Canopy Reflectance of Soybean as Affected by Chronic Doses of Ozone in Open-Top Field Chambers

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ABSTRACT: The relationship between canopy reflectance and ozone (O_3) treatment was investigated in a field experiment with soybean growing in 3-m diameter, 2.4-m high open-top exposure chambers. The objectives were to develop an understanding of the pattern of reflectance changes induced by this air pollutant and to investigate how these changes might ultimately be related to yield. Correlations were obtained between the reflectance data and visual estimates of non-green leaf area, a widely-used technique for rating pollutant injury. Analysis of reflectance spectra taken in early September, early October, and mid October showed O_3 treatments to be highly correlated with reflectances at visible wavelengths and at near-infrared wavelengths up to about 720 nm. Early leaf senescence caused by O_3 was easily measured by reflectance changes. As this would reduce the time during which the plants could produce photosynthate needed for seed development, we hypothesize that multitemporal reflectance data could be used to estimate a rate of senescence that would relate to yield. Correlations between the reflectance data and visual estimates of non-green leaf area were high, indicating that rates of senescence could be estimated by either means.

INTRODUCTION

KNOWLEDGE OF THE EFFECTS of environmental stress on canopy reflectance is essential for interpreting crop condition with remotely-sensed data. Air pollutants are one form of environmental stress and ozone (O_3), a photochemical oxidant, is particularly phytotoxic (Heck *et al.*, 1986). Effects on yield are substantial with a sensitive species like soybean (Heagle *et al.*, 1986).

It has already been demonstrated with reflectance spectrophotometry on detached leaves that experimental treatment with O_3 increases reflectances in the visible portion of the spectrum and at near-infrared (NIR) wavelengths between 700 and 2500 nm (Schutt *et al.*, 1984; Runeckles and Resh, 1975; Gausman *et al.*, 1978). Because O_3 is known to cause foliar chlorosis and necrosis (Heck *et al.*, 1986), reflectance at visible wavelengths would be expected to correlate with pollutant treatments. Effects on reflectance at these wavelengths would be associated with changes in pigment levels. The concentration of chlorophyll extracted from leaves of plants exposed to O_3 has been correlated with ratings of leaf injury (Knudson *et al.*, 1977).

In this report, we will discuss the effects of O_3 stress on canopy reflectance from soybean, one of the most ozone-sensitive of the major crop species (Heck *et al.*, 1983). Our objectives were to study the pattern of reflectance changes associated with O_3 stress at visible and NIR wavelengths (400 to 1100 nm) and to determine how reflectance measurements could be related to soybean yield. Reflectance data were compared with visual estimates of non-green leaf area to establish the degree of correlation between these two measures of crop condition.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

The experiment was conducted 8 km south of Raleigh, North Carolina. Soybeans, *Glycine max* L. Merr. 'Davis', were planted 29 June 1982 in a 0.8-ha field in north-south rows spaced 1-m apart. Plots were selected for plant uniformity and thinned to 18 per metre of row. Open-top exposure chambers, each 3 m in diameter and 2.4 m high, were placed over two rows of plants (Heagle *et al.*, 1973). Ozone exposures began 20 July and continued for 7 hours daily (0900–1600 EST) until 17 October, except on rainy days. Plants were harvested 8 November. Plants in all plots were irrigated when soil tensiometers (Irrometer Co.)*, installed at a depth of 30 cm in each plot, showed readings greater than -0.5 bars for 50 percent of the plots. Between planting and crop maturity, 33.2 cm of rain fell and 19.4 cm of irrigation water was applied.

Eight O_3 treatments were randomly assigned to plots within each of two blocks (randomized complete block design). Six treatment plots within each block were maintained at O_3 concentrations greater than ambient by the addition of O_3 during the daily 7-hour exposure period. In three of the treatments, O_3 was added in proportion to that present in the ambient air - either 1.3, 1.6, or 1.9 times ambient levels (referred to as the 1.3, 1.6, or 1.9 treatments). Three different but constant amounts of O_3 (0.02, 0.04, or 0.06 μLL^{-1}) were added to the other 3 treatments (the 20, 40, or 60 treatments). No O_3 was added to one plot within each block (the nonfiltered or NF treatment), and in the other, all air entering through the chamber air distribution system was charcoal-filtered (the CF treatment). In the CF plots, O_3 concentrations ranged from one-third to one-half the ambient levels due to ingress of ambient air through the open chamber top. Ozone concentrations were monitored at canopy height in each plot using two monitors on a time-sharing basis (Heagle *et al.*, 1979). Ozone treatment concentrations

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(μLL^{-1}) were expressed as the mean over the exposure portion of the growing season of the average O_3 concentration during each daily 7-hour treatment period (0900–1600 EST).

Reflectance measurements were made in the CF, NF, 20, 40, and 60 treatment plots between 1030 and 1330 EST on cloudless or near cloudless days 6-8 September, 2-6 October, and 15-20 October (the early September, early October, and mid October data sets, respectively). Cloudy or partly cloudy skies prevented measurements at other times during the growing season and precluded data collection in all plots of both blocks during each time period. Only data from the CF, 20, 40, and 60 treatments were used in the statistical analysis to compare treatment effects on reflectances in early September and early October. By mid October, leaf loss from plants receiving elevated O_3 treatments limited data collection to the CF and NF plots. Dates of measurements are summarized in Table 1.

Instrumentation for Reflectance Measurements

A 50-m length of optical fiber was extended from over the plants in the chambers to a scanning monochromator and detector housed within a small trailer outside of the study area. A second fiber of the same type was fixed 1.2 m above a reference reflecting surface placed about 10 m from the trailer. The fibers were fused silica and characterized by a nominal loss factor of 5 db/km (0.6-mm core diameter type QSF-600A single fiber manufactured by Quartz Products, Inc.). They were inserted into a 6.4-mm diameter polyvinyl chloride tube for protection. The fiber over the plants was held within a self-leveling holder positioned 1.2 m above the top of the canopy. The holder and fiber were moved among the chambers selected for study.

The opposite end of either fiber could be fixed to the input of a 0.22-metre focal length Czerny-Turner type single monochromator equipped with a stepper-motor drive system (Model 1670, SPEX Industries, Inc.). With the grating-slit combination used, measurements at an indicated wavelength encompassed a 20-nm-wide band centered at that wavelength. Design and fabrication of the connection of the fibers to the monochromator and of the mounting and testing of the silicon photodiode detector were done by Math Associates, Inc. The detector signal was amplified and recorded with a chart recorder. Scans were made at the rate of 10 nm/second. The field-of-view of the entire system was estimated to be 14 degrees. The detector thus "saw" a circle of vegetation about 30 cm in diameter. The canopy was closed and plant rows were in excess of a metre wide.

Table 1. Dates of Reflectance Measurements.

Ozone Treatment	Block	Sampling Period Early Sept. Days 6 to 8	Early Oct. 2 to 6[2]	Mid October[1] 15	16	17	19	20
CF	1	X	X	X	X		X	
	2	X	X	X		X	X	
NF[1]	1	X	X	X		X		X
	2	X			X		X	
20	1	X	X					
	2							
40	1	X						
	2	X	X					
60	1	X	X					
	2	X	X					

[1]Data not used in the statistical analyses comparing treatment effects in early September and early October.

[2]Only data collected 2 and 3 October were used in the statistical analyses.

To compensate for transmission differences between the two fibers, calibration factors were obtained by positioning them side by side 1.2 m above the reference reflecting surface and measuring reflected light intensity with each fiber between 400 and 1100 nm. A second set of calibration factors related reflected light intensity from the reference surface used in this study, a 0.6-m by 1.2-m section of flat-white aluminum siding material, to a painted $BaSO_4$ surface. Reflected radiation from both surfaces was measured with the same optical fiber at solar elevations of 38, 55, and 65 degrees above the horizon between 400 and 1100 nm. Only slight and apparently random differences were noted in the calibration factors calculated for each of the three solar elevations at any given wavelength. The three were thus averaged and applied regardless of solar elevation.

Reflectance Measurements

Reflected radiation from four non-overlapping portions of the plant canopy toward the center of the chamber, two from each of the two rows, was recorded each time reflectance was measured. The optical fiber was centered on the plants in each row to view only the canopy surface.

Scans from 400 to 1100 nm (400 to 800 nm in the September data) were first made of the light intensity reflected from the plant canopy and then of that reflected from the reference surface by exchanging fibers at the input to the monochromator. The process required about 5 minutes for each of the four portions of canopy within a chamber. All chart recordings were digitized at 10-nm intervals. A reflectance factor, expressed as a percent, was calculated, using the appropriate calibration factors, as the ratio of light intensity reflected from the plant canopy to that reflected from the reference surface at the same wavelength. The four spectra obtained from the plants in a chamber on a given day were averaged at 10-nm intervals, resulting in a single spectrum that was used for analysis.

Visual Estimates of Non-Green Leaf Area

Percent chlorotic and/or necrotic leaf area was estimated in 5 percent increments (0 to 100 percent) for the top eight trifoliates arising from the main stem of four randomly chosen plants in each chamber (one per quadrant). Estimates were made 31 August, one week before the early September reflectance measurements, and 23 September, one week before the early October reflectance measurements. All visual estimates were made by the same individual.

Statistical Analysis

An analysis of variance for block and O_3 treatment effects was performed separately with each reflectance factor as a dependent variable. The arcsin-square root transformation was first applied to the reflectance factors to stabilize the variances (Steele and Torrie, 1980). Because transmittance of the optical fibers was very low at wavelengths less than 500 nm and detector response poor at those greater than 1000 nm, only reflectance factors between 500 and 1000 nm (800 nm in the early September data) were included in these analyses. F-values based upon Type III sums of squares were used to judge the wavelengths at which reflectance was sensitive to O_3 treatment effects. Block and O_3 treatment effects on visual estimates of non-green leaf area were assessed by an analysis of variance with arcsin-square root transformed estimates of non-green leaf area made 31 August and 23 September. Comparisons between reflectance values and ratings of non-green leaf area were based upon correlation coefficients. Correlations were calculated between the 31 August estimates of non-green leaf area and all reflectance data collected in early September. The 23 September estimates of non-green leaf were compared with reflectance measurements made on 2–

3 October. For both sets of calculations, only reflectance values measured at 560, 620, 720, and 780 nm were included. All calculations were made with procedures within SAS, the Statistical Analysis System.

RESULTS AND DISCUSSION

Soybean yields were reduced substantially by O_3 treatment (Figure 1) and were similar in magnitude to those observed in other experiments (Heagle *et al.*, 1983). The method of O_3 addition, constant versus proportional, did not significantly effect yield (Heagle *et al.*, 1986).

Two important features were apparent in the visual estimates of non-green leaf area (Figure 2). First, on 31 August, the proportion of non-green leaf area was negligible for plants growing at ambient (NF) or lower (CF) O_3 levels. Differences among treatments, except for those at the highest concentrations, were not great though the O_3 effect was statistically significant ($p<0.01$). Second, by 23 September ratings showed an increase in the non-green leaf area for all plants but especially for those receiving the highest O_3 treatments. This pattern of changes suggests that O_3 treatment hastens the natural process of senescence, an effect consistently observed with other plant species (Heck *et al.*, 1986) as well as with water stress in soybean (Constable and Hearn, 1978). Additional evidence of premature leaf senescence was found in the more rapid reduction in the amount of light intercepted by plants receiving the higher O_3 treatments (Table 2).

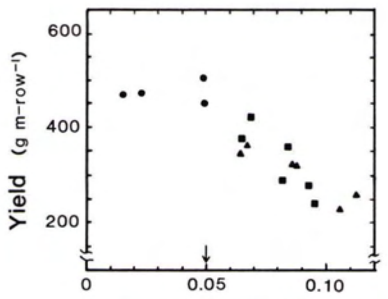

FIG. 1. Soybean yields plotted as a function of the 7 hd^{-1} seasonal mean O_3 treatment concentration. Arrow indicates the 7 hd^{-1} seasonal mean O_3 concentration in ambient air. Squares (■) indicate treatments in which O_3 was added in proportion to ambient levels while triangles (▲) indicate those in which a constant amount was added to ambient air. Circles (●) indicate data from the CF and NF plots to which no O_3 was added.

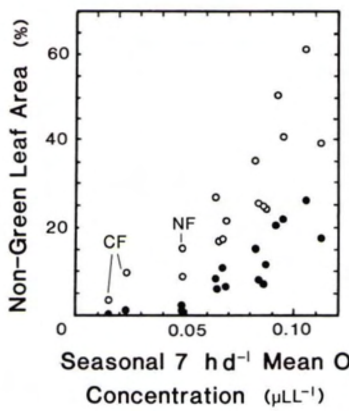

FIG. 2. Visual estimates of non-green soybean leaf area at different O_3 treatment levels. Estimates were made 31 August (●) and 23 September (○).

Reflectance data at selected wavelengths in the visible portion of the spectrum, both in early September and early October, were highly correlated with the visual estimates of non-green leaf area (Table 3). Additionally, the relationships between reflectances and visual estimates were similar for both time periods (Figure 3). Reflectances on the NIR plateau at 780 nm were generally poor surrogates for the visual estimates (Figure 3) while those at 720 nm on the long wavelength side of the chlorophyll absorption band were highly correlated. The slight reduction in the correlations for reflectances at 720 nm, relative to those at visible wavelengths in October compared with September, was likely caused by confounding effects associated with the collapse of the NIR plateau in these rapidly senescing plants (Figure 4). This collapse would be expected with the loss of integrity in leaf mesophyll structure (Gates, 1970; Collins, 1978).

The relationship between the O_3 treatments and the reflectance spectra can best be understood by first considering the two extreme treatments, the CF and the 60 (Figure 4). At visible wavelengths, spectra show plants in early September in the 60 treatment to be chlorotic compared to those grown in charcoal-filtered air. A month later, as would be expected from the visual estimates of non-green leaf area, spectra of the plants in the CF treatment were much the same while reflectances from plants in the 60 treatment had increased considerably at visible wavelengths.

This pattern of more rapid senescence induced by O_3 was also apparent at NIR wavelengths. Reflectances on the long wavelength side of the chlorophyll absorption band, beginning at around 700 nm (the red edge), shifted more rapidly to shorter wavelengths for plants in the 60 treatment relative to those in the CF treatment. This shift has been associated with the loss of chlorophyll (Collins, 1978; Horler, 1983), but the direction of movement of the red edge is the reverse of that reported for leaves of maturing plants (Gates *et al.*, 1965). Thus, this shift is

TABLE 2. SUMMARY OF OZONE TREATMENT AND GROWTH DATA (CONSTANT ADDITION TREATMENTS ONLY) FOR SOYBEAN GROWING IN OPEN-TOP CHAMBERS.

Ozone Treatment	Treatment Mean Ozone Concentration (μLL^{-1})[1]	Leaf Area Index (early Sept)[2]	Fraction of Solar Radiation Intercepted by Canopy[3]		
			Early Sept	Early Oct	Mid Oct
CF	0.019	10.5	0.90	0.89	0.83
NF	0.049	10.0	0.88	0.88	0.80
20	0.066	9.3	0.88	0.88	0.71
40	0.086	10.5	0.86	0.83	0.65
60	0.109	7.5	0.85	0.79	0.43

[1]Seasonal 7 hd^{-1} mean for the period 20 July through 17 October. The seasonal 7 hd^{-1} mean in ambient air was 0.050 μLL^{-1}.

[2]Estimated from Figure 7 of Unsworth *et al.* (1984) for day 63 (31 August).

[3]Estimated from Figure 5 of Unsworth *et al.* (1984) and Unsworth (unpublished data) for days 63, 93 (30 September), and 105 (12 October). Values are based upon measurements made in one block only. Tube solarimeters were used to estimate the difference between radiation incident on the top of the canopy and that per unit of ground area below.

TABLE 3. CORRELATION COEFFICIENTS AMONG REFLECTANCE VARIABLES AND VISUAL ESTIMATES OF NON-GREEN LEAF AREA[1]

	Early September Reflectances at[2]:			
	560 nm	620 nm	720 nm	780 nm
31 Aug Visual Estimates	0.835 (0.005)	0.794 (0.011)	0.897 (0.001)	−0.607 (0.083)
560 nm	–	0.992 (<0.001)	0.972 (<0.001)	−0.308 (0.421)
620 nm	–	–	0.950 (<0.001)	−0.255 (0.509)
720 nm	–	–	–	−0.414 (0.267)
	Early October Reflectances at[3]:			
	560 nm	• 620 nm	720 nm	780 nm
23 Sep Visual Estimates	0.,951 (0.001)	0.949 (0.002)	0.940 (0.002)	−0.373 (0.410)
560 nm	–	0.987 (<0.001)	0.937 (0.002)	−0.494 (0.260)
620 nm	–	–	0.887 (0.008)	−0.464 (0.295)
720 nm	–	–	–	−0.339 (0.456)

[1]Probabilities of greater correlation coefficients under H_0: $R=0$ are underneath in parentheses.
[2]Based upon nine observations per comparison.
[3]Based upon seven observations per comparison.

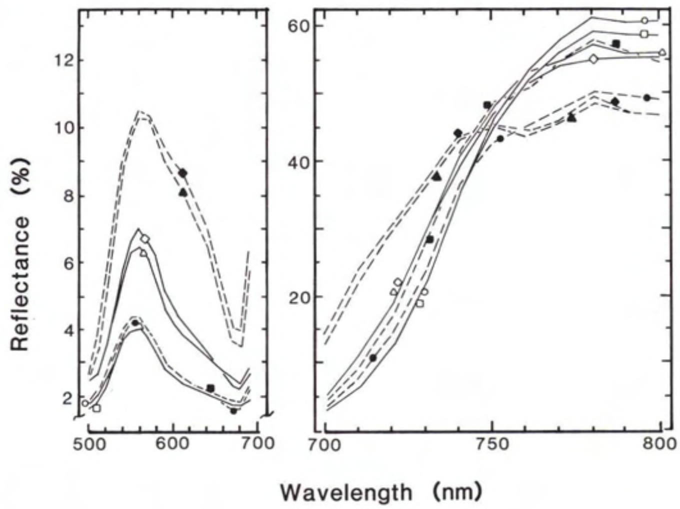

FIG. 4. Digitized reflectance spectra at visible and NIR wavelengths for soybean growing in charcoal-filtered (CF) air and at a mean O_3 concentration of 0.109 μLL⁻¹ (60 treatment) in early September (—) and early October (---). Open symbols (○ and □) indicate the two replicates of plants in CF air in early September; open symbols (◇ and △) indicate the two replicates of the 60 treatment in early September. Solid symbols (● and ■) show the two replicates of the CF treatment in early October; solid symbols (◆ and ▲) show the two replicates of the 60 treatment in early October.

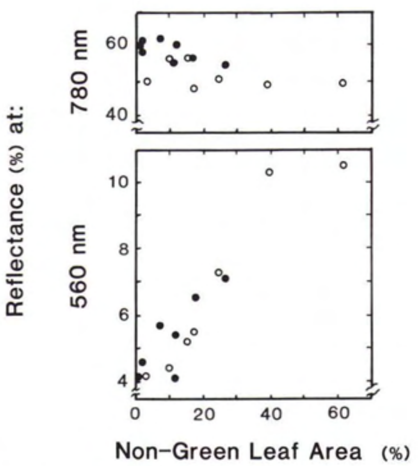

FIG. 3. Relationships between reflectances at 560 and 780 nm and visual estimates of non-green soybean leaf area in the early September (●) and early October data sets (○).

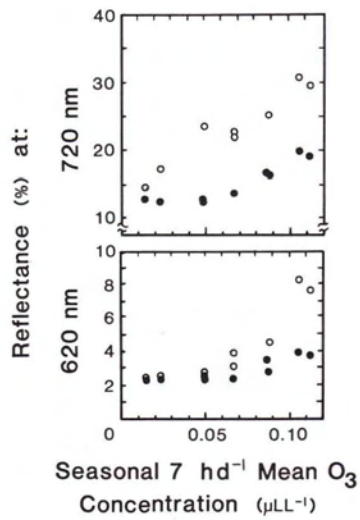

FIG. 5. Canopy reflectances at 620 and 720 nm in early September (●) and early October (○) from soybean growing at different O_3 concentrations.

useful as well for monitoring season-end spectral changes associated with senescence of soybean as well as other crops (Collins, 1978). Soybean plants growing at O_3 levels between those of the CF and 60 treatments showed an intermediate response (Figure 5).

While analysis of variance for treatment effects indicated that reflectances between 700 and 800 nm were almost all good predictors of O_3 effects in early September (Figure 6), this was not the case in early October. Collapse of the NIR plateau eliminated the usefulness of those beyond 720 nm. The disparate responses of the two CF plots in early October at wavelengths beyond 740 nm (Figure 4) indicates that the variability in reflectance measurements at these wavelengths from rapidly senescing soybean plants severly limits their diagnostic value.

The rapidity of the senescence process in terms of reflectance changes, and its acceleration at the very end of the season, are obvious when data from early September through mid October are graphed for plants growing in a single chamber (Figure 7). Of particular note is the abrupt and short-term increase in reflectances on the NIR plateau from 15 to 17 October even though the red edge continued to shift to shorter wavelengths. A similar pattern was observed with plants growing in the CF treatment (data not shown) and has been reported for detached

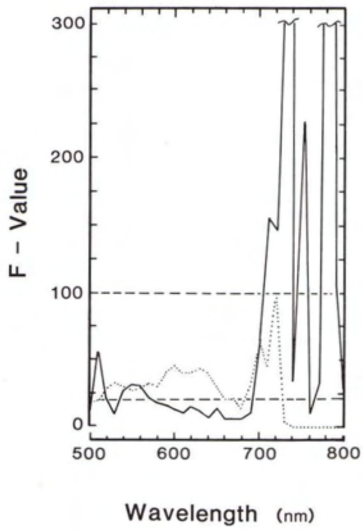

FIG. 6. F-statistics comparing O_3 treatment effects as a function of wavelength in early September (——) and early October (..........). Lower dashed line corresponds to significance at the 0.05 level for an F-test with three and two degrees of freedom; upper dashed line corresponds to the same F-test at the 0.01 level of significance.

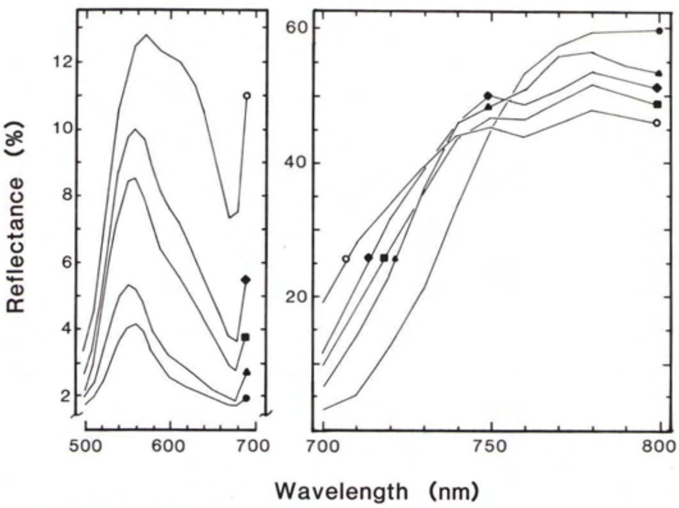

FIG. 7. Digitized reflectance spectra from plants in one of the NF treatment plots at visible and NIR wavelengths. Spectra were obtained 6 September (●), 6 October (▲), 15 October (■), 17 October (◆), and 20 October (○).

birch leaves (Daughtry and Biehl, 1985). This short-lived change would further limit the diagnostic value of reflectances on the NIR plateau measured during soybean senescence.

Premature senescence in this determinate soybean cultivar, by reducing the amount of time during which plants can photosynthesize and produce dry matter for seeds, is probably the principal mechanism by which O_3 effects yield. Characterization of the rate of senescence in soybean, easily visualized in Figure 7, would thus seem to be a more reliable approach to developing correlations between reflectance and yield than using measurements made at one point in time. With the high correlation among reflectances (Table 3) and the broad range of wavelengths over which treatment effects were significant (Figure 6), multitemporal reflectance data at any number of visible or NIR wavelengths, up to about 720 nm, should yield reliable estimates. While reflectance measurements in early September and early October correlated well with O_3 treatment (Figure 6) and, thus, yield, models predicting yield from reflectance developed separately for these two dates would differ because of the more rapid change in color associated with senescence in the plants under greater stress (Figure 5). It would, thus, be necessary that any yield-reflectance model based upon measurements at a single point in time be applied at an equivalent developmental stage. A multitemporal modeling approach minimizes these problems and has proven useful in studies with winter wheat and barley (Idso *et al.*, 1980; Pinter *et al.*, 1981). It should be noted, however, that, although characterizations of senescence with reflectance data might relate well to yield from soybean or other crops, it would not be possible to assign a specific factor as a cause of the stress.

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