Leaf optical properties in higher plants: linking spectral characteristics to stress and chlorophyll concentration[1]

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A number of studies have linked responses in leaf spectral reflectance, transmittance, or absorptance to physiological stress. A variety of stressors including dehydration, flooding, freezing, ozone, herbicides, competition, disease, insects, and deficiencies in ectomycorrhizal development and N fertilization have been imposed on species ranging from grasses to conifers and deciduous trees. In all cases, the maximum difference in reflectance within the 400–850 nm wavelength range between control and stressed states occurred as a reflectance increase at wavelengths near 700 nm. In studies that included transmittance and absorptance as well as reflectance, maximum differences occurred as increases and decreases, respectively, near 700 nm. This common optical response to stress could be simulated closely by varying the chlorophyll concentration of model leaves (fiberglass filter pads) and by the natural variability in leaf chlorophyll concentrations in senescent leaves of five species. The optical response to stress near 700 nm, as well as corresponding changes in reflectance that occur in the green–yellow spectrum, can be explained by the general tendency of stress to reduce leaf chlorophyll concentration.

Key words: absorptance; chlorophyll; leaf optics; light; reflectance; stress; transmittance.

The spectral quality of light reflected from leaves, manifested in leaf color, has long been relied upon as an indicator of plant stress. However, spectral characteristics of radiation reflected, transmitted, or absorbed by leaves can provide a more thorough understanding of physiological responses to growth conditions and plant adaptations to the environment. In the late nineteenth and early twentieth centuries, technological advances began to allow the examination of changes in leaf spectra that occur with stress (Sorby, 1873; Coblentz, 1912; Shull, 1929). Investigation of such spectral characteristics has intensified greatly since the 1960s, along with the development of instrumentation and interest in the potential of remote sensing for stress detection. Largely as a result of interests in remote sensing, leaf reflectance has been studied more extensively than transmittance or absorptance responses to stress. Pioneering efforts in this field have been reviewed elsewhere (Myers et al., 1983; Jackson, 1986).

Throughout this research history, the extent to which differing causes of stress within a species may yield correspondingly different spectral signatures has remained in question. Also in question is the degree to which the spectral response to a particular stressor may vary among species. A mounting body of evidence, described here in part, indicates that leaf reflectance is altered by stress more consistently at visible wavelengths (~400–720 nm) than in the remainder of the incident solar spectrum (~730–2500 nm) (Carter, 1993, 1994). These changes were spectrally similar among many common stressors and vascular plant species. Increased reflectance in the far-red 690–720 nm spectrum is a particularly generic response, providing an earlier or more consistent indication of stress than reflectance in other regions of the incident solar spectrum (Carter, 1993, 1994; Carter, Cibula, and Miller, 1996).

It has long been suggested that alterations of reflectance in the visible spectrum by stress conditions result from the sensitivity of leaf chlorophyll concentrations to metabolic disturbance (Knipling, 1970). Indeed, several studies have shown that indices based on reflectance in the far-red can precisely estimate leaf chlorophyll concentration (Chappelle, Kim, and McMurtrey, 1992; Gitelson and Merzlyak, 1994, 1996, 1997; McMurtrey et al., 1994; Carter, Rebbeck, and Percy, 1995; Gitelson, Merzlyak, and Lichtenthaler, 1996; Lichtenthaler, Gitelson, and Lang, 1996; Schepers et al., 1996; Datt, 1998, 1999). Thus, leaf optical properties in a relatively narrow spectral band near 700 nm are crucial for plant stress detection and the estimation of leaf chlorophyll concentration.

The consistency with which leaf optical properties near 700 nm change in response to stress among causes of stress and species indicates a general mechanism by which such changes occur. The goal of this paper is to elucidate this mechanism by simulating general patterns in optical responses to stress in the 400–850 nm wavelength range. Leaves having unusual anatomical characteristics such as heavy pubescence or succulence or colors other than green in the healthy, mature state were not included. The objectives were to (1) use previously unpublished results and data from the literature to demonstrate that stress-induced changes in reflectance, transmittance, and absorptance tend to be greatest at wavelengths near 700 nm; (2) simulate this stress response for reflectance using chlorophyll in vitro; and (3) simulate reflectance, transmittance, and absorptance responses to stress using the natural range of chlorophyll concentrations found in senescent leaves of several species. This combination of results from a variety of approaches should provide a clearer understanding of the basis for changes in leaf optical properties that occur commonly with stress at visible and near-infrared wavelengths.

MATERIALS AND METHODS

Examples of leaf optical responses to stress—Changes in leaf reflectance that occurred with early infestation of mature loblolly pine (*Pinus taeda* L.)

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by the southern pine beetle (*Dendroctonus frontalis* Zimm.) (Entcheva, Cibula, and Carter, 1996) and insufficient N fertilization in seedlings of radiata pine (*P. radiata* D. Don) (Thorn, 1993) were used as examples of typical reflectance responses to stress. In the southern pine beetle (SPB) study, ~500 current-year needles were sampled from the upper, sun-exposed canopy of each of several trees that were felled during an SPB outbreak in the woodlands of Stennis Space Center, Mississippi during June 1995. The trees represented several damage classes that ranged from uninfested to severely damaged. Here, we report data only for undamaged trees and trees that were recently infested but which still maintained green needles. Reflectance was measured for each of six composite needle samples representing three undamaged and three recently infested trees. A sample was arranged in a bundle and placed on a black platform under a high-intensity tungsten lamp as described previously (Carter et al., 1992). Reflectance of each bundle was measured at 1 nm wavelength intervals using a spectroradiometer attached by a fiber optic cable to a telescope/microscope body (LI1800UW with LI1800-06 body, LI-COR, Lincoln, Nebraska, USA). Radiance reflected from the needle bundle was divided by radiance reflected from a white reference (Spectralon SRT-99-05, Labsphere, North Sutton, New Hampshire, USA) to compute reflectance in units of percentage. True spectral bandwidth produced by the 0.5-mm slitwidth of the monochromator was 4 nm. Data were recorded at 1-nm intervals throughout the 400–850 nm range.

In the N fertilization study of radiata pine, seedlings grown in 4-L pots in the greenhouse were exposed to a range of N fertilization treatments by applying ammonium nitrate to the soil as described earlier (Thorn, 1993). In this paper, seedlings that received N at 0.5 mmol/L (controls) were compared only with seedlings that received no supplemental N. These treatments yielded mean total N concentrations of 10.34 and 5.54 mg/g needle dry mass, respectively. Reflectance was measured for an individual needle selected from each of three seedlings per treatment using procedures described previously in detail (Thorn, 1993; Carter, Rebbeck, and Percy, 1995). Briefly, each needle was placed atop a flat-black surface and irradiated with a tungsten lamp. A 7× microscope objective (LI1800–06E 7× objective) was attached to the same microscope body as described above and microscope aperture set to limit field-of-view within a single needle width. Once focused, light reflected from the needle travelled through the fiber optic to the spectroradiometer where spectral radiance was measured. Radiance reflected from the needle was multiplied by 100 and divided by reference radiance to obtain percentage reflectance.

Manipulation of chlorophyll concentration to simulate stress responses—Glass microfibre circular filters (Whatman GF/F, 25 mm diameter) and mixtures of chlorophylls *a* and *b* (Sigma Chemical Co., St. Louis, Missouri, USA) in 100% methanol were used to construct in vitro leaf models that simulated typical leaf reflectance responses to stress. Chlorophyll mixtures were added to a series of the white filter pads to simulate leaf total chlorophyll ($a + b$) concentrations of 380, 342, 304, 266, 228, and 190 μmol/m². Reductions in the pigment content of model leaves were accomplished via dilution of stock solutions prior to applying a constant volume (0.22 mL) to all filters. The chlorophyll *a/b* ratio remained constant at 3.75 as total concentration was altered. The maximum pigment concentration of the filters approximated concentrations that are found commonly in well-nourished leaves (Porra, Thompson, and Kriedemann, 1989).

Reflected radiance of the leaf models ($N = 3$ for each chlorophyll concentration) was measured immediately after pigment application using a spectroradiometer (Model 2000, Ocean Optics, Dunedin, Florida, USA). Reflected radiance was also measured for $N = 5$ reference filter pads to which only 0.22 ml of 100% methanol was added. A 1300-ms integration time optimized resolution among samples without saturating the detector. Data were output at intervals of ~0.3 nm throughout the 400–850 nm range. Reflectances (in percentages) were computed by multiplying sample spectra by 100 and dividing by the mean reference spectrum. Mean reflectance spectra for each chlorophyll concentration ($N = 3$) were normalized to correct for scattering effects by dividing reflectance at all wavelengths by reflectance at 730 nm and again multiplying by 100. To evaluate the wavelengths at which the reflectance change was largest with a change in chlorophyll concentration, re-

flectance differences were computed by subtracting pad reflectance at 380 μmol/m² chlorophyll from reflectance at each lesser chlorophyll concentration.

Use of senescent leaves to alter chlorophyll concentration—Leaf optical responses to a broad range in leaf chlorophyll concentration were examined also for leaves that were at various stages of senescence in five species. 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. For $N = 42$ leaves per broadleaved species, leaf reflectance and transmittance 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, Lincoln, Nebraska, USA) and methods described earlier (Daughtry, Biehl, and Ranson, 1989). A leaf was clamped into position over the sample port on the sphere wall and a 1.65-cm² leaf area was 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, North Sutton, New Hampshire, USA). Spectral reflectance 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 percentages. Leaf transmittance 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 percentage transmittance. For longleaf pine, reflectance and transmittance were measured for 42 samples. Each sample was composed of 5–6 needles spaced ~1 mm apart and arranged in parallel across the sample port of the integrating sphere. Reflected and transmitted radiances were recorded as above. An additional transmittance scan was taken without needles in the sample holder to enable the correction of radiance values for light that passed between needles (Daughtry, Biehl, and Ranson, 1989). In contrast to the earlier method, a high-resolution digital camera and image processing software (ENVI v. 3.1, Research Systems, Boulder, Colorado, USA) were used to determine the percentage of irradiance that was not intercepted by the needles. In all species, percentage leaf absorptance was computed as 100 − (reflectance + transmittance).

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, Thompson, and Kriedemann (1989). Chlorophyll concentration of the extract and the total disk surface area of 1.84 cm² were used to compute leaf chlorophyll concentrations per unit projected area. Total projected leaf areas for computing chlorophyll concentration in pine needles were determined by the digital camera and image analysis.

Statistical analysis—Significant effects ($P = 0.05$) of SPB damage and N fertilization on reflectance at each 1 nm wavelength interval were determined by analysis of variance (ANOVA) (SAS 6.0, SAS Institute, Cary, North Carolina, USA). For the senescent leaves of five species, coefficients of determination (r^2) were used to evaluate simple linear relationships of reflectance, transmittance, or absorptance with leaf total chlorophyll concentration at 1.6-
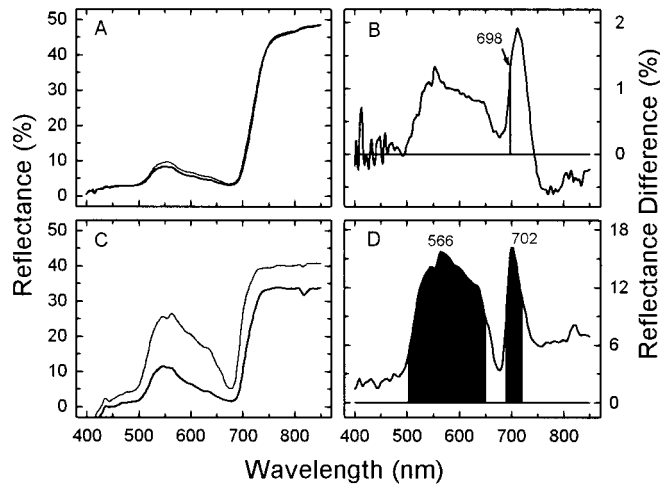
Fig. 1. Typical responses of leaf spectral reflectance to plant stress as illustrated by (A, B) recent infestation of loblolly pine by the southern pine beetle (from Entcheva, Cibula, and Carter, 1996) and (C, D) N deficiency in radiata pine (from Thorn, 1993). The thicker curves in (A) and (C) represent mean needle reflectances ($N = 3$) for relatively nonstressed control trees. The thinner curves represent mean reflectances for the stressed condition in each case. Reflectance difference curves (B, D) were computed by subtracting mean reflectance of the control state from that of the stressed state. Darkened regions under the difference curves denote significant differences at $P = 0.05$. Wavelengths at significant difference maxima are indicated in (B) and (D). Note the different vertical axis scales in (B) vs. (D).

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 and sample size (SAS 6.0; Table Curve 2D v. 4.0, SPSS, Chicago, Illinois, USA).

RESULTS

Changes in needle reflectance that corresponded with SPB damage in loblolly pine and N deficiency in radiata pine serve as examples of typical reflectance responses to plant stress in the 400–850 nm wavelength range (Fig. 1A, C). In both cases, increased reflectance was observed although the increase was much greater for N deficiency in radiata pine. Also observed in radiata pine was a more obvious shift of the red to near-infrared transition spectrum, or red edge, toward the blue end of the spectrum. When control reflectances were subtracted from reflectance curves representing the stressed states, the resulting difference curves indicated the wavebands in which reflectance changed most greatly with stress (Fig. 1B, D). Although N deficiency in radiata pine seedlings yielded much greater reflectance differences than early SPB damage in loblolly pine, both stressors yielded similar patterns in difference curves.

The above reflectance difference patterns could be reproduced closely in vitro by adding chlorophyll $a + b$ solutions of sequentially decreasing concentrations to white glass filter pads (Fig. 2). Shifts of the reflectance red edge toward shorter wavelengths were observed although these are difficult to resolve visually (Fig. 2A). The shifts are much easier to visualize as differences (Fig. 2B). The wavelength of peak reflectance difference for each chlorophyll concentration was 683 nm rather than 700–720 nm as usually observed for leaves (Figs. 1, 2). Reflectance at 683 nm, normalized to reflectance at 730 nm to correct for scattering effects, correlated exponentially
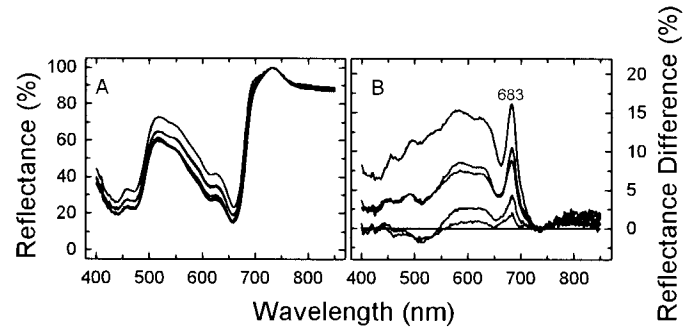

Fig. 2. Simulation of stress-induced changes in leaf reflectance by the reflectances of glass fiber filters to which were added solutions of chlorophylls $a + b$ in 100% methanol. Mixtures of chlorophyll a with chlorophyll b were added to a series of filter pads to simulate leaf total chlorophyll ($a + b$) concentrations of 380, 342, 304, 266, 228, and 190 µmol/m². The chlorophyll a/b ratio remained constant at 3.75 as total concentration was altered. The thicker reflectance curve (A) represents filter pad reflectance at maximum chlorophyll concentration. Sequentially greater reflectances represent correspondingly lesser chlorophyll concentrations. Reflectance in each curve was normalized to correct for scattering effects by dividing reflectance at all wavelengths by reflectance at 730 nm and multiplying by 100. Reflectance difference curves (B) were computed by subtracting normalized reflectance at maximum chlorophyll concentration from reflectance at each lesser concentration. This in vitro simulation yielded difference peaks at 683 nm rather than near 700 nm as in Figure 1.

with the per-unit-area concentration of total chlorophyll added to the filter pads (Fig. 3).

Coefficients of determination (r^2) for linear regressions of reflectance, transmittance, or absorptance with total chlorophyll concentration in senescent leaves generally were maximum near 700 nm in all five species (Fig. 4). Maximum r^2 in all but two cases occurred within the 701–723 nm range of the far-red spectrum. In red maple, the r^2 for reflectance at 533 nm was slightly greater than at 709 nm (Fig. 4D). In longleaf pine, r^2 peaks for reflectance at 567 and 709 nm were equally high (Fig. 4M). Other than these exceptions, r^2 peaks in the green–yellow spectrum were secondary maxima in the 548–599 nm range. Means across species (± 1 SD) of wavelengths in the green–yellow and far-red spectra at which r^2 maxima occurred were 550 ± 12 and 712 ± 4 nm for reflectance, 580 ± 14 and 706 ± 7 nm for transmittance, and 561 ± 8 nm

$$y = (a + b/x)^{1/2}$$
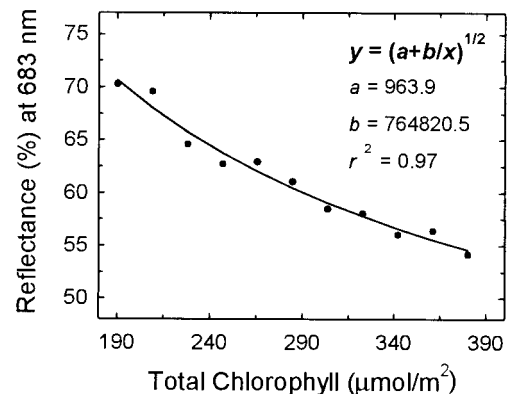$a = 963.9$
$b = 764820.5$
$r^2 = 0.97$

Fig. 3. Reflectance at 683 nm (see Fig. 2B) of glass fiber filter pads soaked with chlorophyll solutions vs. chlorophyll concentration per unit area. The total chlorophyll concentrations (chlorophyll $a + b$) are representative of those found typically in well-nourished leaves.
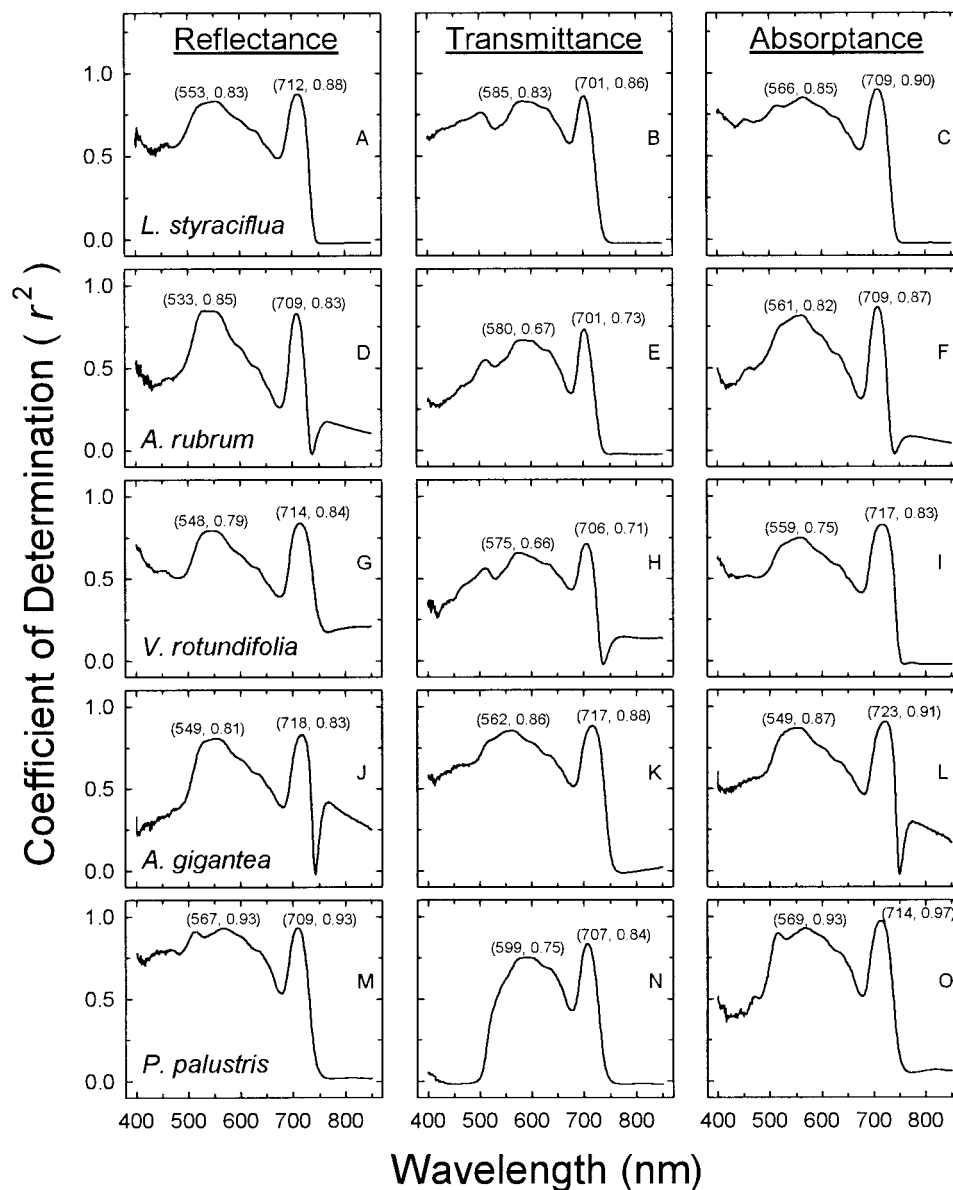
Fig. 4. Coefficient of determination (r^2) vs. wavelength for simple linear relationships of leaf reflectance, transmittance, or absorptance with total leaf chlorophyll concentration in senescing leaves of five species. Coefficients were derived at each 1.6 nm wavelength interval for $N = 42$ leaves per species. Leaves ranged in color from green through yellow for each species. The wavelengths at which best-fit relationships were identified and corresponding r^2 are indicated in parentheses. Means across species (± 1 SD) of wavelengths in the green–yellow and far-red spectra at which r^2 maxima occurred were 550 ± 12 and 712 ± 4 nm for reflectance, 580 ± 14 and 706 ± 7 nm for transmittance, and 561 ± 8 and 714 ± 6 nm for absorptance.

and 714 ± 6 nm for absorptance. Maximum r^2 tended to be greatest in species that possessed the greatest range in leaf chlorophyll concentrations (Fig. 5).

DISCUSSION

Present results combined with data from the recent literature (Table 1) clearly indicate that increases in reflectance and transmittance and a decrease in absorptance in the 695–725 nm wavelength range are highly consistent and general responses of leaf optics to plant stress. Results of many earlier studies could not be included with this comparison because of the analytical methods employed. Frequently in earlier studies, reflectance curves representing stressed and control leaves

were plotted vs. wavelength. Gaps visible between these curves then were used to identify spectral regions in which reflectance changed significantly with stress. However, the steep slope of reflectance curves in the far-red spectrum can produce the illusion that stress-induced differences are negligible near 700 nm (Fig. 1). As a result, spectral responses to stress near 700 nm often have been ignored. However, the simple subtraction of control curves from curves representing the stressed condition shows clearly the far-red response (Fig. 1; Table 1, references).

The far-red optical response to stress is explained by the tendency of stressed leaves to lose chlorophyll and the absorption properties of chlorophyll. The general pattern in reflectance differences that occurs typically with plant stress
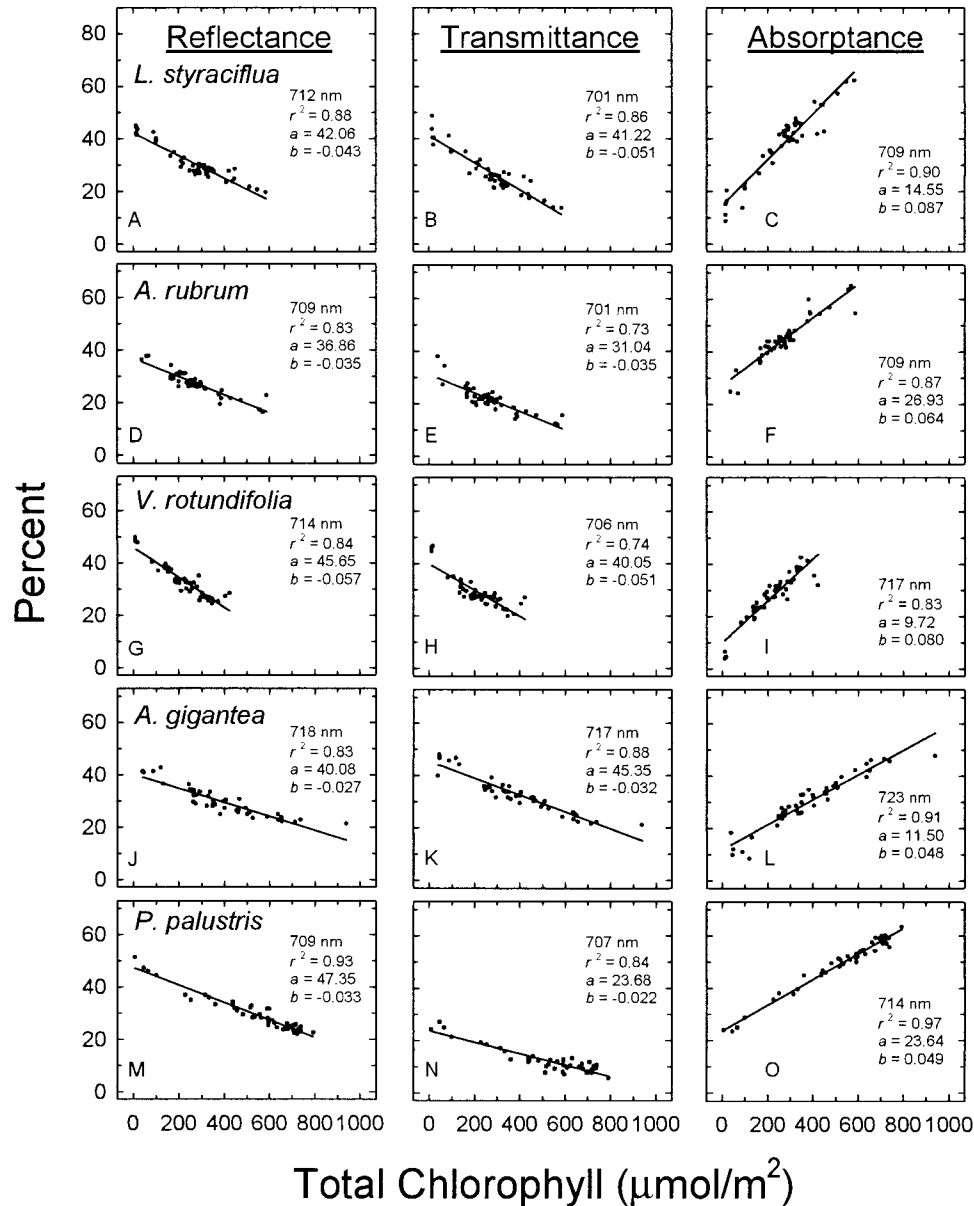
Fig. 5. Best-fit linear relationships of reflectance near 700 nm with total leaf chlorophyll concentration in senescing leaves of five species ($N = 42$ leaves per species).

could be simulated closely in vitro by placing methanol solutions of successively decreasing chlorophyll concentrations on white fiberglass filters (Fig. 2). The spectral locations of difference maxima were shifted ~20 nm toward the blue spectrum compared with leaf data (Fig. 1) because the in vitro molecular environment differed from that of chloroplasts. With respect to wavelength, plotting r^2 for relationships of reflectance, transmittance, or absorptance with leaf chlorophyll concentration against wavelength (Fig. 4) could simulate this pattern more accurately in the green–yellow and far-red spectra. In every case except reflectance in red maple and longleaf pine, the strongest linear relationships with total chlorophyll concentration occurred near 700 nm (Fig. 4). The absorptivity of chlorophyll approaches zero at wavelengths near 700 nm in organic solvents (Lichtenthaler, 1987) and near 720 nm when

it remains associated with chloroplast membranes (Rabideau, French, and Holt, 1946). Thus, reflectance or transmittance may increase and absorptance may decrease significantly in the far-red when leaf chlorophyll concentrations are reduced by relatively small amounts. Indeed, the minor reflectance increase at 698 nm caused by the early stages of an SPB attack on mature loblolly pine (Fig. 1) represents a previsible change in optical properties of the type described earlier (Cibula and Carter, 1992; Carter, Cibula, and Miller, 1996).

Because reflectance generally increases at wavelengths near 700 nm with plant stress, the steep slope of the reflectance curve in the far-red to near-infrared transition spectrum tends to shift toward the blue spectrum (e.g., Fig. 1C). This has become widely known as the *blue shift* of the reflectance curve *red edge* and is quantified by the red-edge inflection point.

TABLE 1. Summary of wavelengths (λ) at which leaf optical properties differed most in stressed vs. control leaves.

Stressor	Species	Variable	λ	Reference
Competition	*Pinus taeda* L.	Reflectance	716	Carter et al., 1989; Carter, 1993
Fungal pathogen	*Euonymus japonica* var. aureo-marginata	Reflectance	708	Carter, 1993
Ectomycorrhizal deficiency	*Pinus elliottii* Engelm.	Reflectance	718	Cibula and Carter, 1992; Carter, 1993
N deficiency	*Pinus radiata* D. Don.	Reflectance	702	Thorn, 1993
Herbicide	*Diospyros virginiana* L.	Reflectance	715	Carter, 1993
Barrier island	*Pinus elliottii*	Reflectance	717	Carter and Young, 1993
Dehydration	*Arundinaria gigantea* (Walter) Muhl.	Reflectance	706	Carter, 1993
Dehydration	*Acer platanoides* L.	Reflectance	707	Carter and McCain, 1993
Flooding	*Myrica cerifera* L.	Reflectance	706	Carter and Young, 1993
Southern pine beetle	*Pinus taeda*	Reflectance	698	Entcheva, Cibula, and Carter, 1996
Freeze/thaw	*Acer rubrum* L.	Reflectance	718	G. A. Carter, unpublished data
Freeze/thaw	*Acer rubrum* L.	Transmittance	719	G. A. Carter, unpublished data
Freeze/thaw	*Acer rubrum* L.	Absorptance	718	G. A. Carter, unpublished data
Elevated ozone	*Pinus taeda*	Reflectance	710	Carter et al., 1992
Elevated ozone	*Liriodendron tulipifera* L.	Reflectance	696	Carter, Rebbeck, and Percy, 1995
Elevated ozone	*Liriodendron tulipifera* L.	Transmittance	697	Carter, Rebbeck, and Percy, 1995
Elevated ozone	*Liriodendron tulipifera* L.	Absorptance	697	Carter, Rebbeck, and Percy, 1995
Elevated ozone and CO_2	*Liriodendron tulipifera* L.	Transmittance	697	Carter, Rebbeck, and Percy, 1995
Elevated ozone and CO_2	*Liriodendron tulipifera* L.	Absorptance	697	Carter, Rebbeck, and Percy, 1995
Elevated ozone and CO_2	*Pinus strobus* L.	Reflectance	711	Carter, Rebbeck, and Percy, 1995

The inflection point is located at the wavelength where the first derivative of the reflectance curve is maximum in the far-red spectrum. This shift of the red edge has long been known to occur with plant stress and corresponds strongly with leaf chlorophyll concentration (Gates et al., 1965; Horler, Dockray, and Barber, 1983; Rock, Hoshizaki, and Miller, 1988; Curran, Dungan, and Gholz, 1990; Buschmann and Nagel, 1993; Vogelmann, Rock, and Moss, 1993; Filella and Peñuelas, 1994; Munden, Curran, and Catt, 1994; Belanger, Miller, and Boyer, 1995; Gitelson, Merzlyak, and Lichtenthaler, 1996; Lichtenthaler, Gitelson, and Lang, 1996; Pinar and Curran, 1996).

With more severe loss of chlorophyll, the absorption spectrum of a leaf continues to narrow and reflectance increases over a broader portion of the visible spectrum (Gates, 1980). The absorptivity of chlorophyll in the green, yellow, and orange spectra (~535–640 nm) is greater than at 700 nm but relatively weak compared with the major chlorophyll absorption bands in the blue spectrum and near 680 nm (Rabideau, French, and Holt, 1946; Moss and Loomis, 1952; Lichtenthaler, 1987). The chronic stress induced by N deficiency in radiata pine resulted in a much greater reflectance increase near 700 nm, but also increased reflectance in the blue–green through orange spectra and produced a yellow–green needle color (Thorn, 1993). Several analyses, some of which have not addressed reflectance in the 700 nm region, concluded that reflectance near 550 nm or its ratio with the near-infrared provides the closest correlation with leaf chlorophyll content (e.g., Thomas and Gausman, 1977; Tsay, Gjerstad, and Glover, 1982; Buschmann and Nagel, 1993). The sensitivity of reflectance near 550 nm to chlorophyll can be similar to that near 700 nm (Buschmann and Nagel, 1993; Blackmer, Schepers, and Varvel, 1994; Gitelson and Merzlyak, 1994, 1997; Blackmer et al., 1996; Schepers et al., 1996). However, reflectance at or near 550 nm appears to be less reliable as a stress indicator than reflectance near 700 nm (Cibula and Carter, 1992; Carter, 1994, 1998; Carter and Miller, 1994; Carter, Cibula, and Miller, 1996). Lower standard deviations for mean wavelengths of peak locations, particularly for reflectance and trans-

mittance, indicated a more precise optical response to stress in the far-red vs. green–yellow spectrum (Fig. 4).

As is often the case, reflectance, transmittance, and absorptance differences in the 400–500 nm, 670–680 nm, and near-infrared spectra tend to be low for stressed vs. healthy leaves. It appears that concentrations of carotenoids and other accessory pigments are usually high enough in stressed leaves that absorption in the 400–500 nm range remains similar to that in healthy leaves (e.g., Merzlyak et al., 1999). In the 670–680 nm range, absorption may saturate due to the strong chlorophyll absorptivity such that relatively large amounts of chlorophyll must be lost from the leaves before a significant optical difference occurs. Beyond ~730 nm in the near-infrared, reflectance is not affected by chlorophyll absorptivity and would be expected to change only if leaf anatomy or water content changed in response to stress (e.g., Sinclair, Schreiber, and Hoffer, 1973).

In conclusion, the highly consistent changes in leaf reflectance, transmittance, and absorptance that occur commonly with plant stress, particularly in the far-red and green–yellow spectra, can be explained by stress-induced decreases in leaf chlorophyll concentration. Stress would generally be expected to result in chlorophyll loss (Hendry, Houghton, and Brown, 1987). Thus, it is likely that stress due to a variety of causes and in most vascular plant species will induce changes in leaf optical properties that are spectrally similar to those described herein. This understanding should provide a basis for modeling responses of plant radiative properties to stress and for remote detection of stress at larger scales. The generality of leaf optical responses to stress implies that diagnosis of the cause of stress by remote sensing will not be possible in most cases. Continued investigation will be required to evaluate the extent to which exceptions to this generality will enable the identification of specific stressors by remote sensing.

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