

Early Detection of Plant Stress by Digital Imaging within Narrow Stress-Sensitive Wavebands

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Digital images of soybean canopies/Glycine max (L.) Merrill] were obtained within selected narrow wavebands (6-10 nm bandwidths) to determine their capability for early detection of plant stress. Images and physiological measurements of stress were acquired 2 days, 4 days, and 7 days following application of control, drought, and herbicide [(3,4-dichlorophenyl)-l, l-dimethylurea, or DCMU] treatments. As a result of frequent rainfall, drought stress never occurred. However, exposure to herbicide rapidly induced plant stress. By day 4, the ratio of variable to maximum leaf fluorescence (F_v/F_m) decreased and leaf water potentials (Ψ_w) increased in the herbicide treated soybean, indicating damage to the photosynthetic apparatus and stomatal closure. Also, Munsell leaf color had increased from approximately 5GY 4.6/5.7 to a lighter green-yellow value. Canopy reflectances at 670 nm, 694 nm, and in the 410-740 nm band (R_{vis}), as well as reflectance at 694 nm divided by reflectance at 760 nm (R_{694}/R_{760}), detected stress simultaneously with the physiological measurements and increased consistently with stress through day 7. Reflectances at 420 nm and 600 nm, together with R_{600}/R_{760} and R_{vis}/R_{760} , did not increase until leaves were yellow or brown and wilted and canopies had begun to collapse on day 7. None of the reflectance or reflectance ratio images detected stress prior to visible color changes. This was attributed primarily to the rapid inducement of chlorosis by the herbicide. Reflectance in narrow wavebands within the 690-700 nm region and its ratio with near-infrared reflectance should, provide earlier detection of stress-induced chlorosis compared with broad band systems or narrow bands located at lesser wavelengths.

Early detection of vegetation stress by passive remote sensing depends largely on identifying the spectral regions in which vegetation reflectance is most responsive to unfavorable growth conditions. For individual leaves, increased reflectance at visible wavelengths (400-700 nm) is generally the most consistent response to stress within the 400-2500 nm range (Carter, 1993). Narrow wavebands within the 480-680 nm range have been recommended for early detection of forest damage (Hoque et al., 1990; 1992). However, reflectance within the 690-700 nm range is particularly sensitive to early, stress-induced decreases in leaf chlorophyll content (Carter, 1993), and represents the "blue shift" of the reflectance curve "red edge" that frequently accompanies stress (Horler et al., 1983; Rock et al., 1988; Hoque and Hutzler, 1992). Photographs taken through a narrow bandpass interference filter centered at 700 nm enabled visualization of this phenomenon in pine seedling canopies, and provided a "previsual" indication of stress (Cibula and Carter, 1992). The above results suggested that digital imagery within key, spectrally narrow wavebands, particularly near 700 nm, could provide earlier detection of plant stress for most causes of stress and species than is possible using broad-band (greater than 10 nm) instrumentation.

The purpose of this study was to determine the capability of digital images measured in narrow (6-10 nm), stress-sensitive wavebands to detect plant stress. These bands were identified previously using the reflectance difference and reflectance sensitivity approach for individual leaves (Carter, 1993) and seedling canopies (Cibula and Carter, 1992). Specifically, our objectives were to: 1) produce digital reflectance images of stressed and nonstressed plant canopies within wavebands that were determined to be stress-sensitive or stress-insensitive (Carter, 1993); 2) compare stress-detect-

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0034-4257 / 94 / \$7.00
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tion capabilities among these images and among reflectance ratio images that also were selected based on previous work (Carter, 1994), and 3) determine if any of these images provided previsual indications of stress.

METHODS

Experimental Design and Treatment Applications Outdoor planting beds were located at the Harrison Experimental Forest, 25 km north of Gulfport, Mississippi. The beds were prepared for planting in April 1993 and soybean [*Glycine max* (L.) Merrill] seeds were sown into three separate beds on 30 April. Each bed contained a 1.2 m x 6 m block which was divided into three, 1.2 m x 2.0 m plots for later applications of control (C), herbicide (H), and drought stress (D) treatments. During germination and early growth, the soybeans were watered daily to prevent high soil temperatures and drought stress. By 14 June, closed canopies had been produced; that is, the soil surface could not be seen when the canopies were viewed from above. At 1600 h, the soybeans in one plot per block were exposed to a single application to the soil of 8 g/liter DCMU [(3,4-dichlorophenyl)-1,1-dimethylurea; Diuron, du Pont de Nemours, Wilmington, Delaware] powder in water. The herbicide was sprayed sufficiently to thoroughly wet the soil surface. Subsequently, soybeans assigned to the C and H treatments were watered daily. Supplemental watering was withheld from plants assigned to the D treatment. However, all plants received ambient rainfall. Image data together with plant physiological measurements were acquired 2 days (16 June), 4 days (18 June), and 7 days (21 June) following treatment application. Data were not obtained after day 7 because the herbicide had caused considerable damage by this time.

Imaging of Plant Stress

Each block of soybeans was imaged to detect treatment effects on canopy reflectance and selected reflectance ratios. The measurement system consisted of a black and white CCD camera (500 lines horizontal x 488 lines vertical resolution; Model CCD-500, CCTV Corporation, New York) that was mounted on a telescoping swivel attached to a transportable cart. Video signal from the camera was transferred through a standard VHS video cassette recorder to a video display monitor. The camera, monitor and recorder were powered by 12 V batteries. The camera system was raised approximately 17 m above the ground in the bucket of a boom truck that was positioned approximately 34 m due south of the soybean beds. Thus, view angle of the camera was approximately 25° from the horizontal, and distance from the camera to the soybeans was approximately 38 m. At this angle, no soil could be seen through the soybean canopies. The camera incorporated a 50 mm telephoto lens to allow larger canopy images and force a smaller acceptance angle for scene elements that were imaged furthest from the optical axis of the system. The small acceptance angle is necessary because the spectral

transmissivity of interference filters changes as incidence angle deviates from perpendicular to the filter plane.

Images were acquired from approximately 1130 h to 1230 h solar time. Thus, solar azimuth and elevation angles were approximately 180° and 80°, respectively. Each block was imaged through narrow-band interference filters (Andover Corporation, Salem, New Hampshire) that were held in front of the lens by a custom mount. The blocks were imaged at 420 ± 5 nm, 600 ± 5 nm, 670 ± 5 nm, 694 ± 3 nm, and 760 ± 5 nm. These filters were selected because leaf reflectances at 420 nm and 760 nm were found earlier to change relatively little with plant stress, whereas reflectances at 600 nm and 694 nm were quite sensitive to stress; reflectance at 670 nm was intermediate in stress sensitivity (Carter, 1993). Additionally, the soybeans were imaged through an infrared-blocking filter that transmitted primarily in the panchromatic visible spectrum (410-740 nm; Andover Corporation, Salem, New Hampshire). Approximately 15 min were required to videotape all three blocks through all filters. Along with each block, a gray scale composed of five cards that ranged from white to black was imaged through each filter. Each card was calibrated to percent reflectance in sunlight using a scanning spectroradiometer (Model LI-1800UW with LI-1800-06 telescope, Li-Cor, Inc., Lincoln, Nebraska) and a white reference (Spectralon SRT-99-05, Labsphere, Inc., North Sutton, New Hampshire). Spectral radiance reflected by each gray card was multiplied by 100 and divided by that from the white reference to yield percent reflectance. These reflectances were used to calibrate the soybean images to percent reflectance (see Image Processing and Analysis, below). Reflectances for the gray cards at 600 nm, the wavelength of maximum sensitivity of the CCD detector, were 89%, 27%, 10%, 5%, and 3%. These values also were the average reflectances for the cards in the 410-740 nm range with the exception of the brightest card, which averaged 91%.

Physiological Measurements

During and immediately after the canopies were videotaped, leaf fluorescence, and water potentials (ψ_w) were measured to provide physiological indications of plant stress. Fluorescence induction kinetics were measured for three leaves in each treatment plot using a field-portable fluorometer (Model CF-1000, Morgan Instruments, Andover, Massachusetts). A leaf was selected from the upper canopy surface, and a 0.8 cm² area of

the leaf was dark-adapted for 15 min by clamping on a cuvette. Induction kinetics then were measured for the adaxial surface as the dark-adapted area was exposed to $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ of actinic light via a fiber optic probe. The ratio of variable fluorescence (F_v) to maximum fluorescence (F_m) was recorded as a measure of photosynthetic quantum yield (Adams et al., 1990; Karukstis, 1991). For three additional leaves per plot, ψ_w were measured using a pressure chamber (Model 1001, PMS Instrument Co., Corvallis, Oregon) to indicate plant water stress. Immediately prior to each ψ_w measurement, leaf color was recorded using the Munsell Color system (Munsell Color Charts for Plant Tissues, Kollmorgen Instruments, Baltimore, Maryland) as a measure of visible responses to stress.

Image Processing and Analysis

The videotape of the soybean canopies was returned to the laboratory where images of each block taken through each optical filter were captured onto a personal computer. This was accomplished by using a video cassette recorder with single-frame advance capability and a video display and capture board (Win/TV, Hauppauge Computer Works, Hauppauge, New York). Digital images then were processed and analyzed using the FIGMENT (Miller, 1993) and ELAS (Beverly and Penton, 1989) image processing software. Random noise associated with individual video frames was reduced by producing an image that was the average of five frames captured in sequence. This averaged image was produced for the gray cards and for each block within a given waveband and day. Images of each block were geometrically registered to a common point and written to a six channel (e.g., waveband) file. These images were calibrated to reflectance by calculating the gamma function (digital value versus brightness response) of the imaging system for each waveband and day. The gamma function was derived by regressing the average digital value of a gray card within an image against the measured percent reflectance of the gray card. A quadratic model was used and r^2 values for all six wavebands were greater than 0.98. Reflectance ratio images were generated by the quotient of the appropriate reflectance images. Average reflectance or ratio values were computed from approximately 200 pixels within each of three polygons drawn manually within each treatment plot. These data were then analyzed for treatment effects ($p \leq 0.05$) using analysis of variance and Dunnett's means comparison with the controls (Steel and Torrie, 1961; PC SAS, SAS Institute, Cary, North Carolina).

RESULTS

Reflectance and reflectance ratio images first indicated herbicide-induced stress on day 4, simultaneously with

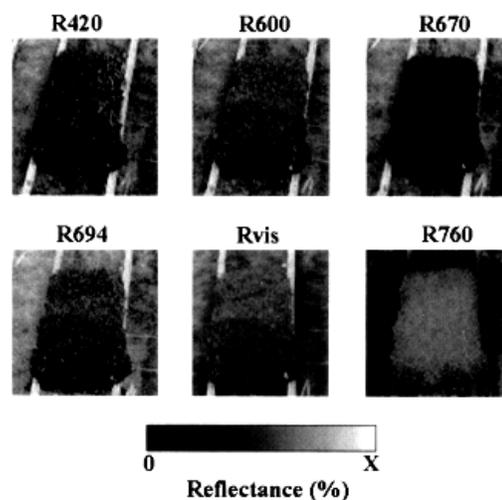


Figure 1. Images of soybean canopy reflectance on the fourth day after treatment application (18 June) taken through optical interference filters. Images represent reflectance at 420 ± 5 nm (R_{420}), 600 ± 5 nm (R_{600}), 670 ± 5 nm (R_{670}), 694 ± 3 nm (R_{694}), 410-740 nm (R_{vis}), and 760 ± 5 nm (R_{760}). Only the data from one of three experimental blocks are shown. In this block, the herbicide treatment was applied across the middle third of the block. The drought treatment was applied to the foreground third, and the control treatment to the background third. Results were similar among the three blocks. Maximum reflectance values (X) were 69% for R_{420} , 94% for R_{600} , 87% for R_{vis} , 100% for R_{670} and R_{694} , and 108% for R_{760} .

the physiological and leaf color measurements. Reflectances at 670 nm (R_{670}), 694 nm (R_{694}), 760 nm (R_{760}), and average reflectance for the 410-740 nm range (R_{vis}) had increased significantly ($p \leq 0.05$) by day 4 in response to the herbicide (Fig. 1, Table 1). Reflectances at 420 nm (R_{420}) and 600 nm (R_{600}) remained unaffected by the herbicide. The reflectance ratios R_{694}/R_{420} and R_{694}/R_{760} also had increased significantly in response to herbicide (Fig. 2, Table 2). Prior to day 4, Munsell leaf color was approximately 5GY 4.6 / 5.7 in all treatments. However, the color of herbicide-damaged leaves had changed to a lighter green-yellow value by day 4 (Table 3). Also, F_v/F_m had decreased, and ψ_w had increased in response to the herbicide (Table 4).

By day 7, R_{420} and R_{600} had increased in the herbicide damaged canopies in addition to R_{670} , R_{694} , and R_{vis} (Table 1). In contrast to results for day 4, R_{760} had decreased rather than increased by day 7 in the herbicide damaged soybean. R_{694}/R_{760} continued to be greater in the herbicide damaged foliage, whereas R_{694}/R_{420} no longer differed among treatments (Table 2). However, R_{600}/R_{760} and R_{vis}/R_{760} were greater in the herbicide damaged soybean by day 7. These reflectance

Table 1. Reflectances (%) of Soybean Canopies Exposed to Control (C), Herbicide (H), and Drought (D) Treatments as Determined in Narrow Wavebands (6-10 nm) Centered at Selected Wavelengths (nm) and in the 410-740 nm Band^a

Wavelength	Treatment	Day		
		2	4	7
420	C	2.8	6.4	3.4
	H	3.5	6.4	6.3*
	D	3.1	5.8	3.0
600	C	7.1	13.1	15.0
	H	6.8	14.7	26.5*
	D	6.1	12.7	14.6
670	C	3.3	2.9	2.8
	H	3.6	4.9*	13.4*
	D	3.8	2.9	2.6
694	C	9.6	12.0	18.1
	H	9.7	16.9*	38.1*
	D	9.6	8.9*	17.5
Vis	C	15.0	21.9	17.0
	H	16.7	26.8*	24.2*
	D	14.8	22.8	16.6
760	C	98.8	103.6	102.4
	H	96.0	106.1*	90.4*
	D	97.8	102.9	102.1

^aMean reflectance values were obtained from image data such as displayed in Figure 1 and as described in Methods. Means denoted by (*) were significantly different from the corresponding control (C) mean ($p \leq 0.05$) according to Dunnett's test.

and reflectance ratio responses to the herbicide corresponded with continued leaf yellowing (Table 3), decreased F_v/F_m and increased ψ_w (Table 4). Although percent ground coverage was not measured for the canopies, visual inspection indicated frequent leaf wilting and partial collapse of herbicide-treated canopies by day 7. This included many brown, dried leaves and some leaf loss. Thus, ground coverage by the herbicide-treated canopies decreased by day 7.

Soybeans exposed to the drought treatment did not receive supplemental watering as did the control and herbicide treatments. However, frequent rainfall prevented ψ_w from decreasing to less than control ψ_w on any sampling day (Table 4). Even so, R_{600}/R_{760} on day 2 (Table 2) and R_{694} on day 4 (Table 1) were less in the drought treatment than in the controls, although stress was not indicated by F_v/F_m or ψ_w (Table 4). Conversely, F_v/F_m decreased under drought treatment on day 7 (Table 4), but there was no corresponding change in reflectances or reflectance ratios (Tables 1 and 2).

Overall, values of R_{420} , R_{600} , R_{670} , R_{694} , and R_{vis} for control canopies were similar to those reported earlier for nonstressed soybean leaves (Gupta and Woolley, 1971). In contrast, R_{760} 's that often exceeded 100% (Table 1) were much greater than soybean leaf reflectances at 760 nm (Gupta and Woolley, 1971). Values

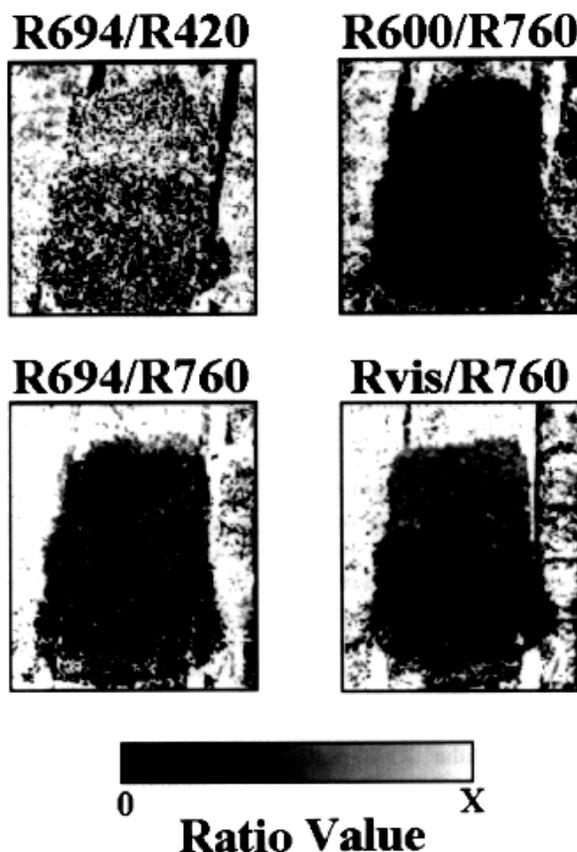


Figure 2. Images of canopy reflectance ratios in soybean on the fourth day after treatment application (18 June). Ratio images were computed from the reflectance data displayed in Figure 1, and represent reflectance at 694 nm divided by reflectance at 420 nm (R_{694}/R_{420}), R_{600}/R_{760} , R_{694}/R_{760} , and reflectance in the 410-740 nm range (R_{vis}) divided by R_{760} . Only the data from one of three experimental blocks are shown. In this block, the herbicide treatment was applied across the middle third of the block. The drought treatment was applied to the foreground third, and the control treatment to the background third. Results were similar among the three blocks. Maximum ratio values (X) were 2.55 for R_{694}/R_{420} , 0.76 for R_{600}/R_{760} , and 0.50 for R_{694}/R_{760} and R_{vis}/R_{760} .

for R_{694}/R_{420} and R_{694}/R_{760} were similar to those reported earlier for nonstressed and stressed leaves of other species (Carter, 1994). The only reflectance images that responded to stress in a consistent manner were R_{670} , R_{694} , and R_{vis} ; that is, these reflectances increased with the onset of herbicide-induced stress (Fig. 1, Table 1), and continued to increase as leaves wilted (Table 1). Similarly, R_{694}/R_{760} was the only reflectance ratio to indicate stress simultaneously with F_v/F_m , C_{wi} , and leaf color, and increase consistently with stress (Fig. 2, Table 2).

Table 2. Reflectance Ratios for Soybean Canopies Exposed to Control (C), Herbicide (H), and Drought (D) Treatments^a

Ratio	Treatment	Day		
		2	4	7
694/420	C	3.8	2.0	5.8
	H	3.1	2.7*	6.4
	D	3.6	1.6	6.0
600/760	C	0.08	0.14	0.15
	H	0.08	0.16	0.32*
	D	0.06*	0.14	0.15
694/760	C	0.10	0.11	0.19
	H	0.12	0.18*	0.46*
	D	0.10	0.10	0.18
Vis/760	C	0.15	0.22	0.17
	H	0.19	0.24	0.29*
	D	0.15	0.20	0.17

^aRatio values were determined from image data such as displayed in Figure 2 and as described in Methods. Means denoted by (*) were significantly different from the corresponding control (C) mean ($p \leq 0.05$) according to Dunnett's test. Ratios were computed for selected numerator and denominator wavebands (central wavelengths in nm), including the 410-740 nm band.

DISCUSSION

The capability of a reflectance or reflectance ratio image to detect plant stress varied substantially with wavelength. The relatively strong response to stress of R_{694} and the weak response of R_{420} were expected based on canopy (Cibula and Carter, 1992) and leaf (Carter, 1993) reflectances for other species. These responses can be explained by the spectral absorptivity of chlorophyll. At wavelengths near 700 nm, the absorptivity of chlorophylls *a* and *b* (Hoff and Amesz, 1991; Chappelle et al., 1992), and of leaves and chloroplast suspensions (Ruhle and Wild, 1979), is weak. If stress is sufficient to inhibit chlorophyll production, increased reflectance is detectable first at wavelengths of weak absorption as chlorophyll content decreases. Thus, reflectance sensitivity to stress-induced chlorosis is high in the 690-700 nm range (Cibula and Carter, 1992; Carter, 1993), and blue shifts of the reflectance curve red edge are closely related to chlorophyll content (Horler et al., 1983; Rock et al., 1988; Curran et al., 1990; Demetriades-Shah et al., 1990; Vogelmann et al., 1993) and visible damage (Ruth et al., 1991; Hoque and Hutzler, 1992). In contrast, chlorophyll and accessory pigments absorb strongly in the violet-blue portion of the spectrum (Ruhle and Wild, 1979; Chappelle et al., 1992), so that pigment content must decrease dramatically for reflectance to increase appreciably at 420 nm.

The absorptivity of chlorophyll is relatively strong near 670 nm and weak near 600 nm (Hoff and Amesz, 1991; Chappelle et al., 1992). This typically results in

Table 3. Munsell Leaf Color in Soybean as an Indicator

of Plant Stress in the Control (C), Herbicide (H), and Drought (D) Treatments^a

Variable	Treatment	Day		
		2	4	7
Hue (GY)	C	5.0	5.0	6.1
	H	5.0	5.0	5.0*
	D	5.0	5.0	6.4
Value	C	4.8	4.1	4.3
	H	4.7	4.8*	5.7*
	D	4.4	4.3	4.9
Chroma	C	5.6	5.9	4.9
	H	6.1	6.0	6.8*
	D	5.6	5.3	5.3

^aHues remained green-yellow (GY) throughout the sampling period. Means denoted by (*) were significantly different from the corresponding control (C) mean ($p \leq 0.05$) according to Dunnett's test.

a high sensitivity of reflectance to stress near 600 nm (Hoque et al., 1988; Carter, 1993) and a lesser stress sensitivity near 670 nm (Carter, 1993). Although an explanation for the relatively weak response to stress of R_{600} is not obvious, the unexpectedly strong response of R_{670} was likely a result of the severity of the herbicide-induced stress. This explanation applies also to the strong response of R_{v-s} . Since the 410-740 nm band contains stress-insensitive as well as stress-sensitive bands, a stress-sensitivity of R_{vis} that was similar to that of R_{694} was not expected. However, exposure to DCMU resulted in rapid inhibition of photosynthesis (Lichtenthaler and Rinderle, 1988), as indicated by decreased F_v/F_m (Adams et al., 1990; Karukstis, 1991) and leaf yellowing. Thus, results for R_{670} and R_{vis} may have been more similar to the expected results if a stress agent that more gradually inhibits plant growth had been used.

Chlorophylls *a* and *b* do not absorb significantly at 760 nm (Hoff and Amesz, 1991; Chappelle et al., 1992).

Table 4. The Ratio of Variable to Maximum Leaf Fluorescence (F_v/F_m) and Water Potentials (ψ_w , bars) of Soybean Leaves Exposed to Control (C), Herbicide (H), and Drought (D) Treatments^a

Means denoted by (*) were significantly different from the corresponding control (C)

Variable	Treatment	Day		
		2	4	7
F_v/F_m	C	0.48	0.50	0.56
	H	0.27	0.28*	0.24*
	D	0.40	0.48	0.50*
ψ_w	C	-11.5	-12.2	-13.6
	H	-10.5	-9.1*	-8.9*
	D	-11.9	-13.2	-12.2

mean ($p \leq 0.05$) according to Dunnett's test.

Although leaf internal reflections serve to increase the absorption pathlength and thus absorption at 760 nm (Ruhle and Wild, 1979), previous work indicated no significant response of reflectance at 760 nm to DCMU or other stress agents for green leaves (Carter, 1993). Thus, increased R_{760} for the herbicide treatment on day 4 probably resulted from a decreased capacity for the heliotropic leaf movements that occur typically in soybean (Rosa et al., 1991); that is, leaf orientation in herbicide-treated soybean differed from that in the controls. Decreased R_{760} on day 7 resulted from severe leaf wilting and partial collapse of the herbicide-treated canopies.

When photosynthetic electron transport is inhibited by DCMU, leaf fluorescence increases within the 650-800 nm range, particularly near 690 nm and 735 nm (Lichtenthaler and Rinderle, 1988). Thus, some proportion of the apparent reflectance increase at 670 nm, 760 nm, and particularly 694 nm in response to DCMU (Table 1) may actually have been fluorescence. However, fluorescence could not be measured by the present methods. Simply viewing plants through a narrow-band interference filter does not allow the separation of emitted from reflected light while plants remain exposed to the incident solar spectrum. To separate fluorescence from reflected light, a method such as the Fraunhofer line-depth principle must be employed (McFarlane et al., 1980; Carter et al., 1990). Nevertheless, increased reflectivity was probably more significant than increased fluorescence as a contributor to increased R_{670} , R_{694} , and R_{760} as evidenced by the leaf yellowing that occurred by day 4 and intensified as the study continued (Tables and 3).

R_{760} 's that were greater than 100% (Table 1) are explained by leaf optical phenomena and errors in the calibration to percent reflectance. In Figure 1, for example, the average digital value for the drought treatment at 760 nm was 125, whereas that for the brightest calibration card (95% reflectance at 760 nm) was 118. Thus, R_{760} was greater than 95%. Application of the quadratic model for calibration of the image to percent reflectance necessitated extrapolation beyond the 95% reflectance point, yielding a canopy reflectance of 108%. However, even a linear extrapolation would have resulted in a canopy reflectance of 101%.

Typical reflectances and transmittances at 760 nm for soybean leaves measured against a black background are 50% and 35%, respectively (Gupta and Woolley, 1971). When such highly reflective and transmissive leaves are assembled in a canopy, light that is reflected and transmitted diffusely at relatively high intensities from the leaves combines with solar irradiance to produce a total irradiance and reflectance of the canopy that is greater than those of the brightest calibration card. This effect would be similar to that which occurs in the leaf interior at 760 nm or other wavelengths where absorption is minimal. Multiple reflections among leaf internal components (Sinclair et al., 1973; Gates, 1980) may result in an internal light intensity that is four times greater than incident irradiance (Vogelmann and Bjorn, 1986). Such additive effects could not have occurred for the simple reflecting surfaces of the reference cards. Neither could this effect have been observable

at visible wavelengths because of substantial absorption by leaf pigments.

The wave-optical phenomena that produce multiple reflections inside leaves depend largely on refractive index differences among intercellular air and wet cell walls (Woolley, 1975). The refractive index of wet soybean cell walls, or of cellulose in general, varies little with wavelength in the visible to near-infrared spectrum (Woolley, 1975). Similarly, it has been shown that internal scattering *per se* is nearly invariant with wavelength (Ruhle and Wild, 1979). Thus, dividing leaf reflectance at a stress-sensitive wavelength by that at a stress-insensitive wavelength, or the inverse, may serve to cancel effects on leaf reflectance caused by internal reflections. This apparently explains the closer relationship of leaf chlorophyll content with reflectance ratios than with reflectances alone (Buschmann et al., 1991; Ruth et al., 1991; Chappelle et al., 1992; Vogelmann et al., 1993; Carter et al., 1994). This also would explain the greater similarity of present results to the stress responses of leaf reflectance ratios, particularly for R_{694}/R_{760} (Carter, 1994), than to leaf reflectance responses *per se* to stress (Carter, 1993).

With respect to wavelength, R_{694}/R_{760} is similar to the leaf fluorescence ratio F_{690}/F_{735} that also is a good indicator of plant stress (Lichtenthaler and Rinderle, 1988). F_{690}/F_{735} increases with decreased photosynthetic electron transport, and, similar to R_{694}/R_{760} , continues to increase as leaf chlorophyll content decreases (Lichtenthaler and Rinderle, 1988). If leaf fluorescence adds with reflected solar irradiance to more greatly increase apparent reflectance at 694 nm and 760 nm in stressed leaves, this would improve the capability of R_{694}/R_{760} to indicate plant stress compared with other reflectance ratios.

At the canopy and larger scales, reflectance ratios tend to correct for geometrical and background effects (Baret and Guyot, 1991), as well as for specular reflectance from leaf surfaces. As a result, R_{694}/R_{760} , along with R_{600}/R_{760} and R_{vis}/R_{760} , was insensitive to decreased ground coverage by leaves. This was indicated by continued increases in ratio values with increased stress on day 7, when R_{760} decreased with partial collapse of the herbicide-treated canopies.

In summary, the reflectance images R_{670} , R_{694} , and R_{vis} , and the reflectance ratio image R_{694}/R_{760} , detected herbicide-induced stress in soybean canopies simultane-

ously with ancillary physiological measurements, and increased in a consistent manner with increasing stress. None of the reflectance or reflectance ratio images detected stress previsually, but this most likely was a consequence of the rapid inducement of chlorosis by the herbicide and the 2-3-day sampling frequency. Re-sults for R_{694} and R_{694}/R_{760} were predicted by previous studies of individual leaves (Carter, 1993; 1994). R_{694}/R_{760} indicated that remote sensing of vegetation within the stress-sensitive 690-700 nm waveband together with the 760 nm region would provide a marked improvement in early stress detection compared with broad band measurements or narrow bands at lesser wavelengths. Continued development of the prototype imaging system will provide an inexpensive tool for the early detection of plant stress. Remote sensing of plant stress using stress-sensitive, narrow wavebands should be applicable to densely vegetated terrain using ground-based, airborne, or spaceborne sensors.

We thank Angele Harrison for assistance in the field and laboratory, Larry Lott and the U.S. Forest Service crew at the Harrison Experimental Forest for field assistance, Chuck Thurman for constructing the camera system, and Dr. William G. Cibula for technical advice and review of the manuscript. This research was supported by the Stennis Space Center Directors Discretionary Fund.

REFERENCES

- Adams, W.W., Demmig-Adams, B. Winter, K., and Schreiber, U. (1990), The ratio of variable to maximum chlorophyll fluorescence from photosystem II, measured in leaves at ambient temperature and at 77K, as an indicator of the photon yield of photosynthesis, *Planta* 180:166-174.
- Baret, F., and Guyot, G. (1991), Potentials and limits of vegetation indices for LAI and APAR assessment, *Remote Sens. Environ.* 35:161-173.
- Beverly, A.M. and Penton, P.G. (1989), *ELAS Users Reference*, Science and Technology Laboratory, NASA, Stennis Space Center, MS, Vol. II.
- Buschmann, C., Rinderle, U., and Lichtenthaler, H.K. (1991), Detection of stress in coniferous forest trees with the VIRAF spectrometer, *IEEE Trans. Geosci. Remote Sensing* 29:96-100.
- Carter, G.A. (1993), Responses of leaf spectral reflectance to plant stress, *Am. J. Bot.* 80:239-243.
- Carter, G.A. (1994), Ratios of leaf reflectances in narrow wavebands as indicators of plant stress, *Int. J. Remote Sens.* 15:697-703.
- Carter, G.A., Theisen, A.F., and Mitchell, R.J. (1990), Chlorophyll fluorescence measured using the Fraunhofer line-depth principle and relationship to photosynthetic rate in the field, *Plant Cell Environ.* 13:79-83.
- Carter, G.A., Rebbeck, J., and Percy, K.E. (1994), Leaf optical properties in *Liriodendron tulipifera* and *Pinus strobus* as influenced by increased atmospheric ozone and carbon dioxide, *Can. J. For. Res.*, forthcoming.
- Chappelle, E.W., Kim, M.S., and McMurtrey, J.E. (1992), Ratio analysis of reflectance spectra (RARS): an algorithm for the remote estimation of the concentrations of chlorophyll a, chlorophyll b, and carotenoids in soybean leaves, *Remote Sens. Environ.* 39:239-247.
- Cibula, W.G., and Carter, G.A. (1992), Identification of a far red reflectance response to ectomycorrhizae in slash pine, *Int. J. Remote Sensing* 13:925-932.
- Curran, P.J., Dungan, J.L. and Gholz, H.L. (1990), Exploring the relationship between reflectance red edge and chlorophyll content in slash pine, *Tree Physiol.* 7:33-48.
- Demetriades-Shah, T.H., Steven, M.D., and Clark, J.A. (1990), High resolution derivative spectra in remote sensing, *Remote Sens. Environ.* 33:55-64.
- Gates, D.M. (1980), *Biophysical Ecology*, Springer-Verlag, New York.
- Gupta, R.K., and Woolley, J.T. (1971), Spectral properties of soybean leaves, *Agron. J.* 63:123-126.
- Hoff, A.J., and Amesz, J. (1991), Visible absorption spectroscopy of chlorophylls, in *Chlorophylls* (H. Scheer, Ed.), CRC Press, Boca Raton, FL, p. 723.
- Hoque, E., and Hutzler, P.J.S. (1992), Spectral blue-shift of red edge monitors damage class of beech trees, *Remote Sens. Environ.* 39:81-84.
- Hoque, E., Hutzler, P.J.S., and Hiendl, H. (1990), Studies on reflective features of Norway spruce and their possible applications in remote sensing of forest damage, *Toxicol. Environ. Chem.* 27:209-215.
- Hoque, E., Hutzler, P.J. S, and Hiendl, H. (1992), Reflectance, colour, and histological features as parameters for the early assessment of forest damages, *Can. J. Remote Sensing* 18:104-110.
- Hoque, E., Hutzler, P.J.S. and Seidlitz, H.K. (1988), Relationship between discoloration and histological changes in leaves of trees affected by forest decline, *Remote Sens. Environ.* 26:171-184.
- Horler, D.N.H., Dockray, M., and Barber, J. (1983), The red edge of plant leaf reflectance, *Int. J. Remote Sens.* 4:273288.
- Karukstis, K.K. (1991), Chlorophyll fluorescence as a physiological probe of the photosynthetic apparatus, in *Chlorophylls* (H. Scheer, Ed.), CRC, Boca Raton, FL, p. 770.
- Lichtenthaler, H.K., and Rinderle, U. (1988), The role of chlorophyll fluorescence in the detection of stress conditions in plants, *Crit. Rev. Anal. Chem.* 19:S29-S85.
- McFarlane, J.C., Watson, R.D., Theisen, A.F. et al. (1980), Plant stress detection by remote measurement of fluorescence, *Appl. Opt.* 19:3287-3289.
- Miller, R.L. (1993), High resolution image processing on low-cost microcomputers, *Int. J. Remote Sens.* 14:655-667.
- Rock, B.N., Hoshizaki, T., and Miller, J.R. (1988), Comparison of in situ and airborne spectral measurements of the blue shift associated with forest decline, *Remote Sens. Environ.* 24:109-127.
- Rosa, L.M., Dillenberg, L.R. and Forseth, I.N. (1991), Responses of soybean leaf angle, photosynthesis and stomatal conductance to leaf and soil water potential, *Ann. Bot. (Lond.)* 67:51-58.
- Ruhle, W. and Wild, A. (1979). The intensification of absorbance changes in leaves by light dispersion, *Planta* 146: 551-557.
- Ruth, B., Hoque E., Weisel, B., and Hutzler, P.J.S. (1991), Reflectance and fluorescence parameters of needles of Norway spruce affected by forest decline, *Remote Sens. Environ.* 38: 35-44.
- Sinclair, T.R., Schreiber, M.M., and Hoffer, R.M. (1973), Diffuse reflectance hypothesis for the pathway of solar radiation through leaves, *Agron J.* 65: 276-283.
- Steel, R.G.D., and Torrie, J.H. (1961), *Principles and Procedures of Statistics*, McGraw-Hill, New York.
- Vogelmann, T.C., and Bjorn, L.O. (1986), Plants as light traps, *Physiol. Plantarum* 68:704-708.
- Vogelmann, J.E., Rock, B.N., and Moss, D.M. (1993), Red edge spectral measurements from sugar maple leaves, *Int. J. Remote Sens.* 14:1563-1575.
- Woolley, J.T. (1975), Refractive index of soybean leaf cell walls, *Plant Physiol.* 55: 172-174.