

Optimal reflectance, transmittance, and absorptance wavebands and band ratios  
for the estimation of leaf chlorophyll concentration

Gregory A. Carter and Bruce A. Spiering  
Earth System Science Office, NASA, Stennis Space Center, MS 39529  
Phone 228-688-1918; e-mail [gcarter@ssc.nasa.gov](mailto:gcarter@ssc.nasa.gov)

**Abstract**

Several recent studies conclude that leaf reflectance is most effective in estimating leaf chlorophyll concentration at wavelengths near 550 and 700 nm. This result can be explained by the *in vivo* absorption properties of chlorophyll. However, some studies have shown exceptions to this general result, and relationships of leaf transmittance or absorptance with chlorophyll have received little attention. The present study utilized regression analyses to identify: 1) wavebands and band ratios within the 400-850 nm range that could be used to estimate total chlorophyll concentration with minimal error, and 2) simple regression models that were most effective in estimating chlorophyll concentration. Optical properties and chlorophyll concentrations were measured for two broadleaved tree species (*Liquidambar styraciflua* L. and *Acer rubrum* L.), a broadleaved vine (*Vitis rotundifolia* Michx.), a needle-leaved conifer (*Pinus palustris* Miller), and a representative of the grass family (*Arundinaria gigantea* (Walter) Muhl.). Overall, reflectance, transmittance, and absorptance corresponded most precisely with chlorophyll concentration at wavelengths near 700 nm, although regressions were strong as well in the 550-625 nm range. Dividing reflectances or transmittances at best-fit wavelengths by maximal values that occurred at approximately 850 nm improved chlorophyll estimation only slightly in most cases. This was true also when absorptances at best-fit wavelengths were divided by maximal absorptances near 400 nm. Regressions based on a power function were superior to simple linear regressions in yielding low standard errors of the estimate (*s*). When data for all broadleaved species were combined, *s* at best-fit wavelengths of 707-709 nm were low at approximately 50  $\mu\text{mol}/\text{m}^2$  out of a 940  $\mu\text{mol}/\text{m}^2$  range. Minimal *s*, ranging among species from 32-62  $\mu\text{mol}/\text{m}^2$  of chlorophyll, were obtained when the power model was used with band ratios having numerator wavelengths that ranged from 693-720 nm. Results indicate that leaf optical properties near 700 nm are most effective in estimating leaf chlorophyll concentration.

**Key words:** leaf optics; light; remote sensing; *Acer rubrum*; *Arundinaria gigantea*; *Liquidambar styraciflua*; *Pinus palustris*; *Vitis rotundifolia*.

## Introduction

Improved methods in the remote sensing of chlorophyll and other pigments may provide greater insight into plant physiological state at a variety of scales (for review see Carter 1998). Several recent studies have evaluated relationships of leaf chlorophyll concentration with leaf reflectance or derived reflectance indices throughout the visible to near-infrared spectrum at high spectral resolution. Results of these studies indicate that the strongest relationships with chlorophyll occur in the green spectrum near 550 nm (Blackburn 1999, Buschmann and Nagel 1993), in the far-red spectrum near 700 nm (Carter et al. 1995, 2000, Chappelle et al. 1992, Datt 1999, Luther and Carroll 1999, Yoder and Pettigrew-Crosby 1995), or that reflectances in these spectral regions are approximately equal in sensitivity to chlorophyll (Carter and Knapp 2001, Datt 1998, Gitelson and Merzlyak 1994, 1996, 1997, Gitelson et al. 1996, Lichtenthaler et al. 1996, McMurtrey et al. 1994). However, others report chlorophyll to be estimated most effectively by indices based on reflectance near 680 nm (Blackburn 1998a, 1998b). Although relationships of chlorophyll concentration with leaf transmittance or absorptance have received far less attention, a few studies indicate relationships with chlorophyll to be most precise near 700 nm (Carter et al. 2000, Carter and Knapp 2001, Yoder and Daley 1990). In order to provide additional insight regarding optimal spectral regions and simple indices for the estimation of leaf chlorophyll concentration, the present study evaluated optical properties and chlorophyll concentrations in mature leaves at various stages of senescence in five species common to the southeastern US. The approach focused on leaf reflectance ( $R$ ), transmittance ( $T$ ), absorptance ( $A$ ), and simple band ratios. Computationally more complex derivative-based techniques such as those described previously (e.g., Datt 1999) are not addressed herein.

## Objectives

The objectives of this study were to: 1) determine the narrow wavebands ( $\sim 1.6$  nm) within the 400-850 nm wavelength ( $\lambda$ ) range in which leaf chlorophyll concentration corresponds most strongly with leaf  $R$ ,  $T$ , and  $A$ ; 2) determine the band ratios which correspond most strongly with leaf chlorophyll concentration, and 3) evaluate variability in these results among species and regression models.

## Materials and Methods

Relationships of leaf chlorophyll concentration with leaf optical properties were examined for leaves that were at various stages of senescence in five species. Mature leaves of sweetgum (Liquidambar styraciflua L.), red maple (Acer rubrum L.), wild grape (Vitis rotundifolia Michx.), switchcane (Arundinaria gigantea (Walter) Muhl.) and longleaf pine (Pinus palustris Miller) that ranged in color from green to yellow were collected from the woodlands of Stennis Space Center during December, 1998 through February, 1999. All leaves had been produced during the 1998 growing season. For  $N = 42$  leaves per broadleaved species,  $R$  and  $T$  were measured throughout the 400-850 nm spectrum using a spectroradiometer (model 1500, Geophysical Environmental Research Corp., Millbrook, New York, USA) attached via fiber optic to an integrating sphere (model LI1800-12S, LI-COR, Inc., Lincoln, Nebraska, USA) and methods described earlier (Mesarch et al. 1999). A leaf was clamped into position over the sample port on the sphere wall and a  $1.65 \text{ cm}^2$  leaf area irradiated by the beam from a tungsten halogen lamp. Light reflected from the leaf was transmitted from the sphere interior through the fiber optic to the spectroradiometer for measurement of reflected spectral radiance. The

spectroradiometer recorded data at wavelength intervals of ~1.6 nm. Similar measurements were made for stray light caused by imperfect collimation of the lamp beam and light reflected from a white reference while the adaxial leaf surface faced the sphere interior (Spectralon SRT-05-99, Labsphere, Inc., North Sutton, New Hampshire, USA). Spectral  $R$  was computed by subtracting stray light radiance from the radiances reflected by the leaf and reference, then dividing leaf reflected radiance by reference reflected radiance. This quantity was multiplied by 100 to yield units of %.  $T$  was measured by illuminating the adaxial leaf surface such that light passed through the leaf into the integrating sphere. Radiance reflected from the white reference was measured while the abaxial surface faced the sphere interior. Transmitted radiance was multiplied by 100 and divided by reference radiance to yield  $T$  in units of %. For longleaf pine,  $R$  and  $T$  were measured for 42 samples. Each sample was composed of 5-6 needles spaced ~1 mm or less apart and arranged in parallel across the sample port of the integrating sphere. Reflected and transmitted radiances were recorded as above. An additional transmission scan was taken without needles in the sample holder to enable the correction of radiance values for light that passed between needles (Mesarch et al. 1999). A high-resolution digital camera and image processing software (ENVI v. 3.1, Research Systems, Inc., Boulder, Colorado, USA) were used to determine the percentage of irradiance that was not intercepted by the needles. In all species,  $A$  was computed as  $100 - (R + T)$ .

**Chlorophyll extraction**-- After leaf optical properties were measured, chlorophyll concentrations of the same leaves were determined. Six circular disks, each 6.25 mm in diameter, were punched from the leaf portion for which optical properties were measured. The disks were placed immediately into 8 mL of 100% methanol, and pigments were allowed to extract in the dark at 30° C for 24 h. Absorbances of the clear extract at 652.0, 665.2, and 750 nm were recorded and concentrations of chlorophylls  $a$ ,  $b$ , and  $a + b$  were computed after Porra et al. (1989). Chlorophyll concentration of the extract and the total disk surface area of 1.84 cm<sup>2</sup> were used to compute leaf chlorophyll concentrations per unit projected leaf area. Total projected leaf areas for computing chlorophyll concentration in pine needles were determined by use of the digital camera and image analysis.

**Statistical analysis**--Coefficients of determination ( $r^2$ ) and standard errors of estimation ( $s$ ) were used to evaluate linear and non-linear relationships of leaf chlorophyll concentration with  $R$ ,  $T$ ,  $A$ , and band ratios at 1.6 nm wavelength intervals throughout the 400-850 nm spectrum. The reported  $r^2$  values were adjusted downward slightly to account for the number of model parameters (SAS 6.0, SAS Institute, Cary, NC, USA; Table Curve 2D v. 4.0, SPSS Inc., Chicago, IL, USA). Analyses were conducted for samples combined among species as well as individual species.

## Results

**Combined Species** -- Because the statistical procedures were identical and results were similar among species, the analytical procedures used for each species are demonstrated below using data combined among the four broadleaved species. When a simple linear function was employed for regressions of leaf total chlorophyll concentration with  $R$ ,  $T$ , or  $A$ , maximum  $r^2$  occurred in the 706-715 nm range (Fig. 1). When curvature of the regression was allowed by using a quadratic function,

maximum  $r^2$  occurred at 709 nm for  $R$  and  $A$  and 603 nm for  $T$ , although the relationship with  $T$  at 703 nm ( $T_{703}$ ) was essentially as strong (Fig. 1). Interestingly,  $r^2$  minima occurred near 675 nm in all cases.

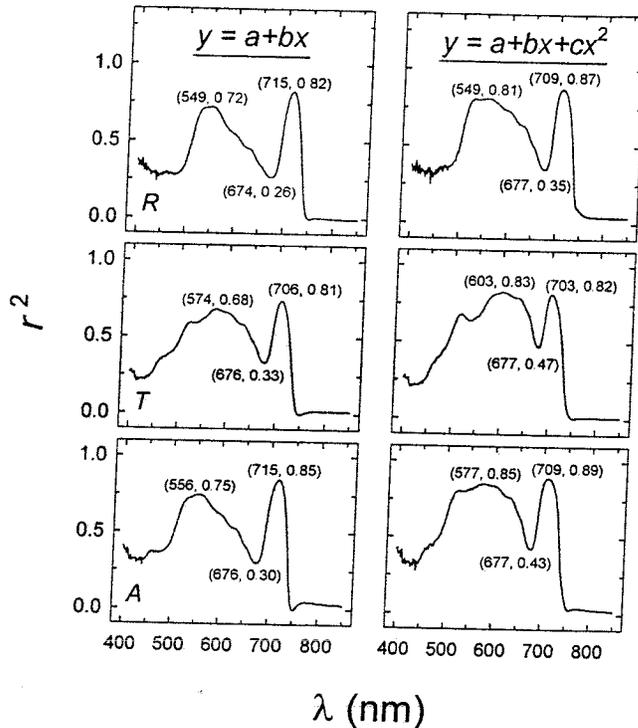


Figure 1. Coefficient of determination ( $r^2$ ) versus wavelength ( $\lambda$ ) for relationships of leaf chlorophyll concentration with leaf reflectance ( $R$ ), transmittance ( $T$ ), and absorbance ( $A$ ). Regressions were based on linear or quadratic models as indicated atop the figure and data combined among the four broadleaved species.

Optimal  $\lambda$  of  $R$ ,  $T$ , and  $A$  to be used in denominators of simple band ratios were identified by dividing  $R$ ,  $T$ , or  $A$  at the best-fit  $\lambda$  indicated in Fig. 1 by  $R$ ,  $T$ , or  $A$  at each  $\lambda$  throughout the 400-850 nm range and regressing total chlorophyll concentration against ratio value. The resulting  $r^2$  were greatest when the denominator incorporated near-infrared  $R$  or  $T$ , or  $A$  from the violet-blue spectrum (Fig. 2). This was true when either the linear or quadratic regression functions were employed.

As a result of the analysis demonstrated in Fig. 2, best-fit ratios were determined by dividing  $R$  or  $T$  at each  $\lambda$  by  $R_{850}$  or  $T_{850}$ , or  $A$  at each  $\lambda$  by  $A_{400}$ . Leaf chlorophyll concentration then was regressed with the resulting ratio values, and  $r^2$  was evaluated with respect to numerator  $\lambda$  (Fig. 3). Maximum  $r^2$  resulting from the simple linear regression occurred at numerator  $\lambda$  of 715-720 nm. Regressions using the quadratic function yielded maximum  $r^2$  at somewhat shorter  $\lambda$  of 713 and 709 nm for  $R$  and  $A$  ratios, respectively. For  $T_{\lambda}/T_{850}$  ratios, the quadratic regression yielded  $r^2$  maxima at 603 and 703 nm. As in Fig. 1,  $r^2$  minima occurred consistently at numerator  $\lambda$  near 675 nm.

Best-fit  $\lambda$  for the quadratic regressions of chlorophyll with  $R$ ,  $T$ ,  $A$ , and ratio values were used as reference points in an iterative process that determined an optimal regression function. In evaluating results from a variety of simple regression functions (Table Curve 2D), it was determined that a power function ( $y = a + bx^c$ ) was superior in yielding precise regression curves among all species and for data combined among the four broadleaved species. The results of this procedure yielded best-fit power regressions when  $\lambda$  for  $R$ ,  $T$ ,

and  $A$  were 706, 698, and 707 nm, respectively, and numerator  $\lambda$  for  $R$ ,  $T$ , and  $A$  band ratios were 709, 707, and 707 nm, respectively (Fig. 4).

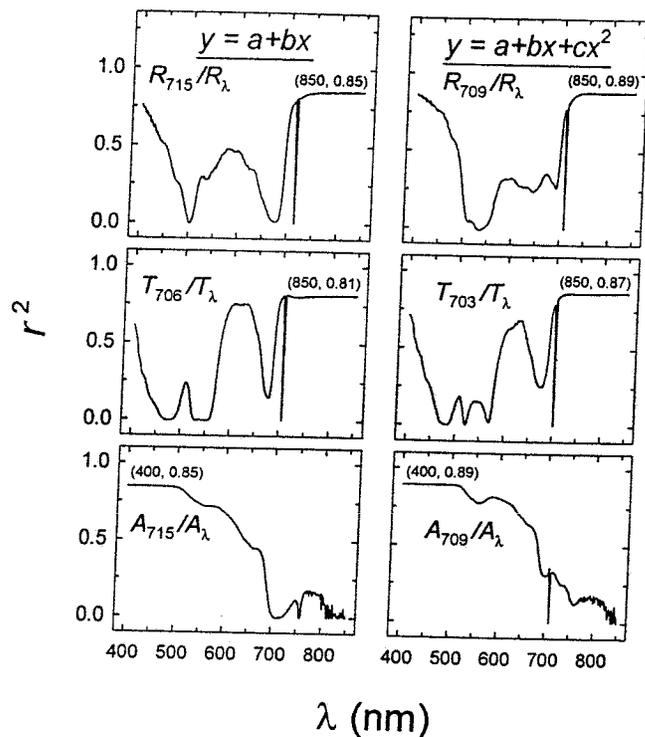


Figure 2. Coefficient of determination ( $r^2$ ) versus wavelength ( $\lambda$ ) for relationships of leaf chlorophyll concentration with leaf reflectance ( $R$ ), transmittance ( $T$ ), and absorbance ( $A$ ) ratios. Regressions were based on linear or quadratic models as indicated atop the figure and data combined among the four broadleaved species. Ratios were computed by dividing  $R$ ,  $T$ , or  $A$  at the best-fit  $\lambda$  indicated in Fig. 1 by  $R$ ,  $T$ , or  $A$ , respectively, at each  $\lambda$  throughout the 400-850 nm spectrum. The  $r^2=0$  spikes near 700 nm resulted when numerator and denominator  $\lambda$  were identical

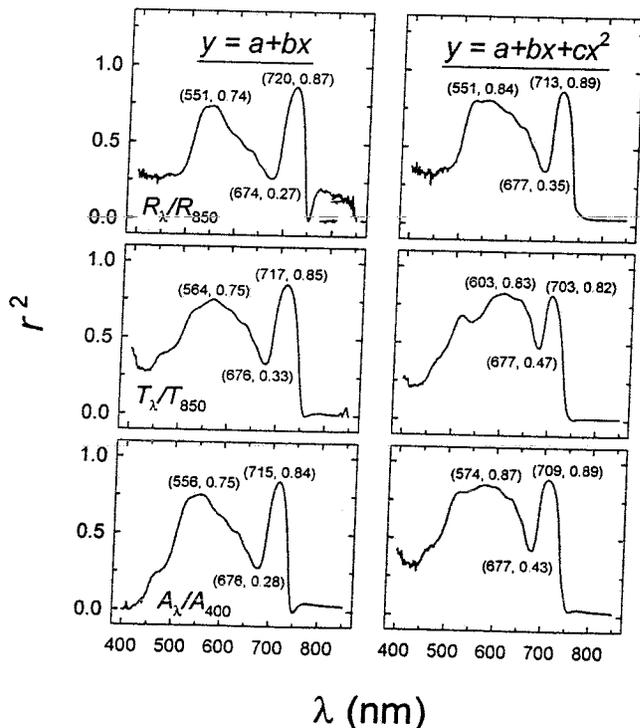


Figure 3. Coefficient of determination ( $r^2$ ) versus wavelength ( $\lambda$ ) for relationships of leaf chlorophyll concentration with leaf reflectance ( $R$ ), transmittance ( $T$ ), and absorbance ( $A$ ) ratios. Regressions were based on linear or quadratic models as indicated atop the figure and data combined among the four broadleaved species. Ratios were computed by dividing  $R$ ,  $T$ , or  $A$  at each  $\lambda$  by  $R$  or  $T$  at 850 nm, or  $A$  at 400 nm.

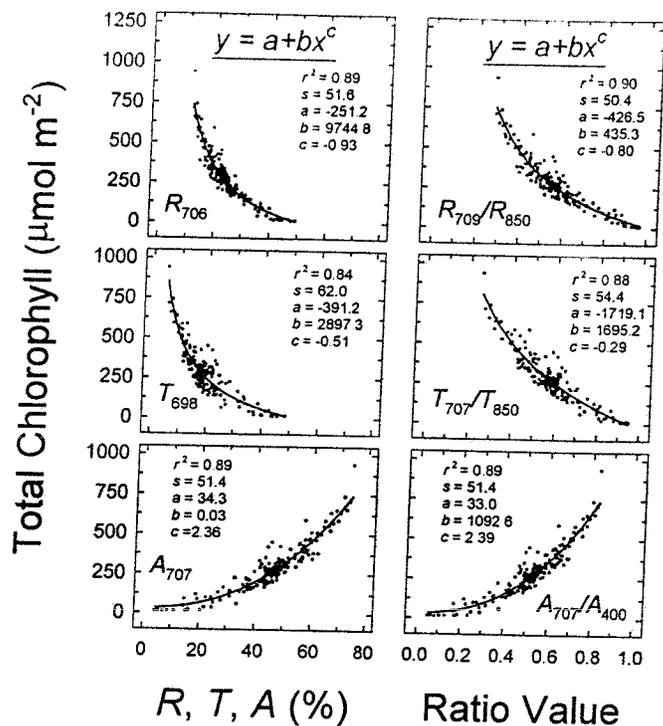


Figure 4. Best-fit regressions of leaf chlorophyll concentration versus leaf reflectance ( $R$ ), transmittance ( $T$ ), absorbance ( $A$ ), and band ratios. Regressions were based on a power function as indicated atop the figure and data combined among four broadleaved species. Regression parameters, including the coefficient of determination ( $r^2$ ) and standard error of the estimate ( $s$ ) are listed for each regression ( $N=168$ ).

**Individual Species** – When linear relationships of leaf chlorophyll concentration versus  $R$ ,  $T$ , and  $A$  were evaluated for individual species, maximum  $r^2$  and minimum  $s$  occurred generally at  $\lambda$  near 700 nm (Table 1). As exceptions,  $R_{533}$  and  $R_{567}$  in red maple and longleaf pine corresponded more linearly with chlorophyll than did  $R$  near 700 nm. The range among species in optimal  $\lambda$  for  $R$ ,  $T$ , and  $A$  in these regressions was greater in the green-orange spectrum (34, 37, and 20 nm, respectively) than in the far-red (9, 16, and 14 nm, respectively).

Linear regressions of chlorophyll with  $R_\lambda/R_{850}$ ,  $T_\lambda/T_{850}$ , and  $A_\lambda/A_{400}$  yielded maximum  $r^2$  and minimum  $s$  when  $\lambda$  was near 700 nm in all cases (Table 2). Among species, best-fit  $\lambda$  ranged only 21, 15, and 20 nm in the green spectrum and 13, 16, and 14 nm in the far-red for  $R$ ,  $T$ , and  $A$ , respectively.

When curvature of chlorophyll versus  $R$ ,  $T$ , or  $A$  regressions was allowed by using a power function, maximum  $r^2$  and minimum  $s$  occurred in the green-orange spectrum as often among species as in the far-red spectrum (Table 3). However, the range among species in optimal  $\lambda$  was much greater in the green-orange than in the far-red (87, 78, and 35 nm versus 11, 14, and 16 nm for  $R$ ,  $T$ , and  $A$ , respectively).

Generally, the greatest  $r^2$  and minimum  $s$  resulted from power regressions of chlorophyll concentration with  $R_\lambda/R_{850}$ ,  $T_\lambda/T_{850}$ , and  $A_\lambda/A_{400}$ . As with the power regressions of  $R$ ,  $T$ , and  $A$ , maximum  $r^2$  and minimum  $s$  occurred in the green-orange spectrum as often as in the far-red (Table 4; Fig. 5). Again, the range among species in optimal  $\lambda$  was much greater in the green-orange than in the far-red (87, 77, and 40 nm versus 22, 27, and 14 nm for  $R_\lambda/R_{850}$ ,  $T_\lambda/T_{850}$ , and  $A_\lambda/A_{400}$ , respectively).

Table 1. Wavelengths ( $\lambda$ ), coefficients of determination ( $r^2$ ), and standard errors of the estimate ( $s$ ) for linear ( $y=a+bx$ ) regressions of leaf total chlorophyll concentration versus reflectance ( $R$ ), transmittance ( $T$ ), or absorbance ( $A$ ).

Species	Green-Orange Spectrum					Far-Red Spectrum				
	$\lambda$ at max	$r^2$	$s$	$a$	$b$	$\lambda$ at max	$r^2$	$s$	$a$	$b$
<b>List</b>										
Acru	553	0.83	58.9	635.8	-23.1	712	0.88	50.8	899.9	-20.6
Viru	533	0.85	47.8	806.0	-35.8	709	0.83	50.8	929.8	-24.0
Argi	548	0.79	45.0	519.5	-16.7	713	0.84	40.0	709.0	-14.8
Pipa	549	0.81	88.2	1008.4	-42.7	718	0.83	83.0	1313.2	-31.1
Combined	567	0.93	54.6	1161.8	-36.1	709	0.93	53.4	1363.3	-28.1
broadleaves	549	0.72	83.3	731.9	-27.5	715	0.82	66.1	1035.4	-23.5
<b>List</b>										
Acru	585	0.83	59.5	581.5	-18.5	701	0.86	53.9	728.2	-16.8
Viru	580	0.67	70.8	602.9	-23.4	701	0.73	63.4	726.4	-21.1
Argi	575	0.66	58.0	453.6	-14.3	706	0.71	53.2	632.0	-14.2
Pipa	562	0.86	76.4	919.4	-32.5	717	0.88	68.7	1287.5	-27.4
Combined	599	0.75	103.1	769.8	-53.7	707	0.83	84.1	991.5	-38.3
broadleaves	574	0.68	88.9	646.8	-22.6	706	0.74	80.9	804.5	-18.7
<b>List</b>										
Acru	566	0.85	55.3	-453.4	10.9	709	0.90	45.0	-125.7	10.4
Viru	561	0.82	52.5	-753.3	15.2	709	0.87	44.5	-328.6	13.5
Argi	559	0.75	49.7	-318.4	8.4	717	0.83	41.1	-63.5	10.4
Pipa	549	0.87	72.6	-1025.3	20.4	723	0.91	61.6	-182.3	19.0
Combined	569	0.93	55.6	-1338.6	24.2	713	0.97	33.0	-459.1	20.0
broadleaves	556	0.75	79.4	-646.6	14.1	715	0.85	61.7	-150.1	13.0

Table 2. Wavelengths ( $\lambda$ ), coefficients of determination ( $r^2$ ), and standard errors of the estimate ( $s$ ) for linear ( $y=a+bx$ ) regressions of leaf total chlorophyll concentration versus reflectance ( $R$ ), transmittance ( $T$ ), or absorbance ( $A$ ) ratios.

Species	Green Spectrum					Far-Red Spectrum				
	$\lambda$ at max	$r^2$	$s$	$a$	$b$	$\lambda$ at max	$r^2$	$s$	$a$	$b$
<b>List</b>										
Acrú	556	0.85	54.8	643.8	-1077.3	715	0.92	40.7	1107.8	-1162.8
Viru	546	0.82	51.5	738.1	-1392.4	717	0.88	42.9	1251.0	-1403.7
Argi	551	0.78	46.0	531.1	-843.7	721	0.85	38.1	1176.4	-1205.6
Pipa	549	0.85	78.9	996.8	-1933.7	728	0.91	60.2	2070.4	-2168.7
Combined	567	0.92	59.6	1164.4	-2001.2	721	0.97	35.0	2605.0	-2767.6
broadleaves	551	0.74	80.4	741.7	-1324.8	720	0.87	57.4	1311.5	-1380.9
					$R_\lambda/R_{850}$					
<b>List</b>										
Acrú	572	0.85	55.0	638.7	-1036.0	710	0.91	43.6	1102.4	-1204.1
Viru	569	0.76	60.3	709.7	-1307.9	712	0.86	45.8	1240.0	-1488.1
Argi	570	0.72	52.8	486.5	-768.9	717	0.82	41.5	1098.4	-1166.1
Pipa	559	0.88	68.7	967.1	-1822.6	726	0.93	53.1	2070.2	-2235.1
Combined	574	0.78	96.4	849.5	-2031.7	721	0.93	55.6	1889.0	-2105.4
broadleaves	564	0.75	78.3	745.2	-1315.7	717	0.85	60.4	1280.2	-1386.3
					$T_\lambda/T_{850}$					
<b>List</b>										
Acrú	566	0.84	57.4	-494.9	1018.2	709	0.90	46.3	-134.9	942.1
Viru	561	0.83	50.9	-848.4	1470.1	709	0.87	44.0	-355.8	1251.7
Argi	559	0.75	49.9	-351.0	792.0	717	0.83	41.3	-68.4	936.6
Pipa	549	0.87	72.8	-1148.3	1953.7	723	0.90	62.5	-194.7	1708.3
Combined	569	0.91	63.4	-1450.7	2308.3	715	0.97	33.3	-490.6	1955.5
broadleaves	556	0.75	78.0	-717.2	1342.5	715	0.84	62.1	-157.7	1166.5
					$A_\lambda/A_{400}$					

Table 3. Wavelengths ( $\lambda$ ), coefficients of determination ( $r^2$ ), and standard errors of the estimate ( $s$ ) for power ( $y=a+bx^c$ ) regressions of leaf total chlorophyll concentration versus reflectance ( $R$ ), transmittance ( $T$ ), or absorbance ( $A$ ).

Species	Green-Orange Spectrum						Far-Red Spectrum					
	$\lambda$ at max	$r^2$	$s$	$a$	$b$	$c$	$\lambda$ at max	$r^2$	$s$	$a$	$b$	$c$
<b>List</b>	570	0.87	43.5	-506.7	3010.5	-0.52	701	0.92	40.3	-352.8	4336.7	-0.65
Acru	519	0.88	42.2	-419.8	3650.6	-0.71	696	0.87	44.3	-78.9	6886.0	-1.15
Viru	606	0.85	38.3	-94.7	2133.4	-0.81	696	0.85	38.3	-158.2	2195.2	-0.67
Argi	564	0.87	70.4	-201.6	7679.2	-1.01	707	0.91	59.1	-108.1	27377.8	-1.35
Pipa	582	0.93	52.6	-3759.2	6305.8	-0.14	701	0.93	52.9	-1855.4	5619.6	-0.29
Combined broadleaves	580	0.84	62.2	-22.4	9516.1	-1.42	706	0.89	51.6	-251.2	9744.8	-0.93
<b>List</b>	641	0.93	38.5	-451.2	1721.9	-0.36	693	0.89	47.9	-1841.4	3101.3	-0.14
Acru	647	0.86	45.9	-107.8	2120.0	-0.82	692	0.85	46.5	-128.6	2878.1	-0.84
Viru	646	0.74	49.5	-140.5	1222.4	-0.57	695	0.75	48.7	-306.4	1930.8	-0.48
Argi	611	0.96	39.5	-601.6	2314.6	-0.38	698	0.95	42.8	-326.5	3006.0	-0.56
Pipa	569	0.53	140.0	-18959.1	19967.2	-0.02	706	0.76	100.3	-8419.9	10110.0	-0.05
Combined broadleaves	609	0.87	55.6	-285.7	2339.6	-0.57	698	0.84	62.0	-391.2	2897.3	-0.51
<b>List</b>	611	0.94	33.3	26.7	$2.08 \times 10^{-8}$	5.34	704	0.92	41.2	-4.9	0.28	1.79
Acru	612	0.91	36.3	46.6	$7.15 \times 10^{-12}$	7.11	693	0.91	37.2	51.4	$5.6 \times 10^{-12}$	7.20
Viru	578	0.81	42.8	-2.8	$9.47 \times 10^{-5}$	3.44	704	0.84	39.3	6.2	0.09	2.00
Argi	578	0.94	48.3	52.4	$1.08 \times 10^{-8}$	5.56	706	0.95	44.6	69.1	0.0009	3.12
Pipa	577	0.96	43.2	-53.4	$1.81 \times 10^{-6}$	4.46	709	0.97	32.7	-116.2	0.34	1.86
Combined broadleaves	601	0.89	52.2	50.9	$3.63 \times 10^{-11}$	6.78	707	0.89	51.4	34.3	0.03	2.36

Table 4. Wavelengths ( $\lambda$ ), coefficients of determination ( $r^2$ ), and standard errors of the estimate ( $s$ ) for power ( $y=a+bx^c$ ) regressions of leaf total chlorophyll concentration versus reflectance ( $R$ ), transmittance ( $T$ ), or absorptance ( $A$ ) ratios.

Species	Green-Orange Spectrum					
	$\lambda$ at max $r^2$	$r^2$	$s$	$a$	$b$	$c$
			$R_\lambda/R_{850}$			
List	575	0.95	30.1	-112.6	89.7	-1.07
Acru	519	0.90	38.0	-233.4	116.7	-0.95
Viru	606	0.84	39.3	-90.7	84.3	-0.86
Argi	572	0.92	57.3	-221.0	176.1	-0.89
Pipa	572	0.95	47.1	-1879.9	1632.6	-0.31
Combined broadleaves	564	0.86	58.9	-171.3	130.2	-1.04
			$T_\lambda/T_{850}$			
List	633	0.89	51.6	-237.8	209.5	-0.58
Acru	642	0.89	39.5	-124.2	100.3	-0.83
Viru	646	0.80	43.6	-125.5	114.5	-0.62
Argi	596	0.96	38.1	-696.0	595.6	-0.37
Pipa	569	0.61	126.8	-12168.8	12057.1	-0.03
Combined broadleaves	606	0.90	48.4	-381.5	326.5	-0.51
			$A_\lambda/A_{400}$			
List	607	0.93	37.2	27.8	559.2	5.76
Acru	612	0.91	35.4	50.3	556.8	8.17
Viru	599	0.82	40.9	10.7	458.1	4.69
Argi	572	0.94	50.5	44.6	817.0	5.32
Pipa	574	0.96	43.1	-55.4	1071.3	4.96
Combined broadleaves	601	0.90	49.1	49.8	608.8	7.26

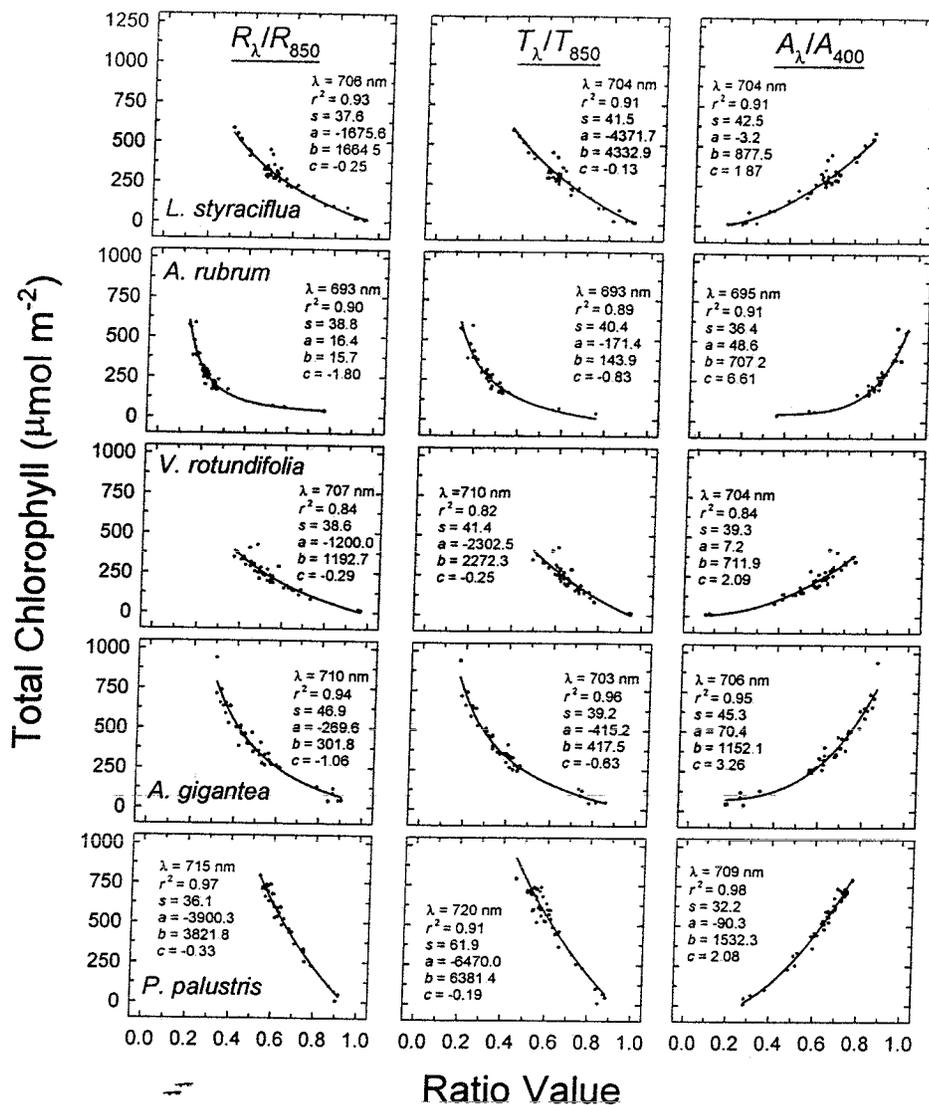


Figure 5. Best-fit regressions in the far-red spectrum of leaf total chlorophyll concentration versus leaf reflectance ( $R$ ), transmittance ( $T$ ), absorptance ( $A$ ), and band ratios for each species ( $N=42$  per species). Regressions were based on the power function  $y=a+bx^c$ . Regression parameters, including the coefficient of determination ( $r^2$ ) and standard error of the estimate ( $s$ ) are listed for each regression along with the wavelength ( $\lambda$ ) for the numerator.

## Discussion and Conclusions

Present results agree generally with those of previous studies in that leaf optical properties in the green-orange and far-red spectra were almost equally sensitive to chlorophyll concentration (Datt 1998, Gitelson and Merzlyak 1994, 1996, 1997, Gitelson et al. 1996, Lichtenthaler et al. 1996, McMurtrey et al. 1994). With the exception of using the 400 nm rather than 850 nm band in the denominator for  $A$  band ratios, results for  $T$  and  $A$  were similar to those for  $R$ .

The high sensitivity to chlorophyll of leaf optics near 550 nm and 700 nm is explained by the relatively low *in vivo* absorptivities of chlorophyll in these spectral regions. The absorptivity of chlorophyll while it remains associated with chloroplast membranes is weak near 550 nm, and approaches zero near 720 nm (Rabideau et al. 1946). Thus,  $R$ ,  $T$ , and  $A$  near 550 nm and 700 nm change measurably with small changes in leaf chlorophyll concentration over a broad range of nearly 1000  $\mu\text{mol}/\text{m}^2$ . In contrast, high absorptivities in the 400-500 nm range and near 680 nm (Rabideau et al. 1946) result in low sensitivities of  $R$ ,  $T$ , and  $A$  to small changes in chlorophyll concentration. This explains the consistently low  $r^2$  for regressions with chlorophyll in these spectral regions (Figs. 1, 3). Low  $r^2$  beyond  $\sim 730$  nm occurred because chlorophyll does not absorb appreciably in the near-infrared spectrum.

It is clear that the specific  $\lambda$  yielding the strongest relationship with chlorophyll can depend substantially on the regression function employed. Linear regressions consistently indicated maximum  $r^2$  and minimum  $s$  at  $\lambda$  near 700 nm. When the power function was used, results for the green-orange spectrum were more nearly equivalent to those near 700 nm. Power regressions usually shifted optimal  $\lambda$  in the green-orange spectrum to longer  $\lambda$ , and optimal  $\lambda$  in the far-red spectrum to shorter  $\lambda$  compared with linear regressions.

Overall, regressions based on the power function and band ratios generally yielded the highest  $r^2$  and lowest  $s$ . However, best-fit numerator  $\lambda$  in the green-orange spectrum occurred throughout a much broader spectral range among species (519-646 nm) than in the far-red (693-720 nm). Thus, the selection of a narrow band for  $R$ ,  $T$ , or  $A$  in the far-red spectrum, such as  $705 \pm 5$  nm, would probably yield the most accurate estimates of chlorophyll concentration among a variety of species.

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13. ABSTRACT (Maximum 200 words)  
The present study identified wavebands and band ratios within the 400-850 nm range that could be used to estimate total chlorophyll concentration with minimal error. Optical properties and chlorophyll concentrations were measured for two broadleaved tree species (sweetgum and red maple), a broadleaved vine (wild grape), a needle-leaved conifer (longleaf pine), and a representative of the grass family (switchcane) Muhl.). Overall, reflectance, transmittance, and absorptance corresponded most precisely with chlorophyll concentration near 700 nm, although regressions were strong as well in the 550-625 nm range. Dividing reflectances or transmittances by maximal values that occurred at approximately 850 nm improved chlorophyll estimation only slightly in most cases. This was true also when absorptances were divided by maximal absorptances near 400 nm. Regressions based on a power function were superior to linear regressions in yielding low standard errors of the estimate (s). When data for all broadleaved species were combined, s at best-fit wavelengths of 707-709 nm were approximately 50 mmol/m<sup>2</sup> out of a 940 mmol/m<sup>2</sup> range. Minimal s, ranging among species from 32-62 mmol/m<sup>2</sup> of chlorophyll, were obtained when the power model was used with band ratios having numerator wavelengths from 693-720 nm.

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