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UV Band Fluorescence (*in vivo*) and Its Implications for the Remote Assessment of Nitrogen Supply in Vegetation

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When excited at 280 nm, intact vegetation produced two overlapping broadband fluorescence emissions; the first centered near 335 nm [ultraviolet (UV) band], and the second centered near 440 nm (blue band). Separation of these two fluorescence bands was achieved by an iterative nonlinear curve fit procedure utilizing the asymmetric double sigmoidal spectral function. The subsequent ratio of the deconvoluted curve intensities exhibited a significant relation between protein concentration and fluorescence. UV band fluorescence from vegetation treated with varying levels of nitrogen fertilization decreased relative to the blue fluorescence as a function of protein levels. These studies indicate that in vivo UV band fluorescence can be utilized as a nondestructive tool to remotely sense variations in protein concentration due to nitrogen supply. Strong similarities were noted in the UV band fluorescence characteristics of intact vegetation to both membrane-bound and soluble plant proteins containing aromatic amino acids. Pure ribulose 1,5-bisphosphate carboxylase in aqueous solution exhibited UV fluorescence characteristics with excitation and emission distributions similar to those of intact vegetation. Because of its high concentration (up to 70% of the soluble leaf proteins), we believe this protein contributes to the UV band fluorescence emanating from the intact leaf. In addition, similar fluorescence characteristics were observed for two other prominent enzymatic plant proteins; namely, adenosine 5'-tri-phosphatase and carboxylase phosphoenolpyruvate carboxylase. These results indicate that UV band fluorescence emanating from the intact leaf could originate from several plant proteins that contain aromatic amino acids. ©*Elsevier Science Inc., 1997*

INTRODUCTION

Vegetation, when exposed to long-wave ultraviolet (UV) radiation of sufficient energy, dissipates a portion of the absorbed excitation energy as fluorescence with emission maxima near 440, 525, 685, and 740 nm (Chappelle et al., 1984). UV radiation from approximately 320 to 380 nm can be used to excite fluorescence emissions in these bands. The broad blue-green fluorescence (BGF) ranges from 360 to 600 nm with a principle maximum at 440 nm and a shoulder near 525 nm. The BGF is a convolution of emissions corresponding to several plant constituents (Chappelle et al., 1991; Lang and Lichtenthaler, 1991; Lichtenthaler et al., 1991, 1993; Subhash et al., 1993).

The dynamic portion of the BGF (i.e., intensity varies in response to short-term physiological changes within the leaf) emanates primarily from mesophyll layers of the leaf. Time-resolved BGF measurements along with the absorption and fluorescence emission characteristics from the water extract of clover give rise to possible BGF plant constituents. Nicotinamide and flavin nucleotides along with the water-soluble compound nicotinamide-adenine dinucleotide phosphate in the reduced form (NADPH) are plant constituents contributing to the dynamic portion of the BGF (Cerovic et al., 1993, 1994; Chappelle et al., 1991; Morales et al., 1994).

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The static portion of the BGF (i.e., variations occur over a much longer time frame) have been attributed to relatively inert structural compounds of the leaf epidermis and cell wall such as polyphenolics and lignin (Chappelle et al., 1991; Lang and Lichtenthaler, 1991; Lichtenthaler et al., 1996). Ultraviolet fluorescence microscopy has been utilized to demonstrate the BGF properties of ferulic acid bound to unlignified cell walls in addition to the fluorescence properties of lignified cell walls (Harris and Hartley, 1976). Furthermore, the blue fluorescent properties of senescent plant material and crop residues have been attributed to lignin and trace compounds associated with cellulose, with the fluorescence emission from these materials decreasing with decomposition (McMurtrey et al., 1993).

Under optimal growth conditions, the majority of light absorbed by plant chlorophylls and carotenoids is utilized in photosynthesis with less than 3% of the absorbed light energy being dissipated as fluorescence emissions near 685 (red) and 740 nm [near infrared (NIR)]. The magnitude of these fluorescence emissions on exposure of a plant to light are governed by chlorophyll concentration and photosynthetic activity. The primary roles played by certain nutrients in photosynthesis and chlorophyll synthesis suggest that nutrient deficiencies could be detected on the basis of changes in these fluorescence emissions (Chappelle et al., 1984). The fluorescence ratio 685:740 nm could relate to changes in the distribution of excitation energy between photosystems I and II (Corp et al., 1996) and has been studied as an indicator of chlorophyll content and stress condition in plants (Lichtenthaler and Rinderle, 1988). In addition, several studies have demonstrated that linear and curvilinear relations exist between certain ratios of fluorescence maxima to pigment concentrations and rates of photosynthesis (Chappelle et al., 1993; Lichtenthaler et al., 1993; McMurtrey et al., 1994). Currently, there are a number of instruments available that utilize remotely sensed chlorophyll fluorescence data for the early detection of plant stress (Mohammed et al., 1995).

Protein fluorescence of the intact leaf has received little attention, even though it has been well established that the aromatic amino acids found in proteins—namely, tryptophan (Trp), tryosine (Tyr), and phenylalanine (Phe)—all exhibit fluorescence characteristics (Barenboim et al., 1969; Teal and Weber, 1957; Wetlaufer, 1962). More recently, the in vitro fluorescent properties of both ribulose 1,5-bisphosphate carboxylase (rubisco) and cucumber microsomal membranes have been demonstrated along with UV-B photodegradative effects (Caldwell, 1993).

The absorption and fluorescence characteristics of aromatic amino acids are summarized in Table 1 (Barenboim et al., 1969; Wetlaufer, 1962). The molar extinction coefficients correspond to the lower energy absorption maxima and are in the ratio of 27:7:1. As a result, exposed Trp residues generally dominate the absorption characteristics of the aromatic amino acids, and Trp is the primary fluorescent chromophore for most proteins. For proteins in which Trp is not present or not exposed to UV illumination, the aromatic amino acids Tyr and Phe exhibit fluorescence emissions. The majority of aromatic amino acids in the leaf are bound to form proteins in which the more buried ones display shorter wavelengths of maximum emission (Lakowicz et al., 1973). Consequently, shifts in the location of fluorescent maxima emanating from various plant proteins can be expected owing to aromatic amino acid composition and structural conformation.

UV band fluorescence characteristics of ten species including representatives of both monocots and dicots have been studied (Corp et al., 1994). Shifts in the location of maximum fluorescence emission were noted between monocot and dicot plant classes. The mode of UV band fluorescence emission maxima for dicot species occurred at 335 nm in contrast with 315 nm for monocots. The blue band fluorescence with emission maxima near 440 nm remained relatively low for all dicot species studies, whereas monocots exhibited more substantial contributions at 440 nm. Similar blue fluorescence characteristics were observed by Chappelle et al. (1984), where a nitrogen laser emitting at 337 nm was used as a blue fluorescence excitation source. The relation between UV and blue bands of fluorescence were better seen through the fluorescence ratio 335:440 nm, where separation of the two plant classes became clear (Corp et al., 1994).

The objectives of this study were to assess in vivo bands of fluorescence to distinguish vegetation treated with varying levels of nitrogen. In addition, we will explore relations between fluoresence and other physiological parameters of the plant, such as rate of photosynthesis, chlorophyll concentration, and total protein content. Because a large portion of the nitrogen in plants is present in the form of amino acids combined to form proteins, it is feasible that fluorescence measurements of intact plant material excited at 280 nm could provide useful estimates of the nitrogen status within the leaf.

METHODS AND MATERIALS

Preparation of Plant Material

Different nitrogen levels were obtained by growing soybeans (*Glycine max* cv. Essex) in the greenhouse with a full complement of nutrients minus N. The plants were grown in a sterile perlite growth medium where no rhizobium species were allowed to infect the roots. Nitrogen was added separately in concentrations that ranged from 0.004 M to 0 M urea to produce levels corresponding to 100%, 75%, 50%, 25%, and 0% of the nitrogen required for optimal growth. Nutrient solutions were applied once a week. The following measurements were

made respectively on the uppermost fully expanded leaf after 8 weeks of growth.

Rate of Photosynthesis

Rates of photosynthesis of soybeans were determined by using an infrared gas analyzer (LI-COR 6200, LI-COR Inc., Lincoln, NE)[1] in the closed mode. The measurements were made in the laboratory with light source consisting of a combination of water-cooled low-pressure sodium lamps and alkaline metal halide lamps, providing an intensity of approximately 1800 μmol/(m^2 s). Measurements were made over a 30-s time frame in which CO_2 concentrations dropped from the ambient concentration of 350 ppm. Temperature within the photosynthesis chamber did not exceed 27°C, and the relative humidity was maintained through the use of desiccant at 38%.

Fluorescence Excitation and Emission Spectra

A spectrofluorometer (Fluorolog II, Spex Industries, Edison, NJ)[1] was used to collect fluorescence excitation and emission spectra. The spectrofluorometer utilized two 0.22-m double monochrometers. The excitation monochrometer was attached to a 450-W xenon lamp, which allowed variation of the excitation radiation. The emission monochrometer was attached to a photon-counting photomultiplier tube corrected to obtain linearity throughout the emission wavelength range of 290 to 850 nm while voltage readings were calibrated to photon counts per second (cps). Fluctuations in lamp intensity were corrected by using a beam splitter to deliver a portion of the excitation radiation to a rhodamine dye cuvette. The fluorescence response of the rhodamine dye was monitored by a silicon photodiode. This response was used to generate correction factors for equalizing changes in lamp intensity as a function of wavelength. Excitation spectra were acquired by setting the emission wavelength (usually at the fluorescence emission maximum) and recording emission intensities while stepping through a shorter wavelength region of the spectrum. Conversely, emission spectra were obtained by fixing the excitation wavelength and recording emission intensity while stepping through a longer wavelength region of the spectrum. Both excitation and emission spectra were acquired at a 1-mm slit width yielding 1.7 nm resolution. Leaf samples were held in place by a nonfluorescent anodized aluminum solid sample holder.

Pigment and Protein Determinations

Leaf disks (2.54 cm^2) were extracted in 3 ml of dimethyl sulfoxide (DMSO), and absoprtion measurements of the extracts were made by using a Perkin-Elmer dual beam spectrophotometer. Chlorophyll concentrations were determined by methods modified from Lichtenthaler (1987). The remainder of the leaf was freeze dried at −45°C and then ground into a fine powder. Total nitrogen was determined by using the Dumas combustion method (Bellomonte et al., 1987). Total protein was estimated by multiplying total nitrogen by 6.25 (Lawlor et al., 1989). Specific proteins utilized in this study were obtained in the purest form supplied by Sigma Chemical.

Deconvolution Analysis

A deconvolution routine based on the Marquardt-Levenberg algorithm (Levenberg, 1944; Marquardt, 1963) was used to separate the UV and blue components of the fluorescence emission data. The software (Peakfit 3.0, Jandel Scientific) includes numerous user functions that were evaluated by both graphical and numerical techniques to determine the equation to best fit the fluorescence data. Of the available functions, the asymmetric double sigmoidal function (ADS) provided the best curve fit routine:

$$y=\frac{a_0}{1+\exp\left[-\left(\frac{(x-a_1+a_2/2)}{a_3}\right)\right]}\left[1-\frac{1}{1+\exp\left[-\left(\frac{x-a_1-a_2/2}{a_3}\right)\right]}\right]$$

a_0=amplitude
a_1=center
a_2=width
a_3=shape 1 (>0)
a_4=shape 2 (>0)

RESULTS AND DISCUSSION

Illumination of vegetation with 280-nm radiation produced a UV fluorescence emission band in addition to the blue, green, red, and NIR emissions (Fig. 1). This observation in vivo leads to the following definition of in vivo UV band fluorescence:

> *A broadband fluorescence emission ranging from 300 to 400 nm with a maximum near 335 nm emanating from plant material when excited within the wavelength range of 220 to 300 nm.*

The wavelength of maximum emission shifts with species has been observed as low as 310 nm with some monocots. The majority of this emission occurs between 300 and 400 nm, while the long tail tapers to negligible amounts after 500 nm. Furthermore reduced emissions from the chlorophyll bands of fluorescence at 685 nm and 740 nm can result when plant material is excited at 280 nm. The excitation spectrum shown in Figure 1 indicates dual excitation maxima—the first centered on 232 nm and the second on 284 nm. The symmetry and proximity of these maxima to the absorption maxima of Trp in aqueous solution (Table 1) provides further evidence to support Trp as the primary chromophore for the *in*

[1] Company and trade names are given for the benefit of the reader and do not imply any endorsement of the product or company by Science Systems and Applications Inc., National Aeronautics and Space Administration, or the U.S. Department of Agriculture.

Table 1. Absorption and Fluorescence Emission Characteristics for Aromatic Amino Acids

Aromatic Amino Acid	*Wavelengths of Maximum Absorption*		*Molar Extinction Coefficient*	*Fluorescence Emission Maxima*	*Fluorescence Quantum Yield*
	I	*II*			
Trp	280.5 nm	221.5 nm	5600	348 nm	0.20
Tyr	293.5 nm	240 nm	800	303 nm	0.21
Phe	258 nm	—	207	282 nm	0.04

Amino acids are in aqueous solution at a neutral pH. The molar extinction coefficients were calculated by using wavelengths of maximum absorption in column I. The quantum yields for these compounds can vary with ionization state of the OH, NH, COOH, and NH_2 groups (Barenboim, 1969).

vivo UV fluorescence emission from soybean vegetation. The fluorescent properties of individual plant proteins depend greatly on the structural conformation and the relative degree of exposure for each of the three aromatic amino acids (Lakowicz et al., 1992). This could explain why the UV band fluorescence from monocots is shifted toward shorter wavelengths.

Illumination of soybean leaf material with 340-nm light yields multiple fluorescence bands (Fig. 2) with maxima in the blue, green, red, and NIR consistent with those reported by Chappelle et al. (1984). The blue band emission excited at 280 nm was less intense than when excited at 340 nm. This observation becomes even more clear after deconvolution (i.e., UV band increases the apparent blue band fluorescence). Some of the compounds that are suspected of contributing to the convoluted blue band fluorescence when excited at 340 nm also exhibit absorption characteristics at 280 nm. Although not optimally excited, these compounds are believed to contribute to blue band fluorescence when excited at 280 nm. Correlation analysis between fluorescence intensities at 440 nm when excited at 280 nm and 340 nm was $r^2=0.98$.

The excitation spectrum shown in Figure 2 indicates that the optimal excitation for in vivo blue fluorescence was 340 nm with more subtle features at 284 and 232 nm. The blue fluorescence excitation spectrum was not symmetrical and was skewed toward shorter wavelengths. The rise in blue fluorescence when excited within the wavelength range of 220 to 300 nm originates from the added intensity by the broad tail of UV band fluorescence. Hence the lack of symmetry in the blue fluorescence excitation spectrum is due to the convolution of UV and blue emission bands of fluorescence.

To identify potential UV fluorescent plant constituents, fluorescence excitation and emission spectra were compared between intact vegetation and the following representatives of enzymatic plant proteins; rubisco, AT-Pase (adenosine 5′-triphosphatase), PEP carboxylase (phosphoenolpyruvate carboxylase). These plant proteins all exhibit dual fluorescence excitation maxima at 227 nm and 280 nm, with emission maxima consistently at 335 nm (Fig. 3). All three proteins exhibit fluorescence characteristics consistent with Trp-containing proteins. The magnitude of fluorescence depends not only on the Trp

Figure 1. Healthy soybean leaf fluorescence characteristics. Excitation spectrum was obtained at a fixed emission wavelength of 335 nm (UV band emission maximum). Emission spectrum was obtained at a fixed excitation wavelength of 280 nm (UV band excitation maximum).

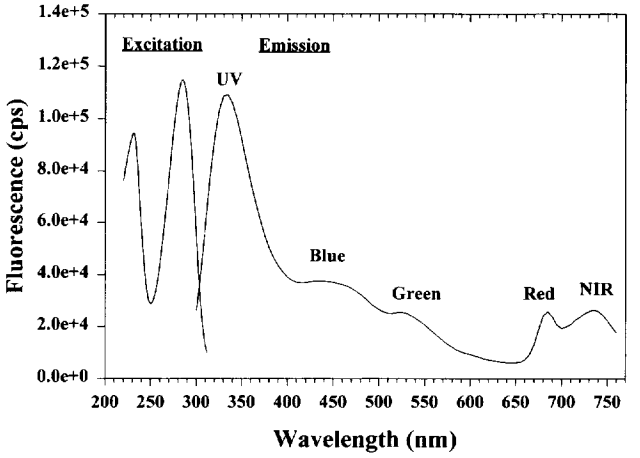
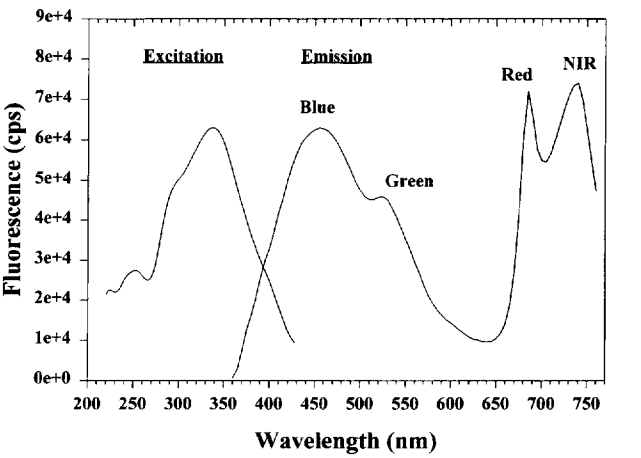


Figure 2. Healthy soybean leaf fluorescence characteristics. Excitation spectrum was obtained at a fixed emission wavelength of 440 nm (blue band emission maximum). Emission spectrum was obtained at a fixed excitation wavelength of 340 nm (blue band excitation maximum).



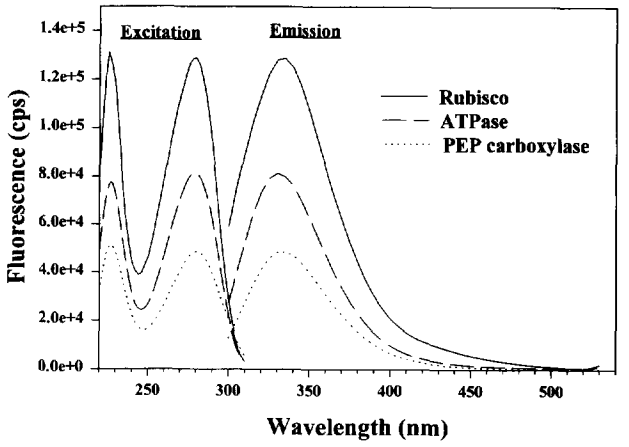


Figure 3. Fluorescence excitation and emission spectra for three prominent plant proteins in aqueous solution (pH= 7.0), each at a concentration of 25 μg of protein/ml.

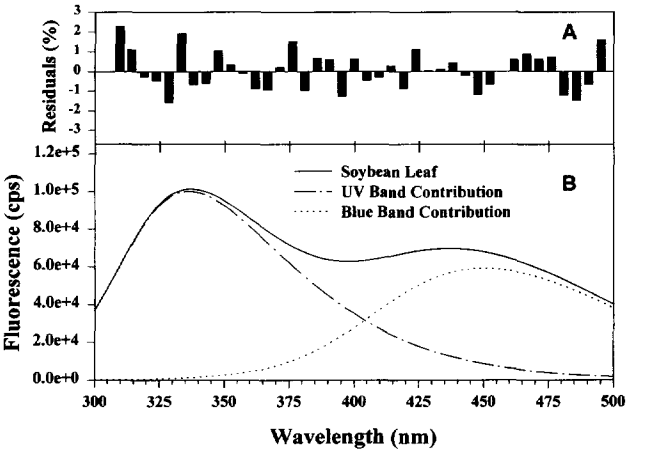


Figure 4. (A) Residuals (observed-predicted) as a percentage of observed values for the ADS spectral function. (B) UV and blue band contributions to the fluorescence spectra of a healthy soybean leaf.

content, but also on the polarity of the solvent and the degree of exposure of the Trp residues within the protein (Pigault and Gerard, 1984). Dicot vegetation exhibits similar characteristics with fluorescence excitation maxima at 232 nm and 284 nm, with emission maxima most frequently at 335 nm (Fig. 1).

A deconvolution routine based on the ASD curve fit function was applied to separate the UV from blue band emission components. UV band curve fit parameters were obtained by applying the least squares curve fit routine to pure rubisco in aqueous solution. Blue band curve fit parameters were obtained by exciting a soybean leaf at 310 nm, where one finds little to no absorption by Trp residues within plant proteins (Teal and Weber, 1957). At this excitation wavelength, the intact leaf exhibited no UV band fluorescence while maintaining a substantial fluorescence emission in the blue. Although evidence suggests that the blue band fluorescence is convoluted with contributions of such compounds as NADPH, polyphenolics, and lignin (Cerovic et al., 1994; Chappelle et al., 1991; Lang and Lichtenthaler, 1991; Lichtenthaler et al., 1996; Morales et al., 1994), at present there is not enough information available regarding the relative contributions of these compounds to construct a deconvolution routine. As a result, the blue band portion of the fluorescence emission was treated as a single entity. The curve fit parameters for the UV and blue bands were combined and applied to the fluorescence spectra of the intact leaf when excited at 280 nm (Fig. 4). From Figure 4, the broad nature of the UV band emission and the magnitude of its contribution to the blue band emission can be visualized.

Fluorescence emission spectra for soybeans treated with varying levels of nitrogen supply were recorded at an excitation of 280 nm (Fig. 5) and at 340 nm (Fig. 6). Clear trends are apparent with the magnitude of fluorescence at both 335 nm and 445 nm decreasing with nitrogen treatment. The foregoing deconvolution procedures were then applied to the 40 fluorescence spectra where:

- Least squares linear fit was achieved within 30 iterations.
- Residuals (observed values-predicted values) remained random and within 2% of the observed values (see Fig. 4*a*).
- ASD center (a_1) remained fairly constant for both bands:
  UV band at 334.4±1.9 nm; blue band at 446.6±5.6 nm.
- Shape indicated a consistent skew toward longer wavelengths.
- The majority of variation occurred with amplitude (a_0) of fluorescence.

After deconvolution, significant differences were apparent between the fluorescence ratios (335:445 nm) of the nitrogen treatments (Table 2). The deconvoluted fluorescence ratio was more sensitive to nitrogen fertilization level than to measurements of chlorophyll concentration or gas exchange. Gas exchange measurements provided significant separation of the high and low treatments, whereas correlation analysis did not indicate a strong relation to the magnitude of fluorescence bands or ratios of these bands. The highest correlation between gas exchange and fluorescence was obtained with the fluorescence ratio of 445:600 when excited at 340 nm (r^2=0.60). This finding is consistent with previous results (Chappelle et al., 1991), although the correlation here was not at strong.

The chlorophyll fluorescence emission maxima at 685 nm and 740 nm with excitation at 340 nm exhibited decreasing trends with reduced nitrogen fertilization (Fig. 6). With the Student-Newman-Keuls multiple

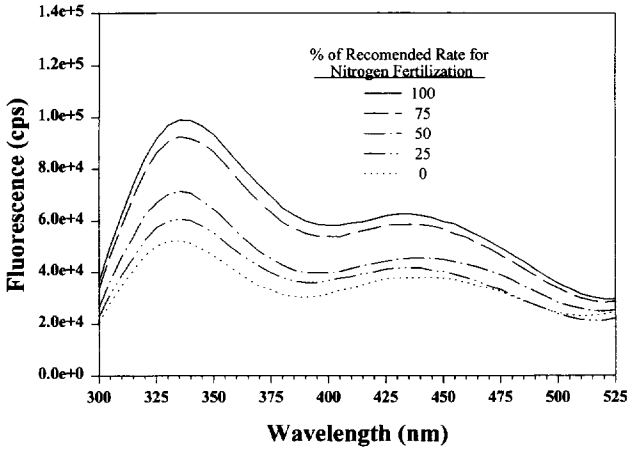


Figure 5. Fluorescence emission spectra (excitation at 280 nm) for soybeans treated with varying levels of nitrogen. Each curve represents the mean of eight replications.

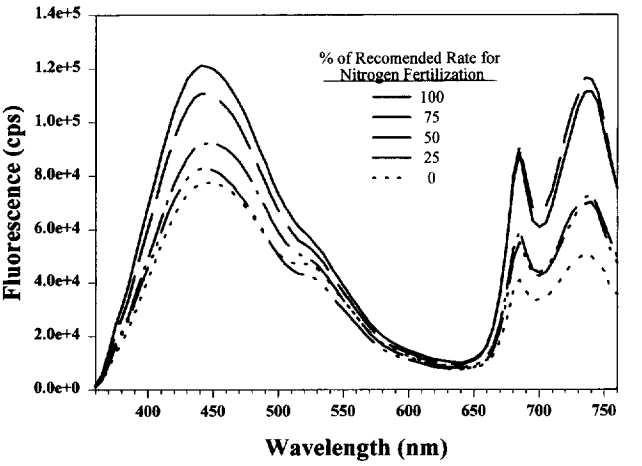


Figure 6. Fluorescence emission spectra (excitation at 340 nm) for soybeans treated with varying levels of nitrogen. Each curve represents the mean of eight replications.

range test, the two highest nitrogen treatments were separable from the lower treatments for each of these two fluorescence bands (Table 3). The ratio of 685:740 nm showed no significant separation of nitrogen treatments. It is possible that a longer wavelength of excitation would improve the relations between nitrogen fertilization and chlorophyll fluorescence.

The most significant finding in this study was the relation between the in vivo fluorescence ratio of 335:445 nm (ex. 280 nm) to the concentration of total leaf protein (Fig. 7). Because 50–80% of leaf nitrogen is allocated to photosynthetic proteins, including rubisco, whose concentration increases with increasing nitrogen supply (Hikosaka and Terashima, 1995), fluorescence could be a more sensitive tool to assay the physiological productivity of the leaf than gas exchange measurements. As evidenced by these studies, UV band and to a higher degree the deconvoluted ratio of UV to blue band fluorescence increases with increasing protein concentration.

CONCLUSIONS

A convoluted UV fluorescence band was observed when intact vegetation was excited with 280-nm monochromatic light. Similarities between the fluorescence properties of isolated plant proteins and the intact leaf were evident. Rubisco, whose function is to catalyze the CO_2 fixation in photosynthesis, can constitute as much as 70% of the soluble plant proteins and consequently has been named the world's most abundant enzyme. Because of its fluorescence properties and high concentration, it is likely that this enzymatic protein contributes to the UV band fluorescence emanating from the intact leaf. To further deconvolute the UV band fluorescence into membrane and soluble protein fractions would be a formidable task owing to the close proximity of fluorescence emission maxima. Purification procedures for membrane-bound proteins generally result in increased exposure to Trp residues, resulting in increased fluorescence making qualitative comparisons difficult.

Recently there has been some debate about the distance UV radiation penetrates into the leaf. The depth of penetration of excitation light affects the feasibility of individual compounds to fluoresce based on their location within the leaf. In these studies, the excitation at 340 nm and to a lesser extent at 280 nm completely penetrated greenhouse-grown soybean leaves because a por-

Table 2. Means for Protein, Fluorescence Maxima, Fluorescence Ratios, and Photosynthesis for the Nitrogen Treatments

% Optimal Fertilization Rate	*% Protein (based on dry leaf weight)*	*Fluorescence at 335 nm*	*Fluorescence at 335 nm Deconvoluted*	*Fluorescence Ratio 335:445*	*Fluorescence Ratio 335:445 Deconvoluted*	*Photosynthesis [μmole CO_2/(m^2s)]*
100	28.85 a	98986 a	98538 a	1.594 a	2.024 a	37.721 a
75	29.16 a	92495 a	91749 a	1.616 a	1.666 b	30.426 b
50	24.64 b	71522 ab	70510 b	1.540 a	1.348 bc	29.926 b
25	21.43 b	60699 b	59479 b	1.464 a	1.130 cd	26.504 b
0	16.51 c	52187 b	50968 b	1.341 a	0.906 d	19.730 c

Student-Newman-Keuls multiple range test was performed at the 95% confidence level.
Means with the same letter are not significantly different (n=40).
Fluorescence measurements were excited at 280 nm.

Table 3. Means for Chlorophyll, Fluorescence Maxima, and Fluorescence Ratios for the Nitrogen Treatments

% Optimal Fertilization Rate	*Chlorophyll a* ($\mu g/cm^2$)	*Fluorescence at 445 nm*	*Fluorescence at 685 nm*	*Fluorescence at 740 nm*	*Fluorescence Ratio 445:600*	*Fluorescence Ratio 685:740*
100	4.427 a	121286 a	88532 a	111345 a	4.246 a	0.7965 a
75	3.427 b	110708 ab	90902 a	115945 a	4.229 a	0.7881 a
50	2.911 b	91717 bc	59506 ab	72851 b	3.807 b	0.8157 a
25	2.713 b	82705 c	54978 b	70078 b	3.723 b	0.7805 a
0	1.335 c	77007 c	40791 b	49813 b	3.037 c	0.8207 a

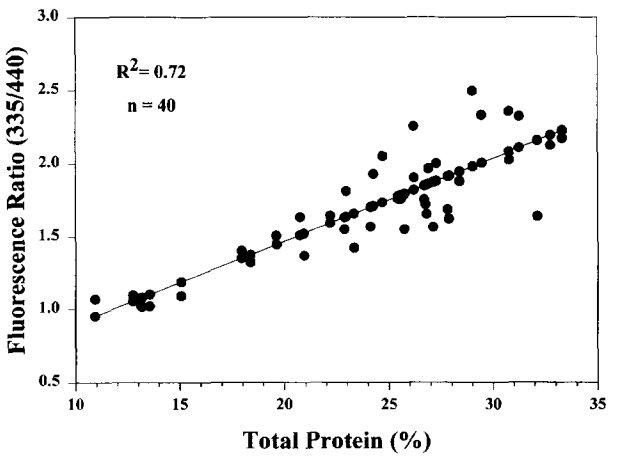
Student-Newman-Keuls multiple range test was performed at the 95% confidence level.
Means with the same letter are not significantly different ($n=40$).
Fluorescence measurements were excited at 340 nm.

tion of the excitation energy was detected at the back side of the leaf. As a result, compounds throughout these leaves had the potential to fluoresce. Field-grown leaves tend to be thicker with higher concentration of UV protective pigments, which in turn reduces the penetration of fluorescence excitation sources. Whether this will have a significant effect on the fluorescence features of field-grown vegetation is yet to be determined.

Nevertheless, the UV band fluorescence emission in vivo adequately separates greenhouse-grown soybean vegetation of varying levels of nitrogen supply. Because a large portion of the nitrogen in plants is present in the form of amino acids existing free or combined to form proteins, it follows that total protein concentrations decrease with decreasing availability of nitrogen. As a consequence the UV fluorescence emission from intact vegetation decreases with decreased availability of nitrogen. The relation between fluorescence and nitrogen fertilization level was improved by deconvolution and the subsequent ratio of UV to blue fluorescence maxima.

In soybeans, rubisco concentrations steadily increase until the leaf reaches full expansion, at which point they decline until senescence (Miller and Huffaker, 1982; Pell et al., 1992). During stages of rapid growth, concentrations of plant proteins are elevated, but heat or cold shock can have varying effects on their rates of synthesis and metabolism. These characteristics along with differential partitioning of protein resources during plant development could well account for the observed variability in UV band fluorescence emanating from the leaf over its growth cycle. Limiting measurements to the uppermost, fully expanded leaf substantially reduces within treatment variability and yields the most significant relation with respect to plant health.

Figure 7. The relation between total protein as a percentage of dry leaf weight and the deconvoluted ratio of UV to blue bands of fluorescence.



Instrumentation for the remote assessment of the UV fluorescence band would be limited insofar as aerial observation depends on the availability of lasers of sufficient power to emit near 280 nm. Portable ground-based instrumentation could easily be fabricated and could prove useful in assessing plant vigor. A rapid quantitative measure of nitrogen status could prove useful to many farming systems where substantial investments are made in the application of nitrogen fertilizers. As far as legume crops are concerned, N_2 from the atmosphere can be fixed and made available to the plant if the roots are adequately inoculated or nodulated with nitrogen fixing bacterium. A rapid procedure for assessing leaf nitrogen would be useful in determining plant deficiencies in the field where organic or chemical supplements are required to improve soil condition and crop health. Both economic and environmental benefits would result in the selective, as needed application of nitrogen fertilizers.

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