a reliable metric for chlorophyll content (Horler et al. 1980, Tsay et al. 1982, Ajai et al. 1983, Demetriades-Shah and Court 1987). In practice, measurements of red reflectance from branches and canopies couple both chlorophyll content and vegetation amount and are very sensitive to the effects of variable irradiance, variable background, the asymptotic form of the red reflectance–chlorophyll relationship, and the geometrical arrangement of the scene, sensor, and surface (Curran 1983, Curran and Milton 1983). A measure of reflectance that is less sensitive to these factors is the point of maximum slope in vegetation reflectance spectra which occurs between wavelengths 690 and 740 nm (Barber and Horler 1981, Ferns et al. 1984, Salisbury et al. 1987). This point defines the "red edge" and marks the boundary between the processes of chlorophyll absorption in red wavelengths and within-leaf scattering in near infrared wavelengths.

The red edge is usually defined on the first or second derivative of reflectance spectra, depending on the signal to noise characteristics of the sensor (Dixit and Ram 1985). The calculation of derivative spectra eliminates additive constants (e.g., illumination changes) and reduces linear functions (e.g., linear increase in background reflectance with wavelength) to constants. This has led researchers to conclude that the red edge is essentially invariant with illumination or the amount of background within the field-of-view of the spectroradiometer. The former is always the case, but the latter conclusion rests on the assumption that the background is spectrally flat, which is usually true in the laboratory (Horler et al. 1983*a*), or increases regularly with wavelength, which is usually true for mineral soils in the field (Demetriades-Shah and Steven 1988, Ustin et al. 1988, 1989). If the reflectance of the background varies nonlinearly with wavelength, then the assumption is invalid and the derivative spectra will not be independent of the background. This point will be developed later in this paper.

The red edge–chlorophyll relationship

Empirical relationships between the red edge and the chlorophyll content of leaves, branches, and canopies have been known for some time (Gates et al. 1965, Howard et al. 1971). Many researchers have used this relationship to explain the movement of the red edge to shorter wavelengths during senescence or stress-induced chlorosis (Collins 1978, Chang and Collins 1983, Collins et al. 1983, Milton et al. 1983, Hare et al. 1984, Rock et al. 1985, Miller et al. 1987, Rock et al. 1988*b*, Milton and Mouat 1989, Milton et al. 1989). The possible range of the red edge is very large compared with other features in vegetation spectra (Curran 1989). For example, a red edge range of 695 to 721 nm was reported for laboratory measurements of English hawthorn leaves as chlorophyll increased from 18 to 66 $\mu g\ cm^{-2}$ (Dockray 1981). The range over which the red edge is sensitive to chlorophyll content is dependent on surface variability, with ranges of around 25, 15 and 5 nm having been reported

for leaves, branches and canopies, respectively (Dockray 1981, Rock et al. 1988*a*, Ustin et al. 1988, Curtiss and Ustin 1989, 1990).

The prevailing explanation for the red edge and its movement is drawn from the Beer-Lambert Law and the principles of gas and liquid spectroscopy. According to the Beer-Lambert Law, a negative exponential relationship exists between chemical concentration (chemical content per unit volume) and absorption (Wiffen 1972, Banwell 1983). The principles of gas and liquid spectroscopy state that the bandwidth of this increased absorption is related to the pressure (i.e., concentration) of the chemical (Goody 1964, Schanda 1986). Therefore, an increase in chlorophyll concentration will cause both deepening and, more importantly, broadening of its absorption feature (and thereby red edge) to longer wavelengths (Dockray 1981, Horler et al. 1983*a*, Rock et al. 1988*a*, Ustin et al. 1988). Therefore, the red edge could be expected to provide an estimate of chlorophyll content up to the absorption maximum, which will occur when the photoreceptive sites are saturated (Gates 1980, Baret et al. 1987).

Problems in measuring the red edge–chlorophyll relationship

Despite a causal relationship between red edge and chlorophyll content, the linear correlation between the two variables is low (Dockray 1981). This has usually been attributed to violation of one of the two implicit assumptions underlying the relationship, or to the effects of a changing leaf chemical content, chlorophyll a/b ratio or fluorescence.

Violation of implicit assumptions

The theoretical relationship between the red edge and chlorophyll content makes many assumptions about the method of measurement. For example, it is assumed that the majority of the radiation received by the sensor from the leaf has been reflected from within the leaf, and the red edge is independent of illumination level. These assumptions hold under usual conditions of illumination, observation and data processing (Salisbury et al. 1987). However, movements of the red edge have been observed that can be attributed to violation of one or both of these assumptions. Typically, these are the result of either measuring a considerable amount of specular radiation, which by definition has not entered the leaf (Vanderbilt et al. 1985, Guyot and Baret 1988), or measuring the movement of the red edge on reflectance spectra rather than derivative reflectance spectra (Schutt et al. 1984, Vanderbilt et al. 1988, Westman and Price 1988). These two examples illustrate the care that is needed if the red edge is to be measured reliably.

Influence of other leaf chemicals on the red edge

Leaves contain a number of chemicals, besides chlorophyll, that absorb radiation in the region of the red edge and could therefore alter the red edge independently of chlorophyll content. The magnitude of this effect is likely to be small unless the chemicals strongly absorb or reflect radiation in the region of the red edge (Curran

1989). This is the case for some red pigments such as amaranthin, which is found in the leaves of *Amaranthus tricolor*. For example, a linear relationship between red edge and chlorophyll concentration for this species was confounded by amaranthin concentrations as low as 0.01 mg g^{-1} (Curran et al., unpublished observations).

Influence of the chlorophyll a/b ratio on the red edge

The absorption maximum of chlorophyll a is at a wavelength that is around 20 nm longer than that of chlorophyll b. Therefore, if the relative proportion of chlorophyll a were to increase there would be a movement of the red edge to longer wavelengths, independent of total chlorophyll content (Guyot and Baret 1988). Likewise, a decrease in the relative proportion of chlorophyll a would result in a movement of the red edge to shorter wavelengths, also independent of total chlorophyll content. The effect of a changing chlorophyll a/b ratio on the red edge is likely to be minor compared to the effect of the total chlorophyll content. Consequently, the effect of a change in chlorophyll a/b ratio on the red edge has proved difficult to observe (Chang and Collins 1983, Horler et al. 1983*a*, 1983*b*). However, its effect has been suspected (Banninger 1989) and in one case quantified (Rock et al. 1988*a*) in a stressed forest canopy.

Influence of fluorescence on the red edge

The chlorophyll fluorescence spectrum has a maximum at 690 nm, which is near to the maximum of a chlorophyll absorption spectrum (Nobel 1983, Lichtenthaler et al. 1986, Rinderle and Lichtenthaler 1989). The fluorescence yield at a wavelength of 690 nm is only a few percent of the absorbed energy and this is partially reabsorbed by chlorophyll before it reaches the leaf surface. However, as chlorophyll content decreases this reabsorption decreases markedly. The result is the slightly enhanced movement of the red edge to shorter wavelengths, but only when the chlorophyll content is very low (Lichtenthaler and Bushmann 1987, Lichtenthaler 1989).

These four effects on the red edge are likely to be minor if measurements are taken with care, and should not severely inhibit our ability to use the red edge to estimate the chlorophyll content of green vegetation (Goetz 1989).

The aim of this study was to explore the relationship between the red edge and the chlorophyll content of foliage in branches and canopies of slash pine (*Pinus elliottii* Engelm.).

Study site

The study site is a 23-year-old (in 1988) slash pine plantation in northern Florida, located approximately 20 km northeast of Gainesville at 29°45′ N and 82°09′ W. The site is essentially flat with a mean elevation of 35 m. The soil is a ultic haplaquod with a low organic matter and nutrient content and a seasonally high water table (USDA 1954). The mean annual rainfall for the area is 1350 mm and the mean annual temperature is 21.7 °C (Gholz et al. 1990). The canopy was open, with a mean

all-sided leaf area index (LAI) of 3.5 (Gholz et al. 1990) at the time the measurements were made. The understory comprised a sparse shrub cover, of under 0.5 LAI, dominated by saw palmetto (*Serenoa repens*) and an even sparser cover of grasses and forbs overlying dead pine needles.

In 1986, sixteen 50 × 50 m plots were established at the study site. Eight of the plots were fertilized quarterly with a complete fertilizer, starting in February 1987, and the other eight plots were left as controls (Gholz et al. 1990).

Red edge and chlorophyll measurements

The red edge and chlorophyll content of intact needles on individual branches and on whole canopies were measured. The relative radiance of the branches or canopy and a white Fiberfrax (ceramic wool fiber) target were recorded with a Spectron SE590 spectroradiometer (Milton 1987). This sensor samples every 2.8 nm from 400–1100 nm. In this case it was used with a 15° field-of-view lens and only the spectral range from 400–850 nm was analyzed. The relative radiance of the branches or canopy and the target were spectrally calibrated, using data from an integrating sphere and narrow waveband filters, then ratioed to provide reflectance spectra. Reflectance spectra were converted to first derivative spectra (Dixit and Ram 1985), to locate the point of maximum change in reflectance with wavelength: the red edge.

Concentrations of chlorophyll a and b were estimated by the colorimetric method of Harborne (1973). Each sample was lyophilized for 48 h, or until a constant weight was reached. Between 10–20 g of needles per sample were ground through a 1-mm mesh screen with a cyclone sample mill and each weighed sample was then ground twice with acetone. The absorption of the resultant liquid at wavelengths of 664 and 667 nm was used to estimate chlorophyll concentration in mg g^{-1} of dry sample. As the weight was related linearly to the area and volume of the leaves, only this measure of concentration was employed.

Two experiments were undertaken using these measurement techniques. The first explored the red edge–chlorophyll relationship for foliage in branches and the second explored the red edge–chlorophyll relationship for foliage in canopies.

The red edge–chlorophyll relationship for branches

One or two branches were shot from the upper canopy of each of the sixteen plots with a Winchester model 43-218 Bee rifle. The foliated portions of nineteen branches were divided into new (< 1 year) and old (> 1 year) to give two samples per branch and a sample size of 38. Each sample was placed at the center of a spectrally flat (white), Fiberfrax target and the relative radiance was measured from a height of 1 m, using a tripod-mounted Spectron SE590 spectroradiometer. A bare Fiberfrax target was also measured. Samples were collected and spectral measurements were made over seven days (February 18–24, 1988), under clear skies and with a solar elevation range of 40 to 47°.

After the spectral measurements had been made, the needles on each sample were stripped from the branch and weighed. They were then bagged and transported on

ice to the University of Florida where they were stored at −74 °C. Once all samples had been collected they were packed in dry ice and flown to NASA/Ames Research Center where they were stored at −18 °C until analyzed for chlorophyll. The chlorophyll concentration was determined and multiplied by the weight of the sample to give a measure of chlorophyll content (mg) per sample. The red edge was determined as described above (Figure 1).

It is unwise to develop a functional or calibration relationship (Curran and Hay 1986) using the data on red edge and chlorophyll content because measurement error exists in both variables (Webster 1989). Replicate measurements indicate that the root-mean-square (rms) error due to imprecision was 2.7 nm (around 3 percent of mean) and 12.3 mg (around 9 percent of mean) for the red edge and chlorophyll content, respectively. Therefore, estimates were made by regression because this allocates all of the error to the estimate of chlorophyll content.

Twenty-eight of the samples were selected at random and used to develop an

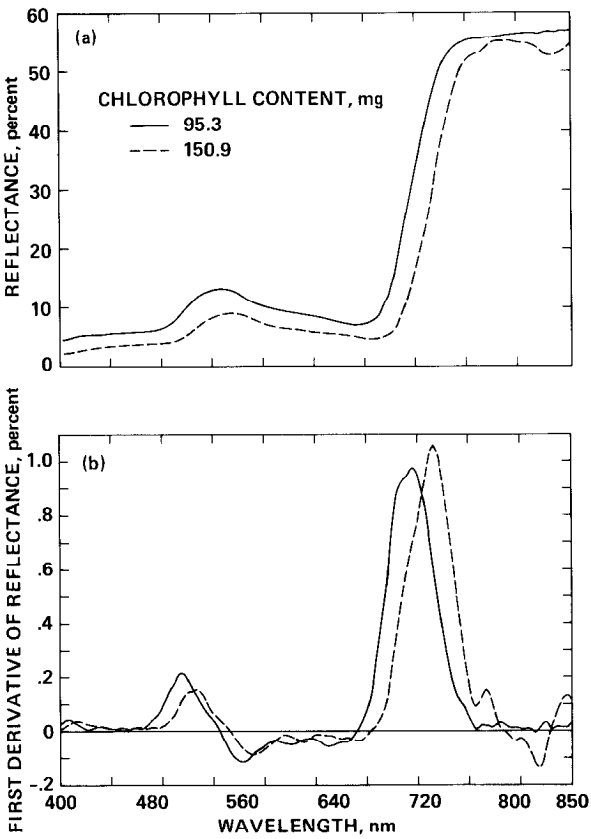

Figure 1. The reflectance spectrum (a) and derivative reflectance spectrum (b) of two slash pine branches, recorded against a spectrally flat Fiberfrax target. Note that the higher chlorophyll content is associated with a red/near infrared boundary that is displaced toward longer wavelengths in (a) and a derivative maximum that is displaced toward longer wavelengths in (b).

estimation equation over the red edge range of 706 to 726 nm (Figure 2).

$$\text{Chlorophyll content (mg)} = -3510.43 + 5.07 \times \text{Red edge (nm)}$$

($n = 28$, $R^2 = 0.91$, standard error of estimate = 10.48 mg)

This equation was used to estimate the chlorophyll content of the remaining ten samples (Figure 3). The rms error of the estimate of chlorophyll content was 7.44 mg (around 6 percent of mean), which was slightly more than half of the error associated with the colorimetric determination of chlorophyll content.

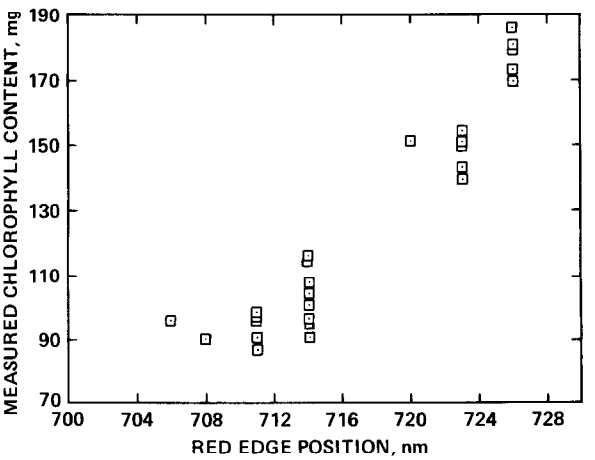

Figure 2. The relationship between red edge and chlorophyll content for 28 slash pine branches, $R^2 = 0.91$.

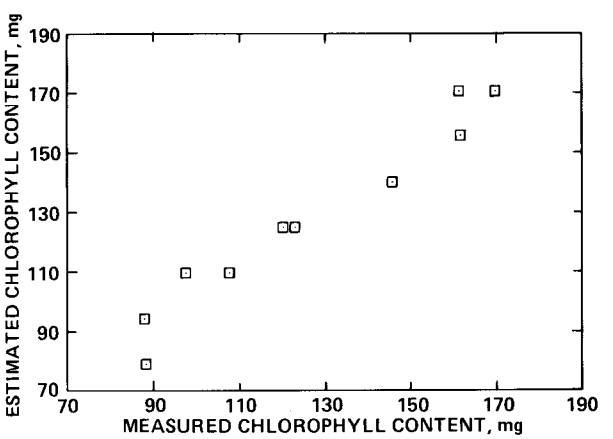

Figure 3. The chlorophyll content of 10 slash pine branches measured in the laboratory and estimated using the red edge in the field.

The procedure was repeated using chlorophyll concentration (mg g^{-1}) in place of chlorophyll content (mg). This resulted in a nonsignificant R^2 (5 percent confidence level) and a near tripling of the standard error of the estimate.

The red edge–chlorophyll relationship for canopies

The relative radiance of a canopy and a Fiberfrax target were measured from four observation towers under the same conditions reported for the branch measurements. The plots surrounding each tower were, 1: fertilized, LAI of 3.69, 2: control, LAI of 3.06, 3: control, LAI of 2.23, and 4: fertilized, LAI of 3.09 (Gholz et al. 1990). Two to eight measurements were made from each tower at up to fifteen different angles of observation and azimuth. This involved pointing the spectroradiometer from vertical to 45° off-vertical over an azimuth range of 0° (upsun) to 180° (downsun).

In each plot, three branches were shot from the measurement-side of the tower. The new and old needles were separated and chlorophyll concentrations (mg g^{-1}) of three new and three old needle samples per plot were determined. As part of a parallel project new and old biomass (kg ha^{-1}) was estimated for each plot by the harvest technique discussed in Gholz et al. (1990). The chlorophyll concentration was multiplied by the biomass to give chlorophyll content for the new and old biomass per unit area (kg ha^{-1}). There was, on average, a higher chlorophyll concentration and content in the fertilized plots (Table 1).

The red edge was very similar for all four plots, with a mean value of around 713 nm when looking vertically, to around 716 nm when looking obliquely. There was no change in the red edge that was attributable to the azimuth of observation. These effects are illustrated using the results from Tower 3 (Figure 4). For the four towers, the maximum red edge had a lower variability than the minimum red edge and these were on average 1.2 and 2.1 nm, respectively. Both the minimum and maximum average red edge were slightly higher (but not significantly, 95 percent confidence level), by 0.6 nm for Plot 4 (fertilized).

Most of the variability in the red edge was attributable to the amount of canopy within the field-of-view of the spectroradiometer, not the chlorophyll in the canopy. The effect of canopy cover on the red edge can be illustrated using a simple spectral mixture model in which multiple scattering is assumed to be invariant with wave-

Table 1. The ranges, at one standard deviation, of chlorophyll concentration and content on the four plots.

Plot number	Treatment	Chlorophyll	
		Concentration (mg g^{-1})	Content (kg ha^{-1})
1	Fertilized	1.36 – 1.68	2.40 – 3.05
2	Control	1.22 – 1.69	1.86 – 2.61
3	Control	0.80 – 1.41	1.13 – 1.80
4	Fertilized	1.16 – 1.85	1.83 – 2.94

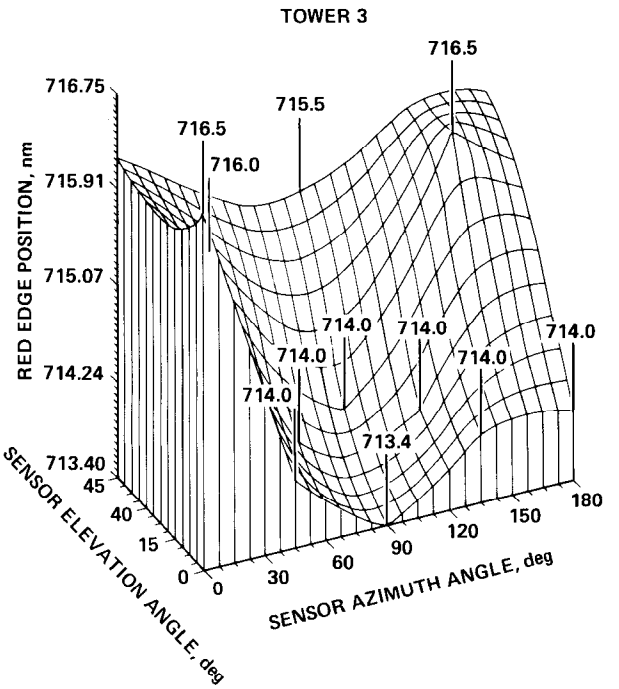

Figure 4. The red edge of a slash pine canopy recorded from Tower 3, one of the four observation towers used in the experiment. Note that the red edge is related to elevation angle but not azimuth angle.

length. The model considers a canopy of live pine needles (red edge of 722 nm) above a background of live palmetto (red edge of 710 nm) and dead pine needles (red edge of 684 nm). A spectrum representative of each of these three cover types is illustrated in Figure 5.

Four models were developed to encompass the range of likely backgrounds (Table 2). For each model the reflectance spectra and derivative reflectance spectra were calculated for the composite scene assuming a canopy coverage of live pine needles between 0 to 100 percent (Figures 6 to 9). The reflectance spectra for each model illustrate the negative relationship between reflectance and canopy cover (Figures 6a–9a) and comparison between the reflectance plots illustrates the positive relationship between reflectance and the proportion of dead pine needles in the background (Figures 6a–9a). The combined effect of these two relationships on the red edge can be seen in the derivative reflectance spectra (Figures 6b–9b).

The red edge of the canopy is dependent on the material in the background. If the background has a flat derivative spectrum in the region of the red edge of the live pine needles in the canopy (e.g., dead pine needles), the red edge of the canopy will dominate at all but low levels of canopy cover. At these low levels of canopy cover the red edge will be that of the background rather than the canopy. If the background has a red edge in the region of the red edge of the live pine needles in the canopy (e.g., live palmetto) then the red edge will be determined by a simple average of the

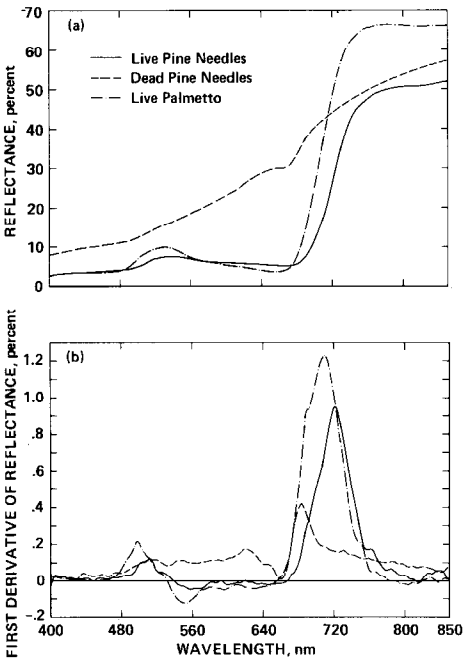

Figure 5. The reflectance spectrum (a) and derivative reflectance spectrum (b) of the three materials used in spectral mixture modeling (Figures 6 to 9).

Table 2. The four spectral mixture models. Each model has a canopy of live pine needles that ranges from 0 to 100 percent cover. The differences between the models are determined by the material in the background, from a complete coverage of dead pine needles in model A to a complete coverage of live palmetto in model D. The spectra developed with these models are presented in Figures 6 to 9.

Model	Material within sensor field-of-view, percent cover		
	Live pine needles	Dead pine needles	Palmetto
A	0 – 100	100 – 0	0
B	0 – 100	75 – 0	25 – 0
C	0 – 100	50 – 0	50 – 0
D	0 – 100	0	100 – 0

two. These trends are illustrated in the derivative spectra of the four models (Figures 6b–9b) and a summary plot (Figure 10). In Figure 10, a high proportion of dead pine needles in the background (models A and B) is associated with an abrupt change in red edge at a canopy cover of between 20 and 50 percent, and a high proportion of live palmetto in the background (models C and D) is associated with a gradual change in red edge over the range of canopy covers. This abrupt change in the red edge at a low canopy cover is related to the definition of the red edge rather than to an abrupt change in the way in which radiation interacts with a forest canopy. This

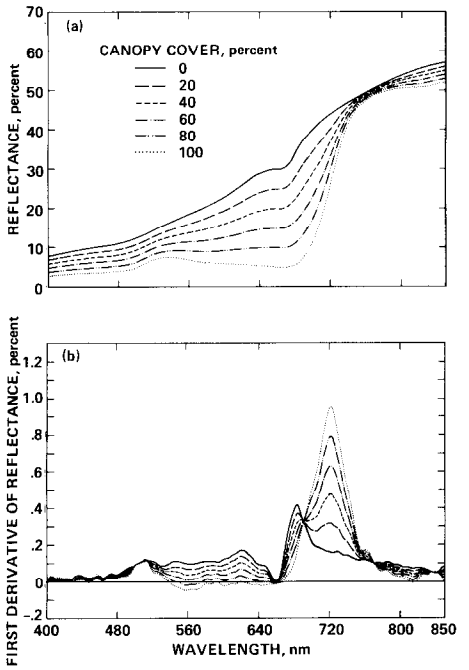

Figure 6. The reflectance spectra (a) and derivative reflectance spectra (b) for model A (Table 2), in which the canopy cover is varied from 0–100 percent over a background of dead pine needles.

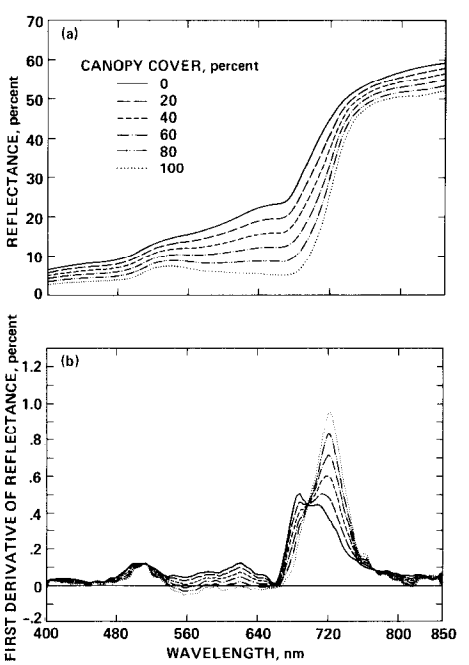

Figure 7. The reflectance spectra (a) and derivative reflectance spectra (b) for model B (Table 2), in which the canopy cover is varied from 0–100 percent over a background of three-quarters dead pine needles and one-quarter live palmetto.

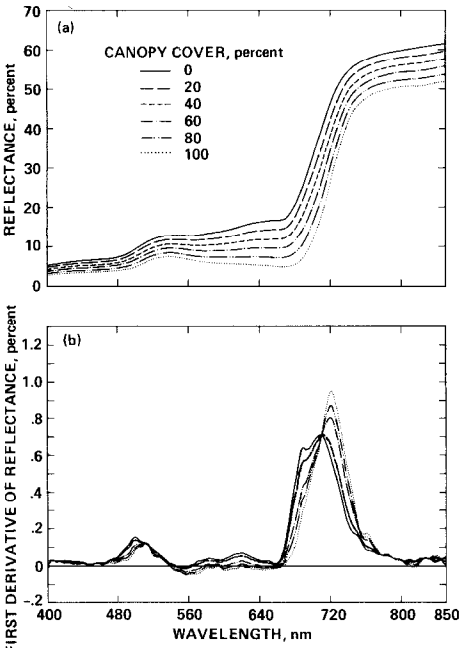

Figure 8. The reflectance spectra (a) and derivative reflectance spectra (b) for model C (Table 2), in which the canopy cover is varied from 0–100 percent over a background of one-half dead pine needles and one-half live palmetto.

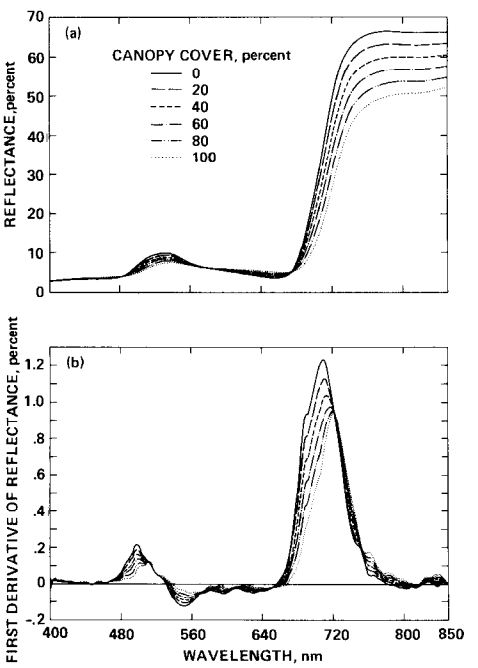

Figure 9. The reflectance spectra (a) and derivative reflectance spectra (b) for model D (Table 2), in which the canopy cover is varied from 0–100 percent over a background of live palmetto.

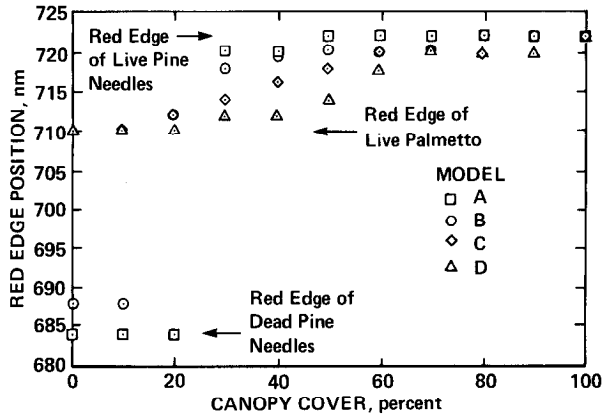

Figure 10. The relationship between canopy cover and red edge for the four models A, B, C and D (Figures 6 to 9).

previously unreported artifact of the red edge definition has implications for the use of the red edge when vegetation cover is low and the background is organic.

These four models led us to the generalization that the red edge of forest canopies can be considered independent of the background in two cases. The first case is when the canopy cover is greater than approximately 75 percent and the background has a red edge in the region of the red edge of the canopy. The second case is when the canopy cover is greater than approximately 25 percent and the background has a flat derivative spectrum in the region of the red edge of the canopy.

In this study, the canopy cover within the field-of-view of the spectroradiometer ranged from 0 to 100 percent when looking vertically to 100 percent when looking at an angle of 45°. The mixture modeling of the three main materials helped to explain why the red edge recorded vertically was both lower and more variable (713 ± 2.1 nm) than the red edge recorded obliquely (716 ± 1.2 nm) and under what conditions the red edge of the background can dominate the red edge of the forest canopy.

Conclusions

There was a linear relationship between the red edge and chlorophyll content of detached branches measured against a spectrally flat background ($R^2 = 0.91$). The red edge was used to estimate the chlorophyll content of branches with an rms error of 7.44 mg, which was slightly more than half the error associated with the colorimetric determination of chlorophyll content.

There was no relationship between the red edge and the chlorophyll content of whole canopies. This was likely due to the overriding influence of the background.

Spectral mixture modeling demonstrated interrelationships between red edge, canopy cover, and background. For example, if the background had a red edge that was similar to that of the canopy then the red edge of the canopy would be a simple

average of the two, but if the background had a red edge that was dissimilar to that of the canopy then the red edge of the canopy would be a discontinuous function with canopy cover.

Acknowledgments

This work was partially funded by the NASA Earth Sciences Applications Division while PJC held a Senior NRC/NASA Research Associateship at NASA Ames Research Center under a grant from the NASA Life Sciences Division and by NSF Grant number 8106678. The authors thank Wendell Cropper Jr., David Delgado, David Nolletti, and Sherry Vogel, Department of Forestry, University of Florida, for assistance in the field; Pam Matson, Tina Billow, and Kelly Dao, NASA Ames Research Center, for assistance in the laboratory; Pierson Chiou and Xoan Trinh, NASA Ames Research Center, for assistance with data processing; Laurie Richardson, NASA Ames Research Center, for providing Spectron calibration measurements, and Dave Peterson and Vern Vanderbilt, NASA Ames Research Center, for their comments on the manuscript.

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