Exploring the relationship between reflectance red edge and chlorophyll concentration in slash pine leaves

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Summary Chlorophyll concentration is related positively to the point of maximum slope in the reflectance spectra of leaves and this point is termed the red edge. The reflectance spectra of slash pine (Pinus elliottii Engelm.) needles were measured in the field and the chlorophyll concentrations of the same needles were measured in the laboratory. The measurement errors for red edge and chlorophyll concentration were determined to be 2.2 nm (3% of mean) and 0.35 mg g⁻¹ (19% of mean), respectively. The red edge–chlorophyll concentration relationship was strong (r² = 0.82, n = 152). A red edge–chlorophyll concentration relationship for n = 100 was used with red edge measurements to estimate chlorophyll concentration with an rms error of 0.31 mg g⁻¹ (17% of mean, n = 52). The entire red edge–chlorophyll concentration relationship for n = 152 was also used with red edge measurements to estimate the chlorophyll concentration of detached needles in the field with an accuracy similar to that obtained by conventional laboratory measurements.

Keywords: Pinus elliottii, reflectance spectroscopy.

Introduction

Measurements of chlorophyll concentration are normally made in a laboratory and involve several physical and chemical extraction procedures followed by measurement of red light transmission through a chlorophyll solution (Yoder and Daley 1990). These laboratory procedures are laborious and result in a delay between field sampling and measurement (Palta 1990). Estimates of chlorophyll concentration can be made in the field with optical sensors that record red light transmission while clamped on to a leaf (Hardacre et al. 1984, Yadava 1986), although these sensors are unsuitable for leaves located several meters above the ground.

Reflectance spectroscopy has been used in the laboratory to estimate the chlorophyll content of leaves (Milton et al. 1991, Chappelle et al. 1992, Hoque and Hutzler 1992) and it may be possible to use this technique in the field (Milton 1987, Mundon et al. 1994). A single measure of reflectance in red wavelengths is unsuitable because it couples chlorophyll concentration with needle mass and is sensitive to the effects of variable irradiance and needle and canopy structure (Curran 1983). A measure that is less sensitive to these factors is the point of maximum slope in a vegetation reflectance spectrum between 690 and 740 nm (Miller et al. 1990, Plummer et al. 1991). This point is termed the red edge and marks the boundary between the processes of chlorophyll absorption in red wavelengths and within-leaf scattering in near infrared wavelengths (Curran et al. 1990, 1991). An increase in chlorophyll concentration causes a broadening of the chlorophyll absorption feature (Banninger 1991), which moves the long wavelength boundary of the chlorophyll absorption feature and thus the red edge to longer wavelengths (Horler et al. 1983, Boocok et al. 1990, Baret et al. 1992). Therefore, the red edge might provide an estimate of chlorophyll concentration up to the absorption maximum that will occur when the photoreceptive sites are saturated (Gates 1980, Bushmann and Nagel 1993). The red edge is calculated on the first (or occasionally second) derivative of the reflectance spectrum because this locates the red edge and eliminates additive constants caused, for example, by changes in irradiance (Demetriades-Shah et al. 1990, Chen et al. 1992).

In a previous study (Curran et al. 1990), we examined the relationship between red edge and chlorophyll content for slash pine (Pinus elliottii Engelm.). We found that the relationship was weak at the canopy level but strong at the branch level (r² = 0.91) despite shortcomings in the experimental design (small sample size, n = 38), a small range of chlorophyll concentrations (0.80–1.85 mg g⁻¹), variable sample weight (70–120 g) and the use of branches rather than needles. Despite these limitations the results suggested that field troradiometric techniques could be used to estimate the chlorophyll content of slash pine needles in the field.

The aim of the experiment reported here was to refine this technique to determine more precisely the nature of the relationship between red edge and chlorophyll concentration.
Materials and methods

Study site

The 24-year-old (in 1989) slash pine plantation was located approximately 20 km northeast of Gainesville, northern Florida, at 29°45′ N and 82°9′ W. In 1986, sixteen 50 × 50 m plots were established. In February 1987, trees in eight of the plots were maintained without fertilization to serve as controls, whereas trees in the remaining eight plots were fertilized by quarterly applications of a balanced N,P,K fertilizer (Gholz et al. 1990).

Methods

Five or six branches (one per tree) were shot from the upper canopy of each of the 16 plots between July 14 and 16, 1989. The foliated portions of 85 branches were divided into new (< 1 year) and old (> 1 year) to give two samples per branch and a sample size of 170. The needles on each sample were stripped from the branch, shaken, weighed and divided into two subsamples. The subsamples were bagged and transported to the University of Florida where they were stored at −18 °C, and the other set of subsamples was flown to the NASA, Ames Research Center in California where they were stored at −74 °C. When all of the samples had been collected, they were packed in dry ice, and one set of subsamples was flown to the NASA, Ames Research Center in California where they were stored at −74 °C and the other set of subsamples was flown to the USDA, Richard B. Russell Agricultural Research Center in Georgia where they were lyophilized and ground.

Spectral measurements

At NASA, Ames Research Center, a 50-g portion of needles was taken from each mixed subsample. The needles were placed at the center of a spectrally flat (white) Fiberfrax target (ceramic wool) (Curran et al. 1990), and the relative radiances of the needles plus target and the target alone were measured within 2 h of solar noon under clear skies with a Spectron SE 590 spectroradiometer (Milton 1987). The sensor, which records from 400–1100 nm with a 2.8 nm spectral resolution, was used with a 15° field-of-view lens from a height of 1 m. For each of the 170 samples, relative radiances of the needles plus target and the target alone over the spectral range 400–850 nm were spectrally calibrated against data from an integrating sphere and narrow waveband filters. The ratio (spectrum for needles plus target/spectrum for target alone) provided a reflectance spectrum. Because there was a slight spectral mismatch between the spectra from the two spectroradiometers used, the spectra from the second spectroradiometer were matched to that of the first based on the water absorption feature located at 970 nm. About half of the spectra recorded by the second spectroradiometer (n = 18) were rejected because they had a mismatch greater than 3 nm, thus reducing the total sample size to 152.

To locate the red edge (the point of maximum slope), the reflectance spectra were converted to first derivative spectra (Dixit and Ram 1985). Because of the way in which the spectroradiometer sampled the spectra, the resulting red edge values were at eleven discontinuous points along the transition region between red absorption and near infrared reflectance.

These points ranged between 704 and 726 nm and their distribution had a slight statistically insignificant negative skew (cf. Curran et al. 1990). For three of the samples, 10 replicate spectra were recorded in the field and these had an average root-mean-square (rms) error of 2.2 nm, which was 3% of the mean.

Water measurements

Immediately after each spectral measurement was made, the 50-g portion of needles was reweighed, dried to a constant weight and then reweighed to determine the water concentration (Table 1). This varied very little with a mean (x̄) = 58.8%, a standard deviation (σ) = 3.9% and a coefficient of variation (cv) = 6.6% for n = 152. This cv is only slightly larger than the precision of measurement which was 4.2% (7.1% of mean) for 10 replicates from five 50-g needle samples.

Chlorophyll measurements

At the USDA, Richard B. Russell Agricultural Research Center, the chlorophyll concentration of a 10-g portion of the lyophilized and ground subsample was determined with an NIRS system 6500 monochrometer. The instrument was calibrated against spinach samples of known chlorophyll concentration. The results were expressed as mg of chlorophyll per g of dry ground leaves (mg g⁻¹). The standard error of the estimate for measures of chlorophyll concentration was 0.35 mg g⁻¹ (19% of the mean). Chlorophyll concentration varied from 0.05 to 3.32 mg g⁻¹ and, like the red edge values, had a distribution with a slight but insignificant negative skew.

Results and discussion

Newly emerged needles on trees in the control (unfertilized) plots had a mean chlorophyll concentration of 1.25 mg g⁻¹ and a mean red edge of 710 nm (Table 1). Mature needles on trees in the fertilized plots had a mean chlorophyll concentration of 2.80 mg g⁻¹ and a mean red edge of 725 nm (Table 1). There was a positive relationship between chlorophyll concentration ([Chl] in mg g⁻¹) and red edge (RE in nm) (Figure 1). The predictive regression had the form (n = 152, r² = 0.82, standard error of the estimate = 0.36 mg g⁻¹):

\[
[\text{Chl}] = -68.26 + 0.098 \text{RE. (1)}
\]

Two analyses were performed to evaluate the predictive value of the red edge–chlorophyll relationship. In the first analysis, the relationship was recalculated for 100 randomly selected samples and used with the red edge measurements to estimate the chlorophyll concentration of the remaining 52 samples. The predictive regression had the form (n = 100, r² = 0.83, standard error of the estimate = 0.32 mg g⁻¹):

\[
[\text{Chl}] = -68.17 + 0.098 \text{RE. (2)}
\]

The rms error of estimated chlorophyll concentration in the 52 samples was 0.31 mg g⁻¹ (17% of mean). This was larger than observed by Curran et al. (1990), but similar in magnitude.
to the error associated with the measurement of chlorophyll concentration in the laboratory.

In the second analysis, Equation 1 \((n = 152)\) was used with the relevant red edge values to estimate the chlorophyll concentration of the 38 samples described by Curran et al. (1990). There were several differences in design between the two experiments, the main one being the measurement of spectra for a whole branch in the 1990 experiment compared with the measurement of spectra for needles only in the current experiment. The rms error of the estimated chlorophyll concentration in the 38 samples was 0.47 mg g\(^{-1}\) (30% of mean). The measurement bias was approximately equal to the amount by which the relationship between estimated chlorophyll concentration and measured chlorophyll concentration departed from a 1/1 relationship. When this underestimation of 0.21 mg g\(^{-1}\) was added to each estimate of chlorophyll concentration, the rms error decreased to 0.32 mg g\(^{-1}\) (21% of mean).

**Conclusions**

There was a linear relationship between red edge and chlorophyll concentration of needles measured against a spectrally flat background \((r^2 = 0.82)\). The scatter of points around this relationship was primarily the result of error in the measurement of chlorophyll concentration (known error of 0.35 mg g\(^{-1}\), 19% of mean) rather than the measurement of red edge (known error of 2.2 nm, 3% of mean).

When used for the estimation of chlorophyll concentration, the relationship between red edge and chlorophyll concentration resulted in an error of 0.31 mg g\(^{-1}\) (17% of mean) for a subsample \((n = 52)\) of the data set and 0.47 mg g\(^{-1}\) (30% of mean) for data from an earlier experiment \((n = 38)\).

The results suggest that the red edge could be used to estimate the chlorophyll concentration of needles in the field. The technique was simple to implement and had an accuracy

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similar to that obtained by traditional assay of chlorophyll in the laboratory.

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References


