

# Leaf Reflectance Spectra of Cereal Aphid-Damaged Wheat

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## ABSTRACT

The efficiency of field monitoring for insect pests would be improved with knowledge of reflected solar radiation from crop canopies during insect outbreaks. The objectives of this greenhouse study were to characterize leaf reflectance spectra of wheat (*Triticum aestivum* L.) damaged by Russian wheat aphids (*Diuraphis noxia* Mordvilko) and greenbugs (*Schizaphis graminum* Rondani) and to determine those leaf reflectance wavelengths that were most responsive to crop stress imposed by these aphid pests. When the ligule was visible on second oldest leaf, wheat plants were infested with four wingless adult Russian wheat aphids, four wingless adult greenbugs, or left uninfested (four replicate plants per treatment). Plants and aphid populations were allowed to grow under greenhouse conditions for 3 wk, after which leaf-reflected radiation (from the adaxial surface across the 350–1075 nm range), dry weight, area, and chlorophyll concentrations were measured. When compared with the control, greenbug feeding damage caused general necrosis in oldest (first) leaves and dramatically lowered the dry weight, leaf area, and chlorophyll concentration of the second, third, and fourth leaves. Russian wheat aphid feeding resulted in a reduction in leaf dry weight and area in the third and fourth leaves, and a reduction in total chlorophyll concentration in all leaves. Leaf reflectance in the 625- to 635-nm and the 680- to 695-nm ranges, as well as the normalized total pigment to chlorophyll *a* ratio index (NPCI), were significantly correlated with total chlorophyll concentrations in both greenbug- and Russian wheat aphid-damaged plants. Thus, both of these wavelength ranges, as well as this reflectance index, were good indicators of chlorophyll loss and leaf senescence caused by the aphid feeding damage.

Russian wheat aphids and greenbugs are major small grain aphid pests in North America (Kieckhefer et al., 1995). Cereal crop damage occurs during feeding when the aphids inject phytotoxic substances into leaves and remove assimilates from leaf vascular tissues (Fouché et al., 1984; Al-Mousawi et al., 1983). Markedly different feeding damage symptoms are caused by these two aphid species. Greenbug feeding damage is characterized by chlorotic and necrotic lesions in and around feeding sites on older leaves (Dorschner et al., 1987). Feeding by large numbers of greenbugs results in general chlorosis (yellow in color) and necrosis of entire leaves (Burton, 1986). In contrast, Russian wheat aphids feed on younger leaves, causing damage symptoms that include longitudinal chlorotic streaking (white in color) and rolling resulting in convolute leaf morphology (Webster et al., 1987). Yield losses of 35 to 60% have been reported in greenbug-damaged wheat (Kieckhefer and Kantack, 1980, 1988) and in Russian wheat aphid-damaged wheat (Webster et al., 1987; Riedell and Kieckhefer, 1993). Wheat producers often apply broadcast insecticides to manage cereal aphid

populations and reduce subsequent yield losses (Kantack et al., 1983).

Crop canopy chlorosis and necrosis in small grain fields infested with greenbugs or Russian wheat aphids could be used as a diagnostic tool by farmers to detect crop damage from cereal aphid population outbreaks (Riedell and Kieckhefer, 1995). If information on the exact field location of aphid outbreaks was available, farmers could target insecticide applications specifically to those portions of the field where the insect pests were present. However, the vastness of the acreage planted to small grains may impede the efficiency of using visual inspection to detect crop damage caused by aphid population outbreaks.

The increased sensitivity, decreased cost, and increased availability of high resolution spectral analysis devices have improved the technology used for remote sensing (Blackmer et al., 1996). Use of remote sensing technology to analyze reflected solar radiation from crop canopies to search for insect outbreaks and integration of these data with geographic information systems would improve the efficiency of field monitoring for insect pests (Everitt et al., 1994). This potential improvement plus an increased interest in site-specific farming and integrated pest management encourage the close examination of optimal reflected radiation wavelengths that could be used to detect cereal aphid population outbreaks.

Leaf reflectance changes in response to numerous stress agents have been documented (Carter et al., 1992, 1996; Malthus and Madeira, 1993). However, the spectral regions or wavelengths at which leaf reflectance is most responsive to stress remains largely undefined, and the extent to which particular stress agents yield spectrally unique leaf reflective responses has not been established (Carter, 1993). Thus, our objectives were to characterize wheat leaf reflectance responses to Russian wheat aphids and greenbugs and to determine the wavelengths at which leaf reflectance was most responsive to crop stress imposed by these insects.

## MATERIALS AND METHODS

### Wheat Plant Culture and Aphid Treatments

Kernels of spring wheat ('Sharp') were germinated between moist paper towels in the dark at 20°C. Three days later, seedlings selected for uniform size were planted in a 2:1:1 mixture of soil/peat/vermiculite in 10-cm clay pots at a rate of one seedling per pot. Pots were placed into the greenhouse under natural lighting supplemented with 12 h d provided by cool white fluorescent bulbs (300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) at 20°C with  $\approx 50\%$  relative humidity. At the two-leaf development stage (ligule visible on second oldest leaf,  $\approx 14$  d after germination), plants were infested with four wingless adult Russian wheat

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**Abbreviations:** ANOVA, analysis of variance; NPCI, normalized total pigment to chlorophyll *a* ratio index; WBI, water band index.

aphids, four wingless adult greenbugs, or left uninfested. Aphids were obtained from aphid colony plants maintained at the Northern Grain Insects Research Laboratory as previously described (Kieckhefer et al., 1995). A 6 cm in diameter by 40 cm high clear plastic vented tubular cage was placed over each plant. Pots were then arranged in a completely random design with four replications on the greenhouse bench.

### Plant Measurements

Plants and aphid populations were allowed to grow under greenhouse conditions for 3 wk, after which leaf reflected radiation, leaf dry weight, leaf area, and leaf chlorophyll concentrations were measured. Leaves were removed from the plant by cutting the leaf blade at the ligule. Aphids were wiped from the leaf with laboratory tissue moistened with distilled water. Leaf radiance from the adaxial surface, across a 350- to 1075-nm range at  $\approx 1.4$ -nm intervals, was measured by clamping a uniform leaf blade portion midway between the leaf tip and base into an External Integrating Sphere (1800-12S, LiCor, Lincoln, NE) attached to a portable spectroradiometer (Personal Spectrometer II, Analytical Spectral Devices, Boulder, CO). Two measurements per leaf were taken and the resulting data were averaged. Reflectance spectra, relative to a barium sulfate standard, were calculated by dividing leaf radiance by reference radiance from a barium sulfate standard for each wavelength. Reflectance standard measurement were made immediately before and after leaf measurements. Reflectance sensitivity at a given wavelength was computed by dividing the reflectance difference (obtained by subtracting the leaf reflectance of control leaves from that of aphid-damaged leaves at each spectroradiometer wavelength channel) by the control reflectance at each channel (Carter, 1993).

After radiance measurements, leaves were measured for leaf area (area meter, Delta-T Devices, Cambridge, England), dried at 60°C in a forced-air oven for 72 h, weighed, and chlorophyll extracted in *N,N*-dimethylformamide. The formulae of Moran (1982) were used for quantitative determination of chlorophyll *a*, chlorophyll *b*, and total chlorophyll concentrations. Leaf chlorophyll *a/b* ratios were determined by dividing the chlorophyll *a* concentration by that of chlorophyll *b*.

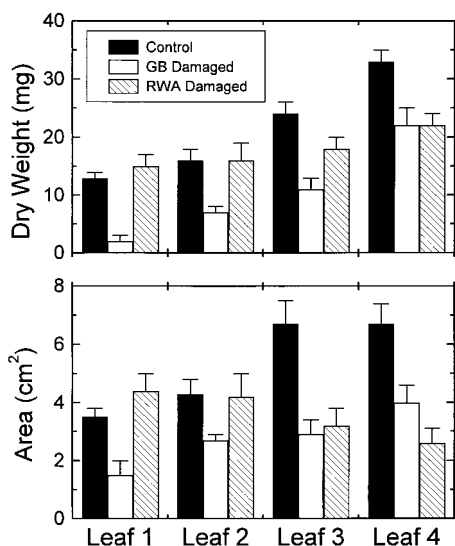


Fig. 1. Leaf dry weight (top) and area (bottom) of wheat subjected to control, greenbug, or Russian wheat aphid treatments. Values represent mean and standard deviation for four replicates per treatment.

### Data Analysis

All crop and reflectance data were analyzed using conventional statistical analysis. Reflectance means and standard deviations for each spectroradiometer channel were calculated across treatments for each leaf and plotted on graphs. Chlorophyll concentration data were subjected to analysis of variance (ANOVA) analysis in SAS (SAS Institute, 1988). Reflectance data for wavelengths identified by sensitivity analysis were averaged across their wavelength ranges (500–520, 625–635, and 680–695 nm) before ANOVA analysis in SAS. Two reflectance indices, the normalized total pigment to chlorophyll *a* ratio index (NPCI),

$$\text{NPCI} = (R_{680} - R_{430}) / (R_{680} + R_{430})$$

and the water band index (WBI),

$$\text{WBI} = R_{950} / R_{900}$$

were also calculated (Peñuelas et al., 1993, 1997) and subjected to ANOVA analysis.

With the occurrence of a significant ANOVA test, means were separated using the LSD option. Potential correlations between total chlorophyll concentrations and the reflectance data at wavelengths identified by sensitivity analysis, as well as the two reflectance indices across treatments, were investigated by calculating Pearson's correlation coefficients using CORR procedures in SAS (SAS Institute, 1988).

## RESULTS AND DISCUSSION

Undamaged control plants grew about six expanded leaves during the experiment. Leaves were dark green in color and had no apparent disease or insect damage. Greenbug feeding damage, which was more intense on the older leaves, caused reductions in the number of leaves per plant (to four per plant), leaf dry weight, and leaf area as compared with the undamaged control plants (Fig. 1). The feeding damage dramatically lowered the total chlorophyll concentration in the oldest (first) leaves (Table 1) and caused these leaves to be

Table 1. Leaf chlorophyll concentrations and ratios of wheat subjected to control, greenbug (GB), or Russian wheat aphid (RWA) treatments.

Treatment	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Chlorophyll total	Chlorophyll <i>a/b</i> ratio
	μg mg <sup>-1</sup> DW			
	<b>Leaf 1</b>			
Control	2.28a‡	1.24a	3.52a	1.84a
GB	0.17c	0.22c	0.39c	0.76b
RWA	1.33b	0.70b	2.03b	1.92a
	<b>Leaf 2</b>			
Control	2.25a	1.28a	3.53a	1.76a
GB	0.28b	0.39b	0.68b	0.85b
RWA	0.62b	0.41b	1.03b	1.53a
	<b>Leaf 3</b>			
Control	2.22a	1.32a	3.53a	1.68a
GB	0.35b	0.31b	0.66b	1.14b
RWA	0.19b	0.12b	0.32b	1.56a
	<b>Leaf 4</b>			
Control	2.11a	1.30a	3.41a	1.62b
GB	0.45b	0.29b	0.74b	1.50b
RWA	0.25b	0.09c	0.34b	2.98a

† Values represent means for four replicate samples per treatment.

‡ Values within columns for each leaf followed by the same letter are not significantly different (LSD test,  $P = 0.05$ ).

mostly brown in color (a general necrosis). The second and third leaves also had significantly less total chlorophyll concentration than control leaves. The feeding damage in these leaves resulted in a general chlorosis (yellow in color) with a considerable portion ( $\approx 50\%$ ) of the leaves covered with isolated necrotic lesions (brown in color). The fourth leaf also showed a general chlorosis (light green-yellow in color) with few necrotic lesions. These damage symptoms are considered to be typical of wheat plant response to feeding by greenbugs (Cook and Veseth, 1991). Chlorophyll *a/b* ratios for leaves severely damaged by greenbug feeding (Leaves 1–3) were significantly lower than those same leaves from the undamaged control plants (Table 1). The leaf chlorophyll *a* concentration appeared to be reduced to a greater proportion than chlorophyll *b* concentration in these greenbug-damaged leaves (Table 1).

Russian wheat aphid feeding also reduced the number of leaves per plant (to four per plant) when compared with the undamaged control plants. The first and second leaves, which did not have any obvious Russian wheat aphid damage, were very similar in dry weight and area but had significantly less total chlorophyll concentration when compared with the control plants (Fig. 1, Table

1). Feeding damage, which was more intense on younger leaves, was accompanied by reductions in leaf dry weight, leaf area, and total chlorophyll concentration in the third and fourth leaves. The third leaf showed longitudinal white streaking from the leaf base to the tip, while the fourth leaf showed considerable longitudinal white streaking and a convolute (longitudinal rolling) growth habit typical of Russian wheat aphid feeding damage (Cook and Veseth, 1991). Chlorophyll *a/b* ratios in Leaves 1 through 3 did not significantly differ from the undamaged control plants, confirming that Russian wheat damage reduced constituent levels of both chlorophylls in a proportionate manner (Burd and Elliott, 1996).

All of the control plant leaves measured had very similar reflectance spectra (Plate 1). Chlorophyll, the major plant pigment in wheat leaves, absorbs energy at 440 to 480 nm and 640 to 660 nm (Verbyla, 1995). Hence, the reflectance peak at 550 nm is probably that portion of the incident radiation that is not absorbed by the chlorophyll pigment (Hinzman et al., 1986; Guyot, 1990). We also observed a dramatic increase in reflectance that started at  $\approx 700$  nm and reached a plateau at  $\approx 750$  nm that extended beyond 1000 nm (Plate 1). Guyot

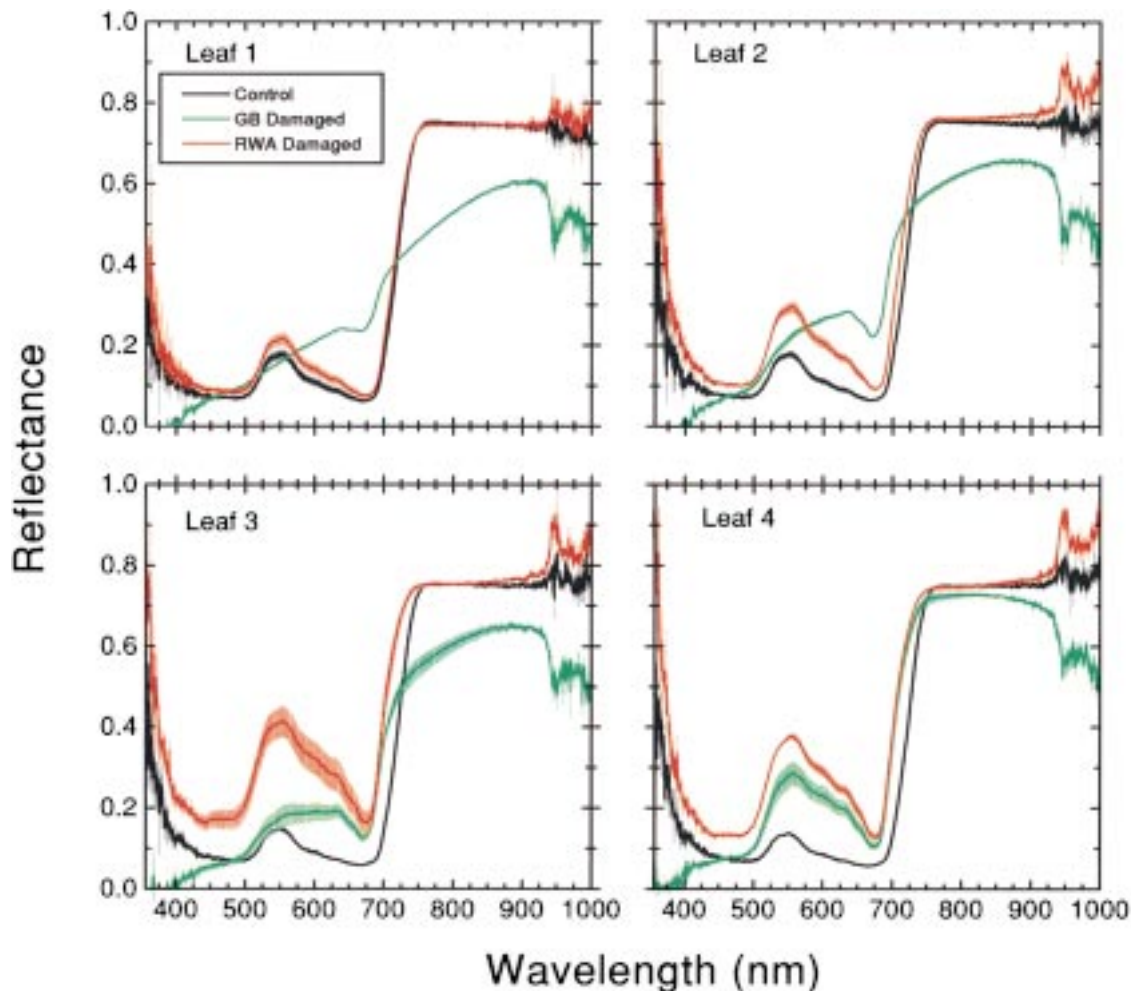


Plate 1. Leaf reflectance spectra relative to a barium sulfate standard for plants damaged by greenbugs or Russian wheat aphids. Values represent mean and standard deviation for four replicates per treatment.

(1990) and Peñuelas et al. (1997), who found a similar reflectance plateau in radiation reflected from plants, suggested that this plateau corresponds with that region of the spectrum where leaf pigments and the cellulose of the cell walls are transparent, and incoming radiation is either reflected or transmitted. The reflectance spectra from our control wheat leaves are consistent in appearance with wheat canopy reflectance spectra published elsewhere (Hinzman et al., 1986; Jackson and Pinter, 1986).

Reflectance spectra (Plate 1) of leaves extensively damaged by greenbug feeding (Leaves 1, 2, and 3) were different from control leaves, possibly because damaged leaves had less total chlorophyll (Table 1) and increased amounts of necrotic lesions. Younger leaves (Leaf 4) with slight greenbug feeding damage reflected greater amounts of incident radiation (at  $\approx 475$  nm up to  $\approx 750$  nm) when compared with the undamaged leaves (Plate 1).

Leaf reflectance spectra from Russian wheat aphid-damaged plants revealed that the oldest leaves (Leaf 1), which were not directly damaged by this insect but had a lower total chlorophyll concentration than the control leaves (Table 1), had reflectance spectra that were very similar to those of control plants (Plate 1). Younger leaves with progressively more Russian wheat aphid-damage (Leaves 2, 3, and 4) reflected greater amounts of incident radiation when compared with undamaged leaves starting at  $\approx 350$  nm up to  $\approx 750$  nm (Plate 1). This increase in reflectance is probably related to decreased total chlorophyll concentrations in these leaves (Table 1).

Leaf damage by both aphid species was accompanied by a shift in the wavelength at which the red-infrared transition region began. In control leaves this transition began at  $\approx 700$  nm as compared to  $\approx 680$  nm in aphid-damaged leaves (Plate 1). This wavelength shift, which is thought to occur in plants undergoing the process of senescence (Guyot, 1990), has also been observed in chlorotic false loosestrife (*Ludwigia palustris* L.) (Peñuelas et al., 1993), and in coniferous forests that have been damaged by air pollution (Rock et al., 1988). Any stress to crop plants that affects photosynthetic electron transport in chlorophyll-containing tissue will change the quantum yield of chlorophyll fluorescence (Krause and Weis, 1984; Burd and Elliott, 1996). Chlorophyll fluorescence has an emission spectrum with a peak at 685 nm (Krause and Weis, 1991). Thus, increased chlorophyll fluorescence in leaves damaged by aphid feeding could be the cause of this recorded shift in the red-infrared transition region. Burd and Elliott (1996) measured increased chlorophyll fluorescence (attributed to detachment of antennal chlorophyll complexes from photosystem II reaction centers) in wheat damaged by Russian wheat aphids. However, the effects of greenbug feeding on wheat leaf chlorophyll fluorescence have not been documented by others. Because the spectroradiometer used in our experiments was barely adequate for measuring chlorophyll fluorescence (J.S. Shepers, 1998, personal communication), we cannot conclude that greenbug feeding damage also increased chloro-

phyll fluorescence. Additional studies would be needed before such a conclusion could be made.

In the near-infrared wavelengths (850–1000 nm), Russian wheat aphid damaged leaves reflected more energy, while the greenbug-damaged leaves reflected less energy than the control (Plate 1). Reflectance at these wavelengths usually increases when leaf drought stress occurs (Ripple, 1986; Verbyla, 1995). Leaves infested with Russian wheat aphid have lower relative water content and more negative water potential than uninfested leaves (Riedell, 1989). Consequently, the reflectance energy increase in the near-infrared wavelengths for Russian wheat aphid-treated plants may be the result of these drought stress symptoms. Greenbug damage has been shown to disrupt leaf cellular structure, which allows vesicles and other cellular contents to fill intercellular spaces (Al-Mousawi et al., 1983). A high intercellular space/cell ratio is one reason why plants reflect radiation highly in the near-infrared region (Gates, 1970; Gausman et al., 1977; Verbyla, 1995). Thus, the reflectance energy decrease in greenbug-treated plants may be the result of cellular lysis and filling of intercellular space with cellular debris.

When the data are presented as reflectance vs. wavelength (as in Plate 1), the effects of stress on leaf reflectance are often difficult to evaluate quantitatively (Carter, 1993). This is particularly true where the slope of the reflectance curve is large (e.g., the red-infrared transition region). Thus, to represent more clearly the leaf reflectance response to aphid feeding damage, reflectance sensitivities (Carter, 1993) were used to identify specific wavelengths in which leaf reflectance was most strongly affected by aphid feeding damage. For both aphid species, the wavelength peaks of greatest sensitivity were located  $\approx 500$  to 520, 625 to 635, and 680 to 695 nm in the visible portion of the electromagnetic spectrum (Fig. 2). Broad sensitivity regions in aphid damaged leaves were also recorded above about 925 nm.

The relationship between leaf reflectance at these sensitive wavelength peaks and damage caused by Russian wheat aphids or greenbugs was investigated. In almost every instance, reflectance at the sensitive wavelengths from aphid-damaged leaves was greater than that seen from undamaged leaves (Table 2). Reflectance at the 625- to 635-nm and the 680- to 695-nm wavelength regions were generally greater in the older leaves of greenbug-damaged plants than these same leaves in Russian wheat aphid-damaged plants. There was a trend toward greater reflectance values in Russian wheat aphid-damaged plants than in greenbug damaged plants in younger leaves at these same wavelengths. Two indices, the NCPI and WBI, which have the potential to reflect the physiological status of plants (Peñuelas et al., 1993, 1997) were also investigated. The NPCI, which quantifies the ratio of total pigments to chlorophyll *a*, were always significantly greater in greenbug-damaged leaves than the other treatments regardless of what leaf was sampled, while these same index values from leaves damaged by Russian wheat aphids were greater than control only in younger leaves (Table 3). Our results tend to support the idea that the NCPI should rise in

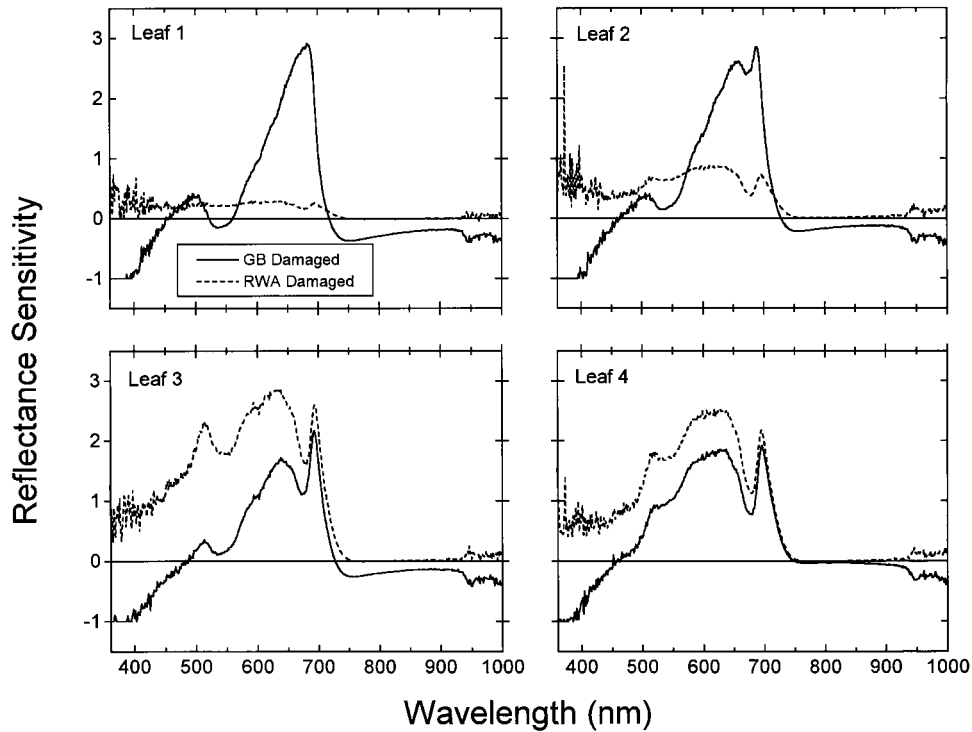


Fig. 2. Spectral sensitivity of leaf reflectance to greenbug or Russian wheat aphid feeding damage. Sensitivities were calculated by dividing the reflectance differences by the control leaf reflectance (zero sensitivity). Values represent means for four replicates per treatment.

senescing plants (Peñuelas et al., 1993). The WBI, which is an indicator of plant water status, was always higher than control in Russian wheat aphid damaged plants and was lower than control in greenbug damaged plants (Table 2). The WBI increases in leaves that have poor water status (Peñuelas et al., 1997), which is consistent with the idea that Russian wheat aphids cause drought stress symptoms in small grain plants (Riedell, 1989).

Table 2. Influence of aphid infestation on leaf reflectance values at wavelengths sensitive to greenbug (GB) and Russian wheat aphid (RWA) damage and reflectance indices that reflect the physiological status of plants.

Treatment	Sensitive wavelengths (nm) <sup>†</sup>			Reflectance indices	
	500–520	625–635	680–695	NPCI <sup>‡</sup>	WBI <sup>§</sup>
<b>Leaf 1</b>					
Control	0.09b <sup>¶</sup>	0.09c	0.08c	-0.03b	0.98b
GB	0.11a	0.24a	0.29a	0.72a	0.79c
RWA	0.11a	0.11b	0.09b	-0.03b	1.04a
<b>Leaf 2</b>					
Control	0.09c	0.09c	0.08c	-0.05b	1.00b
GB	0.12b	0.28a	0.29a	0.72a	0.73c
RWA	0.14a	0.17b	0.12b	-0.01b	1.13a
<b>Leaf 3</b>					
Control	0.08b	0.07c	0.07b	-0.12c	1.04b
GB	0.10b	0.19b	0.19a	0.65a	0.79c
RWA	0.24a	0.28a	0.21a	0.10b	1.15a
<b>Leaf 4</b>					
Control	0.08c	0.07c	0.07c	-0.15c	1.05b
GB	0.13b	0.20b	0.14b	0.39a	0.79c
RWA	0.19a	0.24a	0.16a	0.06b	1.19a

<sup>†</sup> Wavelengths determined by sensitivity analysis (see Fig. 2).  
<sup>‡</sup> NPCI is the normalized total pigment to chlorophyll *a* ratio index.  
<sup>§</sup> WBI is the water band index.  
<sup>¶</sup> Values followed by the same letter within columns and leaves are not significantly different (LSD test, *P* = 0.05).

The reflectance values at the 625- to 635-nm range (which is near the chlorophyll absorption region) were significantly correlated with total chlorophyll concentrations in both greenbug- and Russian wheat aphid-damaged plants (Table 3). Reflectance values at the 680- to 695-nm range (which indicate the wavelength shift in the red-infrared transition zone in aphid-damaged leaves), were also significantly correlated with total chlorophyll concentrations in both greenbug- and Russian wheat aphid-damaged plants. The NPCI was also significantly correlated to total chlorophyll concentration (Table 3). Thus, both of these wavelength ranges, as well as this reflectance index, were good indicators of chlorophyll loss and leaf senescence caused by the aphid feeding damage under laboratory conditions.

Table 3. Correlation coefficients between total chlorophyll concentrations and leaf reflectance at sensitive wavelengths and reflectance indices for greenbug and Russian wheat aphid damaged wheat plants. Data from Leaves 1 through 4 were used in this analysis.

	<i>r</i>	
	Greenbug	Russian wheat aphid
<b>Sensitive wavelengths<sup>†</sup></b>		
500–520 nm	ns	-0.87***
625–635 nm	-0.49*	-0.91***
680–695 nm	-0.62**	-0.84***
<b>Reflectance indices</b>		
NPCI <sup>‡</sup>	-0.52*	-0.84***
WBI <sup>§</sup>	ns	-0.92***

\*, \*\*, \*\*\* Significant at the 0.1, 0.05, and 0.01 probability levels, respectively.  
<sup>†</sup> Wavelengths determined by sensitivity analysis (see Fig. 2).  
<sup>‡</sup> NPCI is the normalized total pigment to chlorophyll *a* ration index.  
<sup>§</sup> WBI is the water band index.

These data are a first step toward the goal of using remote sensing technology to analyze reflected solar radiation from crop canopies to search for insect outbreaks. Because the crop canopy complexity increases under field conditions, we can expect that the magnitude of the spectral responses attributed to aphid feeding damage as outlined above to be attenuated by leaf shadowing, radiant energy absorption by soils, and increased scattering of light. Other crop stresses (such as disease, nutrient deficiency, or drought stress) that result in leaf chlorosis, mottling, necrosis, or leaf temperature changes can also have profound effects on crop canopy reflectance spectra (Gates, 1970; Carter et al., 1992, 1996; Malthus and Madeira, 1993). Thus, the spectral relationships identified in laboratory studies may be very difficult to discern under field conditions at the canopy level (Verbyla, 1995). The successes that others have had in detecting aphid-borne disease and insect infestations (Blakeman, 1990; Everitt et al., 1994) demonstrate the feasibility of using remote sensing to detect insect damage at the field scale. Because the potential economic and environmental benefits of more efficient insecticide applications and the success of this laboratory study in identifying wavelengths sensitive to aphid damage, additional studies under field conditions are warranted.

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