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# Assessment of Vegetation Stress Using Reflectance or Fluorescence Measurements

P. K. E. Campbell,\* E. M. Middleton, J. E. McMurtrey, L. A. Corp, and E. W. Chappelle

## ABSTRACT

Current methods for large-scale vegetation monitoring rely on multispectral remote sensing, which has serious limitation for the detection of vegetation stress. To contribute to the establishment of a generalized spectral approach for vegetation stress detection, this study compares the ability of high-spectral-resolution reflectance (R) and fluorescence (F) foliar measurements to detect vegetation changes associated with common environmental factors affecting plant growth and productivity. To obtain a spectral dataset from a broad range of species and stress conditions, plant material from three experiments was examined, including (i) corn, nitrogen (N) deficiency/excess; (ii) soybean, elevated carbon dioxide, and ozone levels; and (iii) red maple, augmented ultraviolet irradiation. Fluorescence and R spectra (400–800 nm) were measured on the same foliar samples in conjunction with photosynthetic pigments, carbon, and N content. For separation of a wide range of treatment levels, hyperspectral (5–10 nm) R indices were superior compared with F or broadband R indices, with the derivative parameters providing optimal results. For the detection of changes in vegetation physiology, hyperspectral indices can provide a significant improvement over broadband indices. The relationship of treatment levels to R was linear, whereas that to F was curvilinear. Using reflectance measurements, it was not possible to identify the unstressed vegetation condition, which was accomplished in all three experiments using F indices. Large-scale monitoring of vegetation condition and the detection of vegetation stress could be improved by using hyperspectral R and F information, a possible strategy for future remote sensing missions.

SINCE the turn of the 20th century, anthropogenic impacts have significantly altered the natural environment. The current biomass production in agriculture and commercial forestry is achieved primarily by applying nitrogen (N)-enriched fertilizers and raising soil N levels. Tropospheric ozone (O<sub>3</sub>) and carbon dioxide (CO<sub>2</sub>) levels have also increased, as has ultraviolet (UV) radiation reaching the ground level, all of which lead to progressive alteration in natural and cultivated ecosystems (U.S. Climate Change Science Program, 2005). Accurate remote sensing (RS) monitoring, regularly providing synoptic views of the dynamic ecosystem parameters, is required for vegetation monitoring, timely stress detection, understanding the direction of environmental change, and making effective management decisions. To contribute toward the establishment of an

accurate, generalized RS approach for vegetation monitoring, this investigation comparatively evaluates the capabilities of spectral reflectance (R) and fluorescence (F) measurements for the detection of vegetation stress associated with common environmental factors, such as N deficiency and elevated CO<sub>2</sub>, O<sub>3</sub>, and UV.

## Nitrogen, Carbon Dioxide, Tropospheric Ozone, and Ultraviolet Effects on Vegetation Physiology

Plant carbon (C) sequestration and biomass production are driven by N availability because N is involved in photochemical processes and is one of the primary resources regulating plant growth. Corn, a C<sub>4</sub> species, has higher requirement for N than most other plants or crop species (Meisinger, 1984). However, excessive N applications may suppress crop yield and biomass allocation, and N runoff from agricultural and urban areas can cause soil acidification and leaching of essential minerals (e.g., cations P<sup>2+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, and Ca<sup>2+</sup>) (McMurtrey et al., 1994). Because most terrestrial ecosystems evolved in N-limited environments, careful monitoring is needed to understand species and ecosystems dynamics in an N-rich world.

Globally, industry and transportation are adding almost 7 trillion kg yr<sup>-1</sup> of C to the atmosphere, and atmospheric CO<sub>2</sub> concentrations are on the rise (U.S. Climate Change Science Program, 2005). Although this enrichment of the atmosphere portends hazards, it offers the potential benefit to increase plant production. At elevated CO<sub>2</sub>, the photosynthetic efficiency and yield of important crops such as corn, soybean, cotton, peanut, rice, wheat, sorghum, and oats may experience increase (i.e., C<sub>3</sub> plants more than C<sub>4</sub>) (Heagle et al., 1998; Tiedemann and Firsching, 2000). Under natural conditions, limiting factors (e.g., water and N deficits and weather extremes) and the resulting changes to plant physiology would mitigate the positive effects (Tuba, 2005).

Due to anthropogenic activities, O<sub>3</sub> pollution is on the rise in the troposphere, presenting a threat to agricultural crops and human health (U.S. Climate Change Science Program, 2005). Because of its reactive nature, tropospheric O<sub>3</sub> is the primary component of air pollution that causes negative affects on vegetation physiology, growth, and yield (Krupa and Kickert, 1989; Mulchi et al., 1995; Krupa et al., 2001; Kim et al., 2003). During the day, leaf stomata are normally open, permitting the entry of CO<sub>2</sub> for photosynthesis, which enables O<sub>3</sub> to enter the stomata (Reich, 1987; Larcher, 1995). Ozone

P.K.E. Campbell, Joint Center for Earth Systems Technology, Univ. of Maryland, Baltimore County (UMBC), Baltimore, MD 20771, USA. E.M. Middleton, E.W. Chappelle, and P.K.E. Campbell (current address), Biospheric Sciences Branch, Code 614.4, NASA/Goddard Space Flight Center, Greenbelt, MD 20771 USA. J.E. McMurtrey, Hydrology and Remote Sensing Lab., Agricultural Research Service, USDA, Beltsville, MD 20705 USA. L.A. Corp, Science Systems and Applications Inc. (SSAI), Lanham, MD 20706 USA. Received 14 Oct. 2005. \*Corresponding author (pcampbel@pop900.gsfc.nasa.gov).

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677 S. Segoe Rd., Madison, WI 53711 USA

**Abbreviations:** Car, carotenoids; Chl, chlorophyll; ChlF, chlorophyll fluorescence; cps, counts per second; D, derivative; F, fluorescence; K, potassium; LS means, least square means; NDVI, normalized difference vegetation index; PRI, photochemical reflectance index; R, reflectance; REIPw, wavelength position of the red edge inflection point; RS, remote sensing; SLM, specific leaf mass; TM (1–7), spectral bands on LandsatTM; USDA, United States Department of Agriculture; UV, ultraviolet.

can cause a range of adverse effects, including premature leaf aging, early onset of senescence, growth and yield reductions, and altered sensitivity to biotic and abiotic stresses (Skärby et al., 1993; Krupa et al., 2001; Fuhrer, 2003; Jäger et al., 2003). Plants have evolved protective mechanisms to repair O<sub>3</sub> injury before damage is severe, such as increased production of the antioxidant vitamins C and E, polyamines, and specialized enzymes (Melhorn and Wellburn, 1987). Therefore, careful monitoring and timely detection of O<sub>3</sub> damage may enable management decisions that limit the adverse effects.

Numerous investigations addressing the combined effect of CO<sub>2</sub> and O<sub>3</sub> on plants' physiologic responses indicate that the positive effects of CO<sub>2</sub> on vegetation growth are negated, partially or completely, by elevated O<sub>3</sub> concentrations (Krupa and Kickert, 1989; Leblanc, 1998; Kim et al., 2003). Extended foliar exposure to O<sub>3</sub> typically reduces CO<sub>2</sub> fixation, and further inhibition of CO<sub>2</sub> uptake occurs through O<sub>3</sub>-induced reduction in chloroplast function, smaller stomatal opening, or CO<sub>2</sub> loss through respiration (Guzy and Heath, 1993; Jäger et al., 2003). On the other hand, the negative effects of O<sub>3</sub> stress may be partially or fully ameliorated under increased atmospheric CO<sub>2</sub> concentrations (McKee et al., 2000; Kim et al., 2003). However, in the agricultural areas of the midwestern USA, tropospheric O<sub>3</sub> is increasing more rapidly than CO<sub>2</sub>. The corn-soybean agricultural complex is the largest ecosystem of the contiguous USA and is one of the most important for USA exports and international food security. Systematic monitoring of this system would provide significant economic benefits (U.S. Climate Change Science Program, 2005).

Since the 1970s, human activities have disrupted the natural balance between stratospheric O<sub>3</sub> synthesis and breakdown, resulting in chemical depletion of the O<sub>3</sub> layer and an increase of UV-B levels reaching the Earth's surface (especially in the Southern hemisphere). The effects of UV-B radiation on vegetation are mostly damaging, often resulting in toxic or mutagenic DNA products that inhibit plant function and depress biomass allocation (Middleton and Teramura, 1993; Krupa et al., 2001). Common protective responses of field-grown plants to elevated UV-B radiation are the increased synthesis of UV-absorbing compounds in the foliage and the development of thicker leaves as a result of an increase in the thickness of the upper epidermis layer and the spongy parenchyma (Sullivan et al., 2002). Because many of the UV-B-induced changes in physiology and function are linked to reductions in plant growth and productivity, elevated UV-B levels can be considered a significant environmental stress factor for agricultural and natural ecosystems (Middleton and Teramura, 1993; Tegelberg et al., 2001; Sullivan et al., 2003).

### Spectral Detection of Vegetation Stress

As changes in an ecosystem occur, alterations in foliar chemistry and membrane structure result in changes in vegetation spectral signatures (Rock et al., 1986, 1994; Martin, 1994; Martin and Aber, 1997; Entcheva et al., 1999, 2004); thus, spectral observations could offer a

sensitive, physiologically based indicator of vegetation stress. Current methods for monitoring vegetation biophysical and growth parameters rely on broadband ( $\geq 20$  nm bands) satellite data, such as those on Landsat Thematic Mapper, MODIS (Moderate Resolution Imaging Spectroradiometer), and SPOT (Système Pour l'Observation de la Terre) satellites. The commonly used visible and near-infrared (700–1200 nm) R broadband vegetation indices are primarily associated with foliar chlorophyll (Chl) levels at the top of the canopy, not providing information about the function of the canopy as a whole. Therefore, their use has serious limitations for early detection of vegetation stress or for estimation of vegetation biophysical parameters not directly related to Chl content. As compared with broadband, high-spectral-resolution ( $>10$  nm) R parameters are much better correlated with the amount of foliar Chl (Carter, 1994; Carter et al., 1995; Carter and Spiering, 2000; Carter and Knapp, 2001) and with the amount of projected green leaf area of canopies and landscapes observed by RS platforms (Walter-Shea et al., 1992; Entcheva et al., 1999; Carter and Spiering, 2000; Entcheva, 2000). Only the PRI index ( $PRI1 = [R530 - R570]/[R530 + R570]$ ) has been associated with vegetation photosynthetic activity (Gamon et al., 1997). Recent technological developments, such as the launch of the first Earth Observing satellite by NASA, are making space-borne hyperspectral data more widely available. High-spectral-resolution R data can provide a significant improvement over the broadband indices for the detection of changes in vegetation condition and for monitoring vegetation decline (Entcheva, 2000; Entcheva et al., 2004). However, R measurements are not able to directly assess vegetation function.

Unlike R, F emitted from the Chl is directly related to photochemical reactions and has been used extensively for the elucidation of the photosynthetic pathways (Lichtenthaler, 1988; Cerovic et al., 1999; Corp et al., 2003; Kim et al., 2003). Contributions to blue/green F emissions (400–570 nm) come from two sources: (i) the dynamic portion associated primarily with short-term physiologic changes occurring within the leaf mesophyll (Chappelle et al., 1992; Cerovic et al., 1999) and (ii) the static fraction attributed to inert structural compounds, such as polyphenolics and lignin in the leaf epidermis and cell walls (Chappelle et al., 1992; Lichtenthaler et al., 1996; Lichtenthaler and Schweiger, 1998; Cerovic et al., 1999). Fluorescence emissions in the red (685 nm) and far-red (740 nm), which emanate from Chl *a* (referred to as chlorophyll fluorescence [ChlF]), are inversely related to the functionality of the foliar chloroplasts (Adams and Demmig-Adams, 2004). In general, increases in ChlF intensities have been associated with vegetation stress (Lichtenthaler, 1988; Kim et al., 2003). Spectral ratios such as F440/F740, F530/F685, and F685/F740 have been shown to change with decline in photosynthetic efficiency and are more accurate indicators of vegetation condition than individual F bands (Lichtenthaler, 1988; Chappelle et al., 1992; Cerovic et al., 1999; Corp et al., 2003). These F emission ratios have the potential for early detection of disturbances that could inhibit vegetation growth and productivity.

## Research Goal and Objectives

This investigation compares the spectral changes in vegetation R and F over the 400- to 800-nm spectral range, associated with a range of common environmental factors. By focusing on the common spectral trends associated with the different species and stress factors, the goal is to contribute toward the establishment of a generalized, more precise, and timely spectral approach for the detection of vegetation stress. The objectives of the investigation were (i) to compare the sensitivity of R and F measurements to the vegetation treatments and associated changes in foliar constituents and (ii) to determine if the trends in the R and F data differ and can provide complimentary information.

## METHODS

### Experimental Design and Treatments

The current investigation used plant material from the long-term experiments maintained for the study of global climate change conducted by the USDA Climate Stress Laboratory (Beltsville, MD). The experimental treatments, specifically designed to produce a wide range of vegetation stress, included (i) N fertilization on corn (McMurtrey et al., 1994; Middleton et al., 2004), (ii) the combined effects of elevated CO<sub>2</sub> and O<sub>3</sub> on soybean (Mulchi et al., 1995; Leblanc, 1998; Kim et al., 2003), and (iii) elevated UV-B radiation on maple trees (Sullivan et al., 2002, 2003). Plant material from the these three series of experiments was collected at the end of the growing season in 2000 and used to compare the changes in spectral R and F properties, associated with differences in leaf constituents, induced by the long-term treatments.

The effects of nutrient deficiency and nutrient excess on spectral properties were studied in Experiment I on corn foliage (*Zea mays* L.). The spectral data and the foliar samples were collected when the corn was in grain-fill growth stage. The experimental design was a randomized complete block with four blocks, each containing eight N treatments at 150%, 125%, 100%, 75%, 50%, 25%, 12%, and 0% of the optimal N application rate of 162 kg N/ha, prescribed by the University of Maryland Soil Testing Lab. The 100% N treatment was considered optimal, whereas N deficiency was represented by the 0% to 75% treatments, and high N rates were provided by the 125% and 150% groups. Nitrogen deficiency and excess resulted in reduced corn yield (Corp et al., 2003).

The effects of elevated CO<sub>2</sub> and O<sub>3</sub> on spectral properties were studied in Experiment II on leaves from two varieties of soybean (*Glycine max* (L.) Merr.) - Manokin (O<sub>3</sub> sensitive) and KS4694 (CO<sub>2</sub> sensitive), which were planted within the same open-top chambers (3 m in diameter). The plants were grown in Codorus silt loam (fine-loamy, mixed, mesic Fluvaquentic Dystrochrept), under a well-watered regime (0 to -0.05 MPa). The air quality treatments, provided over the entire growing season, are [1] Control (CF or Control), charcoal-filtered ambient air to reduce ambient O<sub>3</sub> and other gaseous pollutants, providing ~350 µL CO<sub>2</sub> L<sup>-1</sup> and ~22 nl O<sub>3</sub> L<sup>-1</sup>; [2] +CO<sub>2</sub>, CF air (345 µL CO<sub>2</sub> L<sup>-1</sup>/20 nl O<sub>3</sub> L<sup>-1</sup>) with the addition of ~137 µL CO<sub>2</sub> L<sup>-1</sup>, elevating CO<sub>2</sub> levels to ~487 µL L<sup>-1</sup> for 18 h d<sup>-1</sup>; [3] +O<sub>3</sub>, ambient nonfiltered air (346 µL CO<sub>2</sub> L<sup>-1</sup>/35 nl O<sub>3</sub> L<sup>-1</sup>) with the addition of 35 ± 5 nl O<sub>3</sub> L<sup>-1</sup>, providing 70 nl O<sub>3</sub> L<sup>-1</sup> for 7 h d<sup>-1</sup>; and [4] +CO<sub>2</sub>+O<sub>3</sub>, ambient nonfiltered air with the additions of the previously mentioned CO<sub>2</sub> and O<sub>3</sub> concentrations, providing elevated levels at ~485 µL CO<sub>2</sub> L<sup>-1</sup> and 70 nl O<sub>3</sub> L<sup>-1</sup>, respectively. Previous USDA studies (Leblanc, 1998; Kim et al., 2003), using the same experiments and treat-

ments, reported that the elevated CO<sub>2</sub> (+CO<sub>2</sub>) treatment enhanced photosynthesis and plant growth compared with ambient conditions, whereas the O<sub>3</sub> treatment, compared with the +CO<sub>2</sub>, caused a negative effect. Therefore, the four treatments are referred to in the text, tables, and figures as [1] Control, [2] optimal treatment (elevated CO<sub>2</sub>+CO<sub>2</sub>), [3] elevated O<sub>3</sub> (+O<sub>3</sub>), and [4] combined treatment (elevated CO<sub>2</sub> and O<sub>3</sub>, +CO<sub>2</sub>+O<sub>3</sub>).

The effect of elevated UV-B radiation on spectral properties was studied on red maple (*Acer rubra* L.) foliage in Experiment III, using experimental treatments and foliage material from an ongoing field experiment conducted during the entire 1999, 2000, and 2001 growing seasons (Sullivan et al., 2002, 2003). Three adjacent sections of a maple tree crown with southern exposure (branch age 20+ years) were provided with (i) ambient UV and visible radiation (control group, Treatment 1), (ii) supplemental UV-A (320–400 nm) radiation (+UV-A, Treatment 2), or (iii) supplemental UV-B (293–320 nm) and UV-A radiation (+UV-B+UV-A, Treatment 3), with maximum daily UV-B exposure of 10 kJ m<sup>-2</sup> to approximate a 15% ozone depletion (Björn and Murphy, 1985). The supplemental UV radiation treatments (Treatments 2 and 3) were provided for a maximum of 6 h daily centered around solar noon at a distance of 1 m by filtered Q-panel UVB-313 lamps, following the procedures outlined in Sullivan et al. (2002, 2003). The lamps in the +UV-A group (Treatment 2) were filtered with polyester film to remove radiation below 316 nm, whereas those in the +UV-B+UV-A group (Treatment 3) were filtered with cellulose diacetate, which transmits visible, UV-A, and UV-B (>293 nm) radiation, and filters were changed weekly. The ambient control group represented the unstressed category. The tree had neither diseases nor insect infestations and was a mature (78 yr old), dominant tree, growing on a sandy loam soil at the edge of a mixed hardwood stand in close proximity (2 m) to a small first-order stream.

### Measurement Procedures

Experimental measurements were conducted within the week of 28 Aug. through 1 Sept. 2000, using freshly excised, fully expanded sunlit leaves. From Experiment I, 16 corn leaf samples per treatment were collected (n = 16, 128 total) at the R3 reproductive grain-fill developmental stage. From Experiment II, 10 soybean leaves per variety and treatment were used for foliar analyses (n = 10, total of 40 per variety). For Experiment III, 10 maple leaf replicates per treatment were collected for measurements (n = 10, 30 total). Leaves were excised, placed immediately in water (soybean and maple petioles), and taken for measurements in an adjacent USDA laboratory. To enable a comparison of the sensitivity of R and F spectral measurements with regard to treatment separation and identification of plant physiologic condition, R and F spectra were acquired on the same foliar samples over the visible (400–800 nm) region, from the upper (adaxial) and lower (abaxial) leaf surfaces. Because under environmental stress vegetation foliage commonly changes its orientation, turning away from the sun the upper and exposing the lower leaf surface, spectra from adaxial and abaxial leaf surfaces were collected.

Reflectance measurements were acquired contemporaneously with the F measurements using a Li-Cor 1800 integrating sphere coupled via a 100-mm-long, single-mode, 5-mm-diameter fiber optic probe to an ASD FieldSpec Pro FR spectroradiometer (Analytical Spectral Devices, Inc., Boulder, CO), yielding a 1-nm sampling interval and approximately 3-nm resolution in the visible range.

Steady-state F emissions were collected using a spectrofluorometer (Fluorolog II; Spex Industries, Edison, NJ) with entrance and exit slits set to 1.5 nm, applying standard measurement



procedures (Corp et al., 1997). To obtain F emissions in the 400- to 800-nm region, an excitation wavelength centered at 360 nm was provided by a 450-W xenon lamp. Steady-state F emissions were collected at a resolution of 2.5 nm (Figure 1A), producing four spectral peaks in the blue (F440), green (F530), red (F685), and far-red (F740), as described by Chappelle et al. (1992).

Foliar Chl, carotenoids, and analytical chemistry (C, N, H) determinations were conducted on the same material used for spectral measurements. For pigment determinations, leaf disks (2.54 cm<sup>2</sup>) were extracted in 3 mL dimethyl sulfoxide. Absorption measurements of the extracts were made at 1-nm resolution using a dual-beam spectrophotometer (Perkin-Elmer, Wellesley, MA). Concentrations of photosynthetic pigments were calculated using absorption coefficients at specific wavelengths and equations described by Wellburn (1994) and expressed on a mass per area basis (mg m<sup>-2</sup>). Air-dried samples

were analyzed by the University of Maryland Soil Testing Lab using the Dumas combustion method for determining levels of C, N, and H by percent dry mass. In this investigation, the pigment, C, N, and H data were used to guide the explanation of the spectral R and F responses. Photosynthesis and additional gas exchange parameters and biomass and yield measurements, auxiliary to the current spectral investigation, were collected by earlier investigators, and the treatment effects on leaf/plant physiologic parameters were reported as a prime focus of their studies (Mulchi et al., 1988; McMurtrey et al., 1994; Mulchi et al., 1995; Leblanc, 1998; Sullivan et al., 2002; Kim et al., 2003; Sullivan et al., 2003; Middleton et al., 2004).

### Analytical Approach

To determine the optimal spectral regions for damage detection, a sensitivity analysis of the R and F spectra was con-

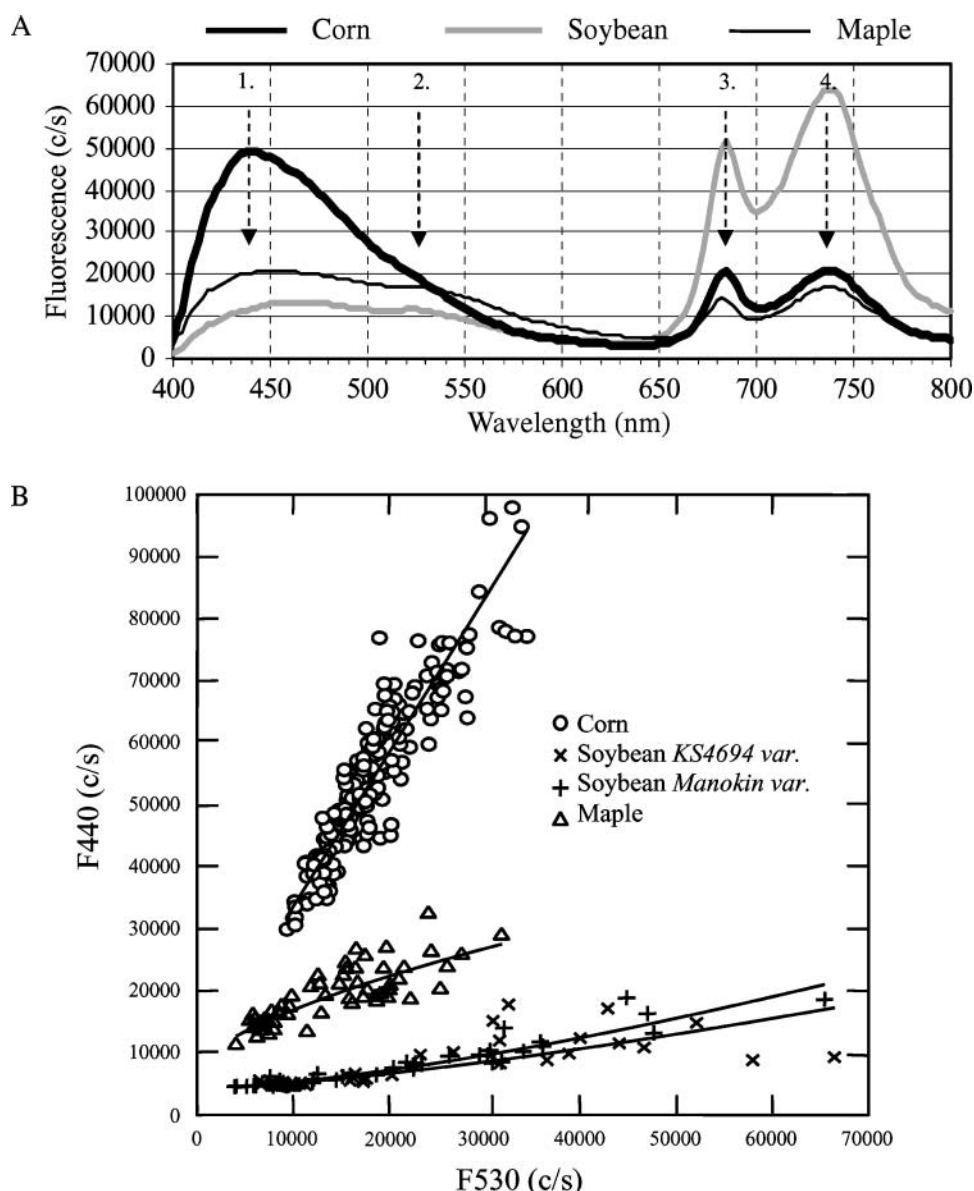


Fig. 1. (A) Typical fluorescence (F) emissions (400–800 nm) for the three species in this study, including corn (black, bold), soybean (gray), and maple (black). F (counts/second [c/s]) were produced using a 360-nm excitation wavelength, exhibiting distinct F features in the blue, green, red, and far-red regions. (B) F emissions from the three species significantly differed in the blue and green regions (F440 and F530, adaxial leaf surface): corn (black circles); soybean, Ks4694 (gray + symbols); soybean, Manokin (gray x symbols); and maple (black triangles).

ducted following a procedure described by Carter et al. (1995). To discriminate between overlapping bands and to determine if there were changes in the shape or position of the spectral features associated with leaf damage, a derivative analysis was conducted (Martin, 1994; Miller et al., 1990). The wavelength of the red edge inflection position (REIPw), which defines the boundary between Chl absorption in the visible region and leaf scattering in the near-infrared wavelengths where maximum value (Dmax) occurs, was computed (Horler et al., 1983). The calculated R derivative (D) indices, suggested as sensitive to stress and to reflect the differences in the shape of the first D curve among damage levels (Entcheva, 2000), included the ratio of the Dmax to D714 and D744 nm (Dmax/D714, Dmax/D744), the Dmax/D705, Dmax/D745, and D715/D705. Previous investigations have identified a large utility of spectral R and F indices related to vegetation physiologic properties (Chappelle et al., 1992; Carter, 1994; Gitelson and Merzlyak, 1996; Middleton et al., 1996; Gamon et al., 1997; Datt, 1998; Merzlyak et al., 1999; Zarco-Tejada et al., 1999; Entcheva, 2000; Entcheva et al., 2004). The current study evaluated approximately 25 of the previously published R and F parameters to determine those most suitable for separation of environmental stress levels. The broadband R indices tested included the commonly used Landsat TM band ratios (e.g., TM5/TM4, TM3/TM1) and the normalized difference vegetation index (NDVI).

The changes in foliar pigments, analytical chemistry, and spectral properties associated with the treatments were evaluated by statistical comparisons to the unstressed (assumed optimal) treatment per experiment (Experiments I–III). The statistical significance levels of the differences ( $p \leq 0.05$ ) were determined by ANOVA and the Tukey-Kramer pairwise mean comparisons test, and the relationships of the spectral indices to foliar pigments and chemistry were evaluated by a correlation analysis using SYSTAT 9.0 (Systat Software Inc., 2000). The coefficient of determination ( $r^2$ ) was used to describe the experiment-level variation in foliar variables attributed to the treatments. Spectral sensitivity was considered significant at the wavelengths where the corresponding difference was statistically significant according to ANOVA (Carter, 1994).

## RESULTS

### Changes in Foliar Compounds Associated with Vegetation Treatment

The ANOVA of foliar properties revealed treatment differences among all three experiments for Chl *a*, Chl *b*, total carotenoids, and N levels (Table 1). Photosynthetic pigment content and N were reduced in most treatments associated with environmental stresses (exception corn excess N). These included the sub-optimal (<100%) N applications for corn, the O<sub>3</sub> exposures of soybean varieties, and the elevated UV exposures for maple leaves. Pigment and N levels were highest for the unstressed (+CO<sub>2</sub>) condition for the CO<sub>2</sub>-sensitive soybean variety (KS4692) and for ambient UV radiation exposure on maple leaves. However, pigment levels were highest for corn plants that were provided excess N application rates, and foliar N content and the N levels applied to the soil were strongly associated ( $r = 0.83$ ).

Corn yield was reduced in association with N deficiency and excess (as established by Corp et al., 2003). For

corn, significant treatment differences in foliar C and H (but not their ratios) were observed between the optimum (100%) and the 150% excess levels (Table 1). The 50% treatment could be separated from the other levels with respect to total Chl content. Foliar N content and the N/H ratio discriminated the optimal 100% N treatment from the higher and lower N application rates. In addition, the 0% N group could be separated from all other groups by the C/N and N/H ratios (Table 1).

Although soybean leaf area and total Chl were maximal in association with elevated CO<sub>2</sub>, they declined in association with the O<sub>3</sub> treatment (Table 1). These findings agree with the previous investigations from the same long-term experiments, reporting a fertilization effect of CO<sub>2</sub> that is partially or completely negated by an O<sub>3</sub> stress effect (Mulchi et al., 1988; Mulchi et al., 1995; Leblanc, 1998; Kim et al., 2003). The “optimal” (+CO<sub>2</sub>) group could be separated from the other treatments using the C/H ratio for the O<sub>3</sub> sensitive variety (*Manokin*), whereas several variables (Chl, Car, N, and the C/N ratio) accomplished this for the CO<sub>2</sub> sensitive variety (*KS4694*; Table 1).

Maple specific leaf weight and foliar C and H content increased in association with elevated UV radiation, whereas total Chl content decreased (Table 1). This trend has been reported as a physiologic change in response to UV stress by Sullivan et al. (2002, 2003). The elevated UV exposures were easy to separate using any of the measured foliar pigment constituents (Table 1). The N/H ratio was the only variable to discriminate among all treatments in the UV experiment.

### Changes in Foliar Reflectance Associated with Vegetation Treatment

In all experiments, R was positively associated with advancement of vegetation stress in the green and red-edge regions for both leaf surfaces (e.g., corn adaxial surface; Fig. 2B). Reflectance intensity differences among treatments were significant in the 695- to 730-nm region. Narrow band ratios were superior to broadband ratios for corn, separating six to seven treatment groups (Fig. 3B). The two common broadband indices, TM3/TM1 (Fig. 3B) and NDVI, had considerably lower discrimination ability, separating only two or three groups per experiment for adaxial or abaxial surfaces (Tables 2 and 3). However, individual narrow R bands were not as successful in separating experimental treatments as the derivative (D) spectra in the red-edge region (Figs. 2C and 3B; Tables 2 and 3).

In each of the three experiments, there were several R indices offering the highest potential for separating treatments and for detecting the unstressed condition (Tables 2 and 3). However, only a few indices could be used as a general spectral index for both leaf surfaces across species and stress responses. The index that consistently performed well for both leaf surfaces in all experiments (I, II, and III) was the D715/D705 ratio (Fig. 3B), which exhibited high values for the unstressed condition and significantly lower values

Table 1. Changes in foliar pigments, carbon (C), nitrogen (N), and hydrogen (H) levels, or some of their ratios, associated with treatment level (ANOVA: least square means and correlation coefficients). Optimal (no stress) treatment responses are in italic type.

Species	Treatment	Chl <i>a</i>	Chl <i>b</i>	Chl <i>a+b</i>	Car <sup>‡</sup>	Chl <i>a/b</i>	C	N	C/N	N/H	C/H	Yield <sup>§</sup> kg ha <sup>-1</sup>	Leaf area m <sup>2</sup>	SLM <sup>¶</sup> mg m <sup>-2</sup>
I. Corn ( <i>Zea mays</i> L.) <i>n</i> = 16	N 0%	255.6d <sup>†</sup>	69.1c	324.1c	66.1d	3.75a	46.46ab	2.32e	20.27a	0.39e		4636e		
	N 12%	268.6d	72.3cd	340.7cd	66.2d	3.75a	46.47ab	2.51d	18.69b	0.42d		5810de		
	N 25%	301.5cd	83.0cd	384.6d	73.0c	3.66b	46.44ab	2.53d	18.89b	0.42d		6522d		
	N 50%	329.0c	90.4c	418.8c	71.6c	3.62b	46.11ba	3.19cd	14.63c	0.54c		8175c		
	N 75%	421.6ab	113.2b	534.6b	89.2ab	3.77a	46.1ba	3.35c	13.90cd	0.56c		9798b		
	N 100%	395.6b	105.3b	500.3b	82.1b	3.80a	45.9b	3.43b	13.76de	0.58b		11485a		
	N 125%	449.9a	131.2a	580.9a	88.1ba	3.62b	46.58a	3.73a	12.63d	0.63a		10229b		
	N 150%	479.7a	140.5a	620.1a	92.7a	3.44c	46.72a	3.77a	12.49d	0.63a		10068b		
	Exp. correlation	0.93***	0.89***	0.92**	0.88***	0.82**	0.62*	0.83***	0.84***	0.85***		0.92***		
	Control	510.4a	160.0a	670.4a	118.8a	3.20ab	52.62a	4.83a	10.80b		7.65ab		0.001578ba	
II. Soybean ( <i>Glycine max</i> ) <i>Manokin</i> Var. <i>O<sub>3</sub></i> sensitive <i>n</i> = 10	+CO <sub>2</sub>	500.0a	161.4a	661.4a	115.5a	3.11a	46.51b	4.52ab	10.38b		7.45c		0.001825a	
	+CO <sub>2</sub> +O <sub>3</sub>	407.9b	121.1b	529.0b	104.5b	3.37b	46.38b	4.24b	10.99b		7.68a		0.001658ab	
	+O <sub>3</sub>	305.8c	92.4c	398.2c	78.1c	3.34b	46.28b	3.61c	12.88a		7.60b		0.001478b	
	Exp. correlation	0.96***	0.95**	0.97***	0.89***	0.68***	0.72ns#	0.78***	0.79***	0.87***		0.68**		
	Control	299.2c	91.9bc	391.0c	86.9c	3.25b	46.07a	3.56b	13.02a		7.43b		0.001392b	
III. Maple ( <i>Acer rubra</i> ) <i>n</i> = 10	+CO <sub>2</sub>	509.9a	163.9a	673.8a	132.6a	3.13b	45.46a	4.06a	11.22c		7.42b		0.001542a	
	+CO <sub>2</sub> +O <sub>3</sub>	396.2b	110.7b	506.9b	111.7b	3.58a	42.79a	3.43b	12.40b		7.65a		0.001426ab	
	+O <sub>3</sub>	306.0c	85.5c	391.4c	87.4c	3.58a	47.05a	3.44b	13.72a		7.45b		0.001365b	
	Exp. correlation	0.97***	0.95**	0.96***	0.94***	0.68***	0.52ns	0.70***	0.79***	0.83***		0.63*		
	Control	391.6a	177.3a	568.9a	95.4a	2.33a	49.14b	2.07a	23.99b	0.36a				84.5c
	+UV-A	272.0b	112.3b	384.3b	77.5b	2.42a	50.83a	1.89b	27.36a	0.29c				102.1b
	+UV-A + UV-B	283.4b	118.0b	401.4b	78.9b	2.40a	50.62a	1.85b	27.14a	0.31b				114.8a
	Exp. correlation	0.80***	0.69**	0.76***	0.73**	0.58*	0.86***	0.55*	0.67**	0.86***				0.91***

\* Different lowercase letters indicate statistically significant differences.

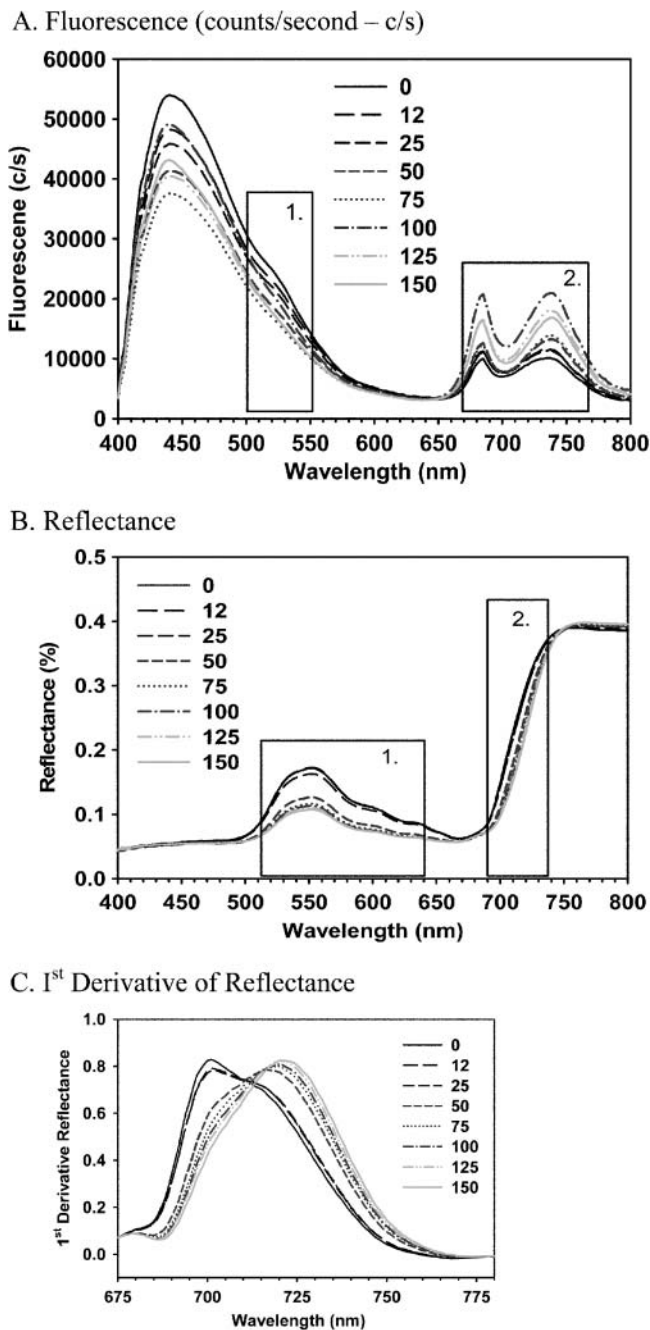
† Car, carotenoids.

‡ Reported in Corp et al. (2003).

§ SLM, specific leaf mass.

# ns, not significant.





**Fig. 2.** The changes in spectral fluorescence and reflectance are shown for corn leaves (Experiment I) that were provided in eight N applications rates between 0 and 150% of the optimal (100%) levels ( $n = 16$  per group). Differences among treatments were highly significant for the two spectral regions in the green and red/far-red (Regions 1 and 2; delineated as dotted rectangles): (A) fluorescence emissions (400–800 nm); (B) reflectance (400–800 nm); and (C) the first derivative of the red-edge reflectance spectra, between 675 and 775 nm. Similar responses were observed across all three experiments.

as vegetation stress increased. Additional indices that performed well were the V2 index,  $R_{750}/R_{700}$ , and the REIPw.

Reflectance spectral trends differed by species, but only the abaxial red/far-red ratios (e.g.,  $R_{695}/R_{760}$  and  $R_{750}/R_{700}$ ) provided statistically significant species

separation (ANOVA,  $r = 0.78$ – $0.95$ ;  $p > 0.001$ ). In the blue/green region, R values from both corn leaf surfaces did not statistically differ. However, significantly higher blue/green R was measured from the abaxial vs. adaxial surfaces of maple and soybean leaves (Fig. 4A). In the red/far-red region, R intensities of either leaf surface for corn and soybean seemed to be similar, whereas the abaxial surfaces of maple leaves produced higher R ( $r = 0.82$ ;  $p < 0.001$ ) (Fig. 5B).

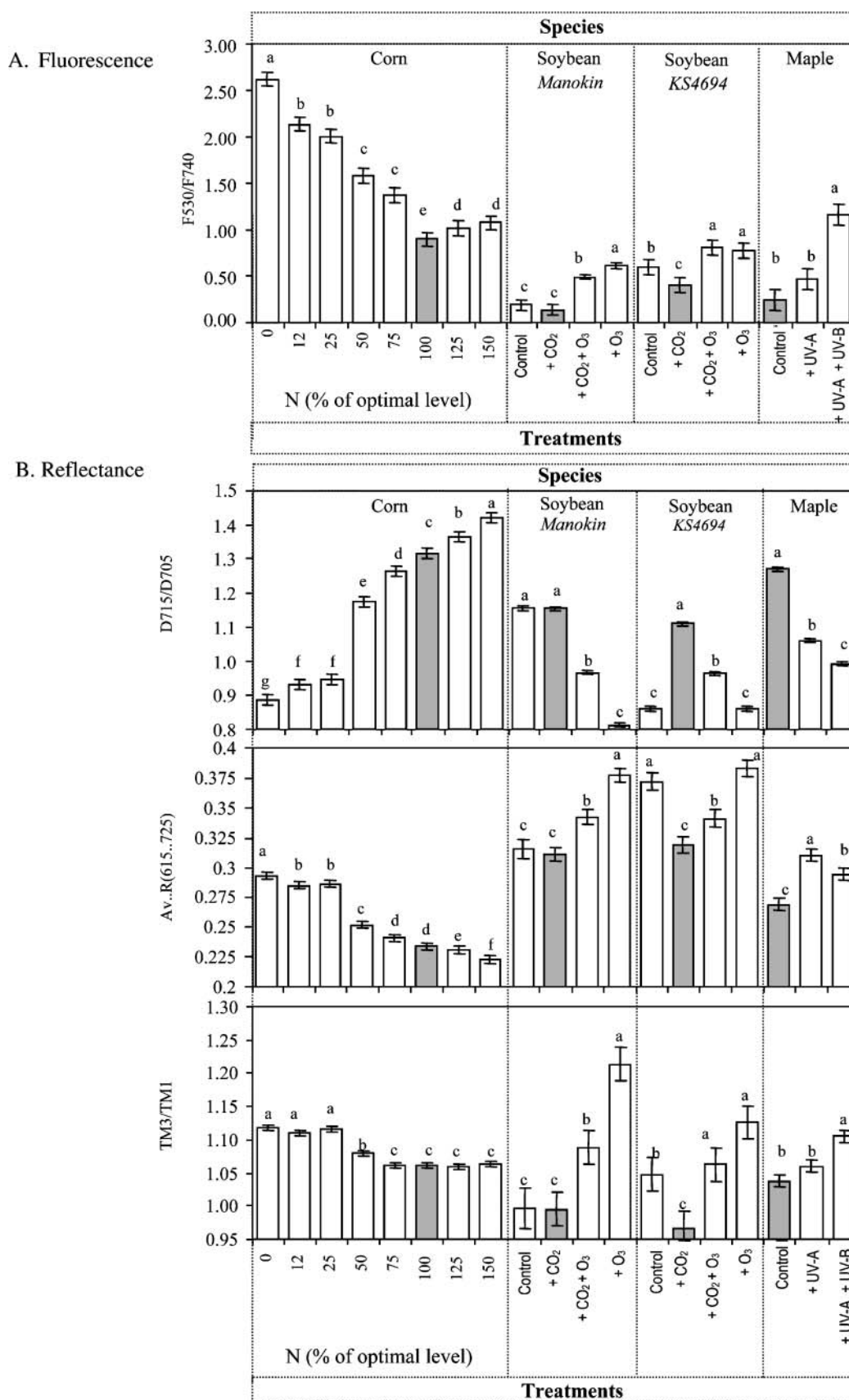
### Changes in Foliar Fluorescence Associated with Vegetation Treatment

Spectral sensitivity analyses indicated significant changes in F intensity associated with the treatments in all experiments, but derivative analyses (first and second F derivatives) did not detect any shift in the wavelength position of the spectral F features. Across all experiments, higher F emissions were measured from the leaf abaxial (lower) vs. adaxial (upper) surfaces (Tables 4 and 5). Fluorescence emissions from the three species significantly differed in the blue and green regions (Figs. 1A and B). For both leaf surfaces, higher blue F was measured from the corn (a  $C_4$  monocot) than from the maple and soybean ( $C_3$  dicots) (Figs. 1A and B; Tables 4 and 5). In all three experiments, blue and green F emissions increased for both foliar surfaces with stress levels (e.g., the lowest blue F intensity was measured from the unstressed group). The opposite trend occurred for the F685 and F740 emissions, which decreased with stress from initially high values, as illustrated for corn (Fig. 2A). The differences among F spectra were minimal at 650 nm, where there were no statistically significant differences among treatment levels in any experiment.

The changes in foliar F associated with N levels in corn leaves were most consistent in the  $530 (\pm 10)$  nm and in the 680- to 760-nm regions (Fig. 2A, regions 1 and 2). The F intensity differences among N treatments were significant for F530 and F740, with the green feature allowing for better treatment separation (Tables 4 and 5). The red/far-red (F685/F740) ratio was not useful for treatment separation in the corn N experiment, but in the other two experiments, it was useful for separating stressed from nonstressed treatments.

Spectra from the adaxial (upper) leaf surfaces (Table 4) provided higher statistical treatment separation as compared with spectra from abaxial surfaces across species and treatments. Overall, F ratios provided better separation than individual F bands. Five groups were discriminated using the adaxial green/far-red (F530/F740) ratio or using the abaxial blue/red (F440/F685) ratio. Several simple band ratios allowed for separation of a wide gradient among N levels (Tables 4 and 5): blue/green (F440/F530); blue/red (F440/685); blue/far-red (F440/F740); green/far-red (F530/F740) (Fig. 3A); and green/red (F530/F685). Among all F indices, the adaxial F530/F740 ratio, which decreased with stress level, provided the best treatment separation and successfully detected the corn 100% treatment





**Fig. 3.** Fluorescence and reflectance for the experimental treatment groups, shown in each panel for the three experiments, left to right: Exp. I, Exp II (Manokin, KS4694), and Exp. III. The means and SE are shown for each group for four spectral variables: (A) F530/F740 (top panel); (B) D715/D705 (second panel); (B) Av. R (615..725) (third panel); and (B) TM3/TM1 (bottom panel).

**Table 2. Changes in foliar adaxial spectral reflectance (R) ratios associated with treatment level (ANOVA: least square means and correlation coefficients). Optimal (no stress) treatment responses are in italic type.**

Species	Treatments	R695/ R760†	R750/ R700	R550/ R675	V2§	PRI 1¶	Av.R(615..725)	REIPw	D715/ D705	TM3/ TM1	NDVI#
I. Corn ( <i>Z. mays</i> L.) <i>n</i> = 16	N 0%	0.269a‡	2.63e	2.67a	-0.044a	-0.049d	0.287a	702.3f	0.88g	1.12a	0.681a
	N 12%	0.258a	2.79de	2.57b	-0.051b	-0.05d	0.279b	705.9e	0.94f	1.11a	0.690a
	N 25%	0.260a	2.81d	2.55b	-0.051b	-0.05d	0.279b	708.3d	0.95f	1.12a	0.687a
	N 50%	0.215b	3.49c	2.08c	-0.083c	-0.055c	0.245c	717.4c	1.19e	1.08ab	0.727b
	N 75%	0.205c	3.73bc	1.95d	-0.097d	-0.056b	0.233d	719.3b	1.27d	1.06b	0.737b
	N 100%	0.202c	3.84b	1.89de	-0.105e	-0.057a	0.226de	720.6b	1.34c	1.06b	0.739b
	N 125%	0.200d	3.91ab	1.84e	-0.113f	-0.056ab	0.223e	721.2ab	1.38b	1.06b	0.741b
	N 150%	0.199d	4.02a	1.76f	-0.124g	-0.052c	0.216f	722.7a	1.45a	1.06b	0.739b
	Exp. correlation	0.81***	0.94**	0.93***	0.91***	0.78***	0.93**	0.93***	0.91***	0.92***	0.93**
II. Soybean ( <i>Glycine max</i> ) <i>Manokin</i> Var. O <sub>3</sub> sensitive <i>n</i> = 10	Control	0.194c	3.89b	1.90c	-0.069c	-0.059a	0.316c	716.0a	1.19a	0.99c	0.78a
	+CO <sub>2</sub>	0.176c	4.16a	1.89c	-0.071c	-0.065a	0.311c	717.0a	1.19a	0.99c	0.79a
	+CO <sub>2</sub> +O <sub>3</sub>	0.217b	3.29b	2.20b	-0.051b	-0.046b	0.342b	707.8b	0.98b	1.10b	0.77a
	+O <sub>3</sub>	0.279a	2.59c	2.47a	-0.035a	-0.029c	0.377a	702.1c	0.82c	1.23a	0.71b
	Exp. correlation	0.97***	0.98**	0.95***	0.97***	0.90***	0.95***	0.99***	0.98***	0.93***	0.93***
	Control	0.242a	2.89c	2.77a	-0.037a	0.005ab	0.372a	701.8c	0.859c	1.05b	0.77b
	+CO <sub>2</sub>	0.165c	4.25a	2.11c	-0.070c	0.018a	0.319c	713.8a	1.109a	0.97c	0.81a
	+CO <sub>2</sub> +O <sub>3</sub>	0.207b	3.37b	2.37b	-0.050b	-0.007ab	0.341b	706.6b	0.963b	1.06ab	0.78b
III. Maple ( <i>Acer rubra</i> ) <i>n</i> = 10	CO <sub>2</sub> sensitive +O <sub>3</sub>	0.249a	2.85c	2.60bc	-0.038a	-0.011b	0.383a	702.0c	0.861c	1.13a	0.74c
	Exp. correlation	0.97***	0.98***	0.78***	0.97***	0.94***	0.96***	0.96***	0.96***	0.94***	0.95***
	Control	0.131b	5.36a	1.64c	-0.146b	-0.67a	0.26c	719.5a	1.31a	1.04b	0.83a
	+UV-A	0.159a	4.36b	1.87b	-0.095a	-0.35c	0.28b	711.6b	1.06b	1.06b	0.81b
	+UV-A + UV-B	0.148a	4.04c	2.09a	-0.084a	-0.64b	0.30a	708.2c	0.99c	1.11a	0.83a
	Exp. correlation	0.72**	0.78*	0.76**	0.76**	0.77**	0.76***	0.74***	0.80***	0.72**	0.64**

\*, \*\*, \*\*\*  $p < 0.05 > 0.01$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively.

† R, reflectance.

‡ Different lowercase letters indicate statistically significant differences.

§ V2 = (R735 - R745)/(R715 + R726).

¶ PRI 1 = (R530 - R570)/(R530 + R570).

# NDVI, normalized difference vegetation index.

group (having the lowest value, Fig. 3A) as well as the “optimal” treatment in the soybean CO<sub>2</sub>-sensitive variety (KS4694) (Tables 4 and 5). The blue/red (F440/F685) ratio was the best for detecting the unstressed condition in the maple UV experiment. Moreover, the

adaxial blue/red F ratio increased four-fold in the UV-B-augmented foliage, as might be expected from a thicker adaxial epidermis where more blue-fluorescing structural compounds enhance blue F while shielding UV-induced ChlF.

**Table 3. Changes in foliar abaxial spectral reflectance (R) ratios associated with treatment level (ANOVA: least square means and correlation coefficients). Optimal (no stress) treatment responses are in italic type.**

Species	Treatment	R695/ R760†	R750/ R700	R550/ R675	V2¶	PRI 1#	Av.R(615..725)	REIPw	D715/ D705	TM3/ TM1	NDVI††
I. Corn ( <i>Z. mays</i> L.) <i>n</i> = 16	N 0%	0.285a‡	2.66e	2.56a	-0.041a	-0.047d	0.290a	701.7e	0.87g	1.12a	0.664a
	N 12%	0.272a	2.78e	2.47ab	-0.047ab	-0.049cd	0.284b	705.1d	0.91f	1.11a	0.671a
	N 25%	0.274a	2.91d	2.38b	-0.052b	-0.049cd	0.280b	707.6d	0.95f	1.10a	0.678a
	N 50%	0.224b	3.44c	2.00c	-0.078c	-0.055b	0.252c	715.8c	1.16e	1.07b	0.713ab
	N 75%	0.212c	3.68b	1.84d	-0.093d	-0.056ab	0.238d	718.3b	1.27d	1.06b	0.721b
	N 100%	0.209c	3.76ab	1.82d	-0.099d	-0.057a	0.233de	720.5ab	1.31c	1.07b	0.725b
	N 125%	0.206cd	3.88a	1.75e	-0.109e	-0.056ab	0.227e	721.6a	1.37b	1.06b	0.728b
	N 150%	0.203d	3.89a	1.77de	-0.112e	-0.051c	0.226f	719.8ab	1.38a	1.06b	0.726b
	Exp. correlation	0.84**	0.87**	0.87***	0.86***	0.89***	0.86**	0.87***	0.86***	0.72***	0.78**
II. Soybean ( <i>Glycine max</i> ) <i>Manokin</i> Var. O <sub>3</sub> sensitive <i>n</i> = 10	Control	0.326c	2.46ab	1.84c	-0.047c	-0.039bc	0.334c	703.0ab	0.96a	1.12c	0.65a
	+CO <sub>2</sub>	0.308c	2.57a	1.94b	-0.047c	-0.045c	0.334c	705.2a	0.96a	1.08c	0.67a
	+CO <sub>2</sub> +O <sub>3</sub>	0.345b	2.30b	1.99a	-0.039b	-0.033b	0.355b	701.4b	0.88b	1.15b	0.63b
	+O <sub>3</sub>	0.419a	1.94c	1.95ab	-0.027a	-0.019a	0.383a	698.8c	0.77c	1.18a	0.55c
	Exp. correlation	0.87***	0.91**	0.43ns§	0.92***	0.93***	0.82***	0.76***	0.94***	0.83***	0.87***
	Control	0.385a	2.05c	2.26a	-0.027a	-0.031b	0.375b	699.0c	0.77c	1.14b	0.59b
	+CO <sub>2</sub>	0.308c	2.53a	2.12b	-0.046c	-0.043c	0.345c	702.2a	0.93a	1.07c	0.66a
	+CO <sub>2</sub> +O <sub>3</sub>	0.341b	2.29b	2.20ab	-0.037b	-0.032b	0.353c	700.4b	0.87b	1.16ab	0.63a
III. Maple ( <i>Acer rubra</i> ) <i>n</i> = 10	CO <sub>2</sub> sensitive +O <sub>3</sub>	0.383a	2.09c	2.15b	-0.029a	-0.026a	0.391a	699.2c	0.78c	1.13a	0.57b
	Exp. correlation	0.78***	0.83***	0.45ns	0.91***	0.72***	0.80***	0.81***	0.82***	0.79***	0.75***
	Control	0.440c	1.97a	1.52a	-0.048a	-0.028a	0.354b	701.0a	0.91a	0.96b	0.46a
	+UV-A	0.482b	1.80b	1.52a	-0.035b	-0.023b	0.383a	699.5b	0.83b	0.97a	0.42b
	+UV-A + UV-B	0.495a	1.75c	1.52a	-0.032b	-0.020b	0.392a	695.1c	0.79c	0.97a	0.41b
	Exp. correlation	0.72***	0.82***	0.72ns	0.76***	0.75***	0.76***	0.53ns	0.79***	ns**	0.64**

\*, \*\*, \*\*\*  $p < 0.05 > 0.01$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively.

† R, reflectance.

‡ Different lowercase letters indicate statistically significant differences.

§ ns, not significant.

¶ V2 = (R735 - R745)/(R715 + R726).

# PRI 1 = (R530 - R570)/(R530 + R570).

†† NDVI, normalized difference vegetation index.

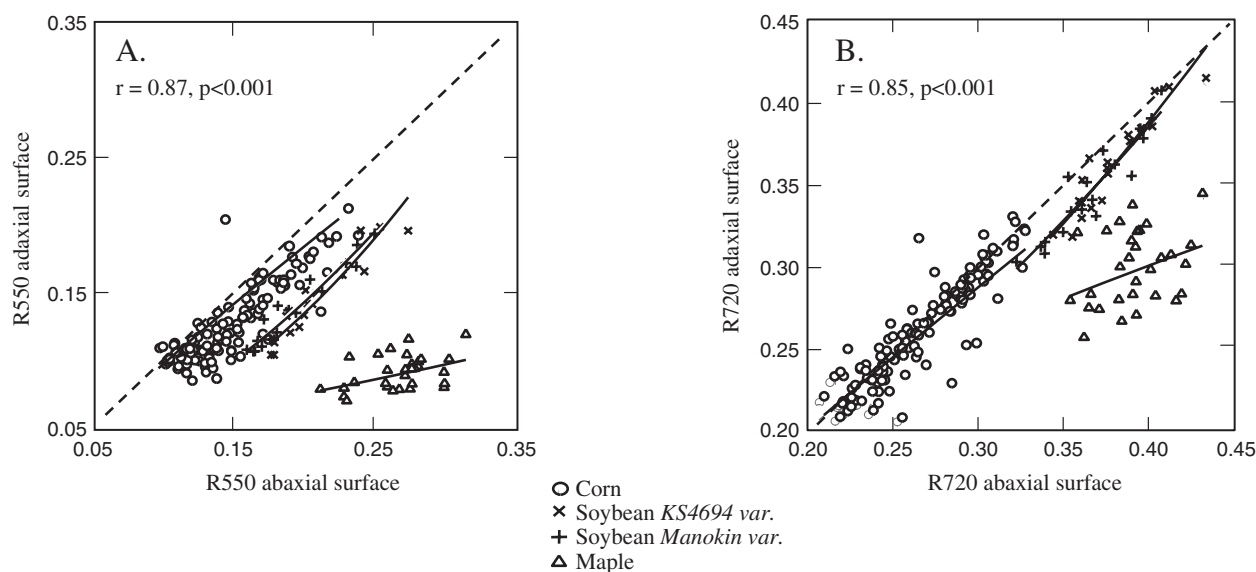


Fig. 4. Reflectance of adaxial vs. abaxial leaf surfaces for the three species are shown: (A) R500; and (B) R720. Species symbols: corn (black circles); soybean, Ks4694 (+ symbols); soybean, Manokin (x symbols); and maple (black triangles).

### Correlation of Spectral Properties and Foliar Compounds

For all experiments, abaxial rather than adaxial F emission spectra were better correlated to foliar pigments and chemical compounds. The association of individual F bands to C/N was lower than to pigments, with the F400 to F500 region exhibiting the highest correlation. Fluorescence emissions in the spectral region from 400 to 620 nm were negatively associated with pigment content, whereas the association was positive in the 680- to 760-nm range (Fig. 5). F600 ( $r \sim 0.78$ ) and F740 to F760 ( $r = 0.65$ – $0.78$ ) were best associated to total Chl. Fluorescence was not associated with foliar pigment content between 640 and 660 nm, the transition

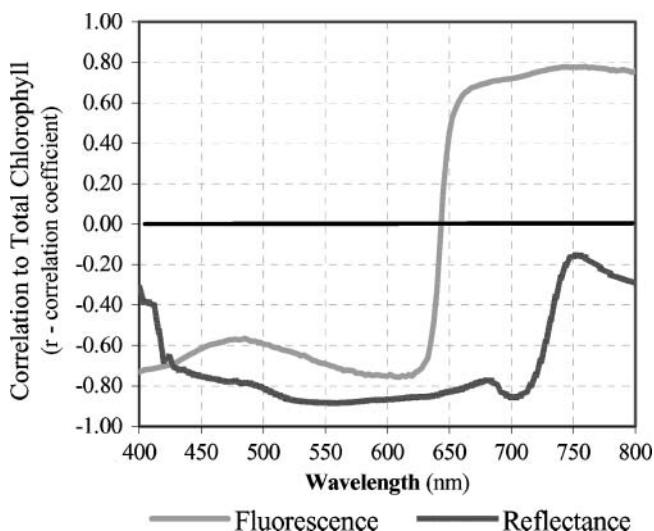


Fig. 5. The correlation of total chlorophyll across the VIS/NIR near-infrared spectrum (400–800 nm) to the mean fluorescence (gray line) and reflectance (black line) spectra for the abaxial surface of soybean leaves (Manokin var.).

zone between negative and positive correlations (Fig. 6). The F ratios having the highest correlation to total Chl were from the abaxial leaf surface: maple, F530/F740 ( $r = -0.65$ ) and F685/F530 ( $r = -0.78$ ); soybean KS4694, F530/F740 ( $r = -0.84$ ) and F685/F740 ( $r = -0.81$ ); soybean (Manokin), F530/F740 ( $r = -0.83$ ), F685/F740 ( $r = -0.85$ ), F530/F650 ( $r = -0.86$ ), and F685/F530 ( $r = -0.86$ ); and corn, F530/F650 ( $r = -0.56$ ). Recalculating the ratios, after subtracting the baseline F650, increased their correlation to all foliar compounds (e.g., [F440–F650]/[ChlF–F650]). Overall, the abaxial F530/F650, F685/F530, and F685/F740 ratios were best correlated to foliar N and the C/N ratio.

Considering all experiments, the correlation of individual R bands to foliar pigments and N varied across the spectra (400–800 nm); those regions having negative correlations to N provided positive correlations to the C/N ratio (Fig. 5). The R525- to R575-nm region and R700 were most strongly associated with total Chl ( $r = -0.84$ ) (Fig. 5). Strong correlations were observed between R700 and N ( $r = -0.80$ ) and between R700 or R550 and C/N ( $r = 0.80$ ). Reflectance indices, using spectra from the red-edge region, were strongly associated with foliar pigments ( $-0.60 < r < -0.90$ ), N ( $-0.75 < r < -0.85$ ), the ratio of Chl *a* to accessory pigments ( $-0.65 < r < -0.75$ ), C/N ( $0.80 < r < 0.85$ ), and N/H ( $0.75 < r < 0.80$ ). The R ratios were not related to foliar C or to the Chl *a/b* ratio.

### DISCUSSION

This study evaluates the advantages of using R and F data for the detection of vegetation stress (unstressed vs. stressed condition) and the separation of treatment effects in three unrelated experiments that examined a wide range of experimental conditions.

Leaf and crop parameters varied considerably over the experimental treatments (Table 1). The levels of pho-



**Table 4. Changes in foliar adaxial fluorescence (F, 360-nm excitation), and F emission ratios associated with treatment level (ANOVA: least squares means and correlation coefficients). Optimal (no stress) treatment responses are in italic type.**

Species	Treatment	F440 F	F530 F	F685 F	F740 F	F440/F685	F440/F740	F530/F740	F685/F740
cps†									
I. Corn ( <i>Z. mays</i> L.) <i>n</i> = 16	N 0%	54 023a‡	21 796a	9995c	9954d	6.99a	6.30a	2.62a	0.93a
	N 12%	48 255b	20 385ab	11 276c	11 419d	5.06b	4.83b	2.14b	0.96a
	N 25%	45 876b	19 347b	11 167c	11 183d	5.17b	4.63b	2.01b	0.95a
	N 50%	41 388c	16 491c	12 599bc	13 184cd	4.47bc	3.89bc	1.58c	0.93a
	N 75%	37 575cd	14 672d	12 316bc	13 784c	3.86c	3.43c	1.37cd	0.89a
	N 100%	49 223b	17 946bc	20 761a	20 926a	2.77d	2.24d	0.90e	0.98a
	N 125%	40 482d	15 281d	16 474b	17 911b	2.80cd	2.57d	1.02de	0.92a
	N 150%	40 494cd	15 069d	17 088ab	17 529b	3.02cd	2.74cd	1.08d	0.95a
	Exp. correlation	0.50*	0.69***	0.44*	0.45*	0.62*	0.67***	0.62***	ns
	Control	13 362b	12 664b	58 028a	71 604a	0.25b	0.20b	0.19c	0.80c
II. Soybean ( <i>Glycine max</i> ) <i>Manokin</i> Var. O <sub>3</sub> sensitive <i>n</i> = 10	+CO <sub>2</sub>	8801d	7636d	46 679b	56 310b	0.20b	0.16b	0.14c	0.82c
	+CO <sub>2</sub> +O <sub>3</sub>	11 379c	9724c	22 949d	26 186d	0.65a	0.58a	0.49b	0.89b
	+O <sub>3</sub>	18 694a	17 345a	28 895c	29 125c	0.66a	0.66a	0.61a	0.99a
	Exp. correlation	0.92**	0.96***	0.88**	0.84***	0.73**	0.78***	0.83**	0.83***
	Control	19 656a	14 884a	32 388a	38 095a	0.92ab	0.78ab	0.59b	0.86c
II. Soybean ( <i>Glycine max</i> ) <i>KS4694</i> Var. CO <sub>2</sub> sensitive <i>n</i> = 10	+CO <sub>2</sub>	16 102b	12 586b	31 287a	36 550b	0.59b	0.50b	0.40c	0.85bc
	+CO <sub>2</sub> +O <sub>3</sub>	11 126d	9377c	10 863c	12 038d	1.09a	0.96a	0.81a	0.90b
	+O <sub>3</sub>	11 436c	11 232b	13 768b	14 681c	0.83ab	0.78ab	0.77a	0.94a
	Exp. correlation	0.83**	0.86***	0.61*	0.78**	0.59*	0.69**	0.68***	0.66**
	Control	11 322b	8927b	41 853a	23 881b	1.40a	0.36c	0.24b	0.26c
III. Maple ( <i>Acer rubra</i> ) <i>n</i> = 10	+UV-A	12 445b	9163b	16 589b	66 010a	1.24b	0.85b	0.46b	0.69b
	+UV-A + UV-B	20 480a	16 838a	14 148b	17 070b	1.23b	2.10a	1.16a	1.73a
	Exp. correlation	0.80***	0.81***	0.61***	0.62**	0.73**	0.90***	0.77***	0.91**
	Control	11 322b	8927b	41 853a	23 881b	1.40a	0.36c	0.24b	0.26c

\*, \*\*, \*\*\*  $p < 0.05 > 0.01$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively.

† cps, counts per second.

‡ Different lowercase letters indicate statistically significant differences.

tosynthetic pigments (Chl *a*, Chl *b*, total Chl, and carotenoids) were correlated to the experimentally induced stress levels in the corn N experiment ( $r = 0.77$ – $0.89$ ), the soybean CO<sub>2</sub>/O<sub>3</sub> fumigation experiment ( $r = 0.91$ – $0.97$ ), and Chl *a* in the maple UV experiment ( $r = 0.68$ ). The observed increase in Chl content in corn leaves, in concert with N application rate, agrees with earlier investigations (McMurtrey et al., 1994; Langsdorf et al., 2000). The foliar C/N and N/H ratios provide an insight

into N use efficiency during the process of C sequestration via photosynthesis (Kull et al. 1995, Patterson et al., 1997), and high C/N and low N/H ratios are indicative of low N use efficiency and physiologic imbalance. In particular, the N/H ratio describes the relative oxidative stress associated with H<sup>+</sup> vs. OH<sup>−</sup> side-chains of the C backbone of proteins (Patterson et al., 1997). Consequently, the N/H ratio of stressed foliage exhibited lower values as compared with the unstressed condition in the UV-

**Table 5. Changes in foliar abaxial spectral fluorescence (F, 360-nm excitation), and F emission ratios associated with treatment level (ANOVA: least square means and correlation coefficients). Optimal (no stress) treatment responses are in italic type.**

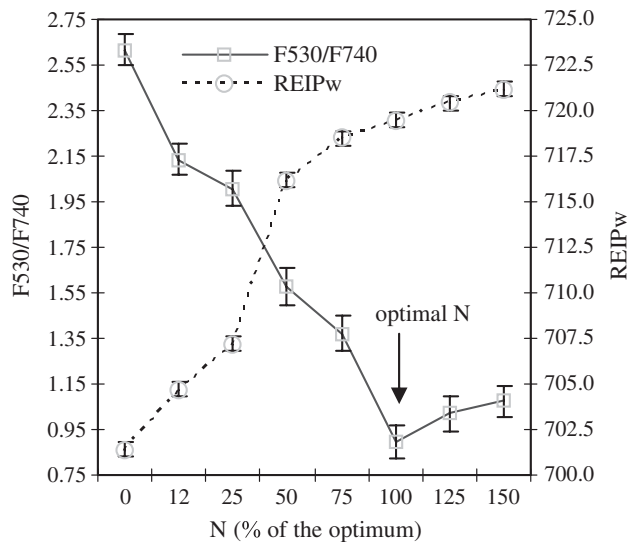
Species	Treatment	F440 F	F530 F	F685 F	F740 F	F440/F685	F440/F740	F530/F740	F685/F740
cps†									
I. Corn ( <i>Z. mays</i> L.) <i>n</i> = 16	N 0%	63 678	24 591ab	23 249c	20 677d	2.59d	3.81a	1.47a	1.37a
	N 12%	59 133	23 663ab	24 861c	22 567d	2.52d	3.38ab	1.36ab	1.32a
	N 25%	66 668	26 125a	29 877c	26 904cd	2.56d	3.01b	1.2b	1.09b
	N 50%	57 526	20 757ab	37 532bc	33 955c	2.77c	1.94c	0.71c	0.69c
	N 75%	54 754	19 185b	48 371b	45 835b	2.83bc	1.33cd	0.48cd	0.47d
	N 100%	51 813	18 870c	48 731ab	46 024bc	2.77c	1.27d	0.45d	0.42d
	N 125%	54 907	19 108bc	56 034a	53 904a	2.87b	1.20d	0.42d	0.41d
	N 150%	56 993	19 564b	63 562a	56 515a	2.91a	1.20d	0.41d	0.38d
	Exp. correlation	0.25ns‡	0.38***	0.35*	0.38*	0.48*	0.59**	0.58**	0.63*
	Control	5888b§	32 302a	562 990a	562 797a	0.08c	0.08c	0.06c	0.06c
II. Soybean ( <i>Glycine max</i> ) <i>Manokin</i> Var. O <sub>3</sub> sensitive <i>n</i> = 10	+CO <sub>2</sub>	4302c	25 665c	407 671b	426 153b	0.09c	0.09c	0.06c	0.07c
	+CO <sub>2</sub> +O <sub>3</sub>	5853b	29 237c	232 989c	217 224c	0.21b	0.22b	0.16b	0.15b
	+O <sub>3</sub>	12 887a	48 897b	194 931d	154 999c	0.44a	0.54a	0.36a	0.29a
	Exp. correlation	0.72**	0.68***	0.75**	0.77***	0.68**	0.74***	0.79**	0.72***
	Control	4518c	42 452b	178 556b	139 108b	0.3b	0.38b	0.29b	0.23b
II. Soybean ( <i>Glycine max</i> ) <i>KS4694</i> Var. CO <sub>2</sub> sensitive <i>n</i> = 10	+CO <sub>2</sub>	5330bc	32 626a	264 524a	263 774a	0.22b	0.21c	0.15c	0.16c
	+CO <sub>2</sub> +O <sub>3</sub>	10 847a	36 355c	141 022c	111 797bc	0.47a	0.58a	0.33b	0.27b
	+O <sub>3</sub>	6452b	32 812b	97 256d	75 796c	0.45a	0.57a	0.44a	0.35a
	Exp. correlation	0.46**	0.25ns	0.42*	0.57**	0.78*	0.88**	0.86***	0.78*
	Control	16 267b	16 937c	96 126a	96 900a	0.24c	0.27c	0.26c	0.97c
III. Maple ( <i>Acer rubra</i> ) <i>n</i> = 10	+UV-A	16 519b	19 397b	30 568b	41 979b	0.63a	0.50b	0.47b	1.14b
	+UV-A + UV-B	18 645a	24 342a	19 148b	27 186c	0.40b	0.95a	1.83a	1.06a
	Exp. correlation	0.68***	0.76***	0.45***	0.42***	0.85***	0.69***	0.77**	0.68**

\*, \*\*, \*\*\*  $p < 0.05 > 0.01$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively.

† cps, counts per second.

‡ ns, not significant.

§ Different lowercase letters indicate statistically significant differences.



**Fig. 6.** The trends of N application levels for a corn crop are compared for two of the most successful spectral indices: F530/F740 (solid gray line) and REIPw (broken black line). The F ratio detects the 100% group as the minimum F530/F740 value.

exposed maple ( $r = 0.76$ ) and corn ( $r = 0.75$ ) leaves. The C/N or C/H ratios were more successful in tracking C uptake efficiency in the soybean experiments where  $\text{CO}_2$  augmentation was a treatment factor.

The trends in R indices, across the range of experimental treatments, were linked primarily to leaf pigment concentrations, and in all experiments R intensity from either leaf surface was positively associated with the advancement of vegetation stress, most clearly shown in the corn experiment associated with nutrient deficiency ( $<100\%$  N). These responses are consistent with the foliar spectral trends commonly associated with forest decline (Rock et al., 1986; Entcheva et al., 2004).

Comparing broad- and narrow-band reflectance indices, high-spectral-resolution indices were found superior to broadband ratios. This result highlights the importance for vegetation stress detection of continuous high-resolution R observations and not just isolated, well placed, single narrow bands. Of the numerous R spectral algorithms tested, the high-spectral-resolution first derivative D715/D705 index consistently separated the largest number of treatment levels across the three experiments. The similarities in R properties of both leaf sides of corn were anticipated because corn, in contrast to soybean and maple, does not show differentiation into palisade parenchyma on the upper leaf side and spongy parenchyma on the lower leaf side.

The F indices provided different information than R indices because they apparently were influenced by structural constituents in addition to pigments. A blue-green/ChlF (F530/F740) ratio was the most successful F index over all three experiments in separating treatment groups, increasing in concert with stress-induced foliar damage. Chlorophyll fluorescence (F685 or F740) increased nonlinearly with N application, producing the highest F at the optimal (100% N) level, which is in agreement with the F trends associated by Langsdorf et al.

(2000) with the effects of N on sugar beet plants (*Beta vulgaris* L. cv. Patricia) and reported for corn by Corp et al. (2003), whereas the green F decreased in response to higher N availability. In addition, the F530/F740 ratio tracked the curvilinear response of corn yields over N levels associated with deficiency and excess stress, allowing the detection of the optimal (100% N) treatment for maximizing crop production (Fig. 6). This advantage, if parlayed into an operational RS capability, could improve regional crop production estimates. Also, adaxial F combinations (e.g., F 440 vs. F530; Fig. 3) enabled discrimination of the three species studied here, whereas species separation using R measurements was only possible with abaxial red/far-red ratios.

Because there were no statistically significant F differences in the red ( $\sim\text{F650}$ ) among treatment levels in any experiment, this band region can serve to normalize the blue F and ChlF. Normalization of the blue-green F and ChlF to the F650 value enabled results that were more consistent across different studies. The current study confirms the previously reported trend in steady-state F (using an UV-A excitation) for corn under N treatments in association with vegetation stress (Corp et al., 2003).

Reflectance spectral algorithms, as compared with F ratios, were capable of separating a larger number of treatments but exhibited a saturation response that prevented the detection of the “optimal” vegetation condition. However, the most effective R indices (e.g., D715/D705, Av.R615..R725) were more successful than the F ratios (e.g., F530/F740) in detecting differences among treatment groups across experiments. The PRI1 index, associated with vegetation photosynthetic activity, produced a nonlinear R response similar to that reported for F emissions at F685 and F740. However, two F ratios (F440/F685 and F440/F740) had higher sensitivity and provided better treatment level separation than the PRI1 index (Tables 4 and 5 versus Tables 2 and 3).

The differences observed in F intensities of adaxial vs. abaxial leaf surfaces could be useful in stress detection and species discrimination because many species alter the orientation of their leaves to minimize adverse environmental effects (e.g., high incoming radiation, water and heat stress). The higher abaxial F (for soybean and maple leaves) could be explained with the lower content of Chl and protective pigments in the spongy parenchyma of leaf undersides as compared with the protective pigments and Chl content in the mesophyll of leaf upper sides (Middleton and Teramura, 1993; Sullivan et al., 2002; Tegelberg, 2002). Intensity differences between the two leaf surfaces are also associated with epidermal structural differences, especially in the blue region. Because leaf display geometry often changes for plants under physiologic stress (Walter-Shea et al., 1992; Larcher, 1995; Kim et al., 2003), these spectral differences are likely to be manifested at the crop and stand level as higher intensities for stressed canopies. Radiation transfer models for vegetation do not address these effects, which could offer improvements for remote estimation of vegetation dynamics and stress.

## CONCLUSIONS

Although R and F variables were successful in detecting vegetation stress responses in each of the three experiments, only a few of the R and F variables performed rigorously across all three experiments, including the D715/D705 R ratio and the green/far-red (F530/F740) F ratio. This study established that high-spectral-resolution reflectance (5–10 nm) can provide a significant improvement over the currently used broadband indices for the detection of changes in vegetation physiologic condition. Narrow-band R indices, especially those using first derivatives, performed best and were found strongly correlated to pigment, C, and N contents and to the C/N ratio. The narrow band R indices successfully discriminated among treatment responses, but they could not identify the unstressed (optimal) plant condition within a stress gradient, as was demonstrated for the F (F530/F740) data. Consequently, F and R data provided complimentary information.

The current RS methods for assessment of photosynthetic function and C sequestration, based on the combined satellite observations and modeling efforts, could be improved by incorporating the physiologic information obtained using hyperspectral R and F observations. More research is needed to extend our findings to other vegetation types and species and to evaluate the association of photosynthetic function to F emissions produced from visible excitation wavelengths (e.g., 400–650 nm) and high-spectral-resolution R properties.

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