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Research Article

Reflectance Signatures Developed from Multi-Spectral Imaging of Peanut (*Arachis hypogaea L.*) Expressing Visible Herbicide Injury

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Abstract

Multi-spectral imaging can be used to define a number of abiotic and biotic stresses associated with crop production and management. Research was conducted during 2004 and 2005 to develop spectral signatures of peanut leaves expressing visual symptoms of herbicide injury. Single leaf measurements using an artificial light source were used to develop spectra ranging from 350 to 2500 nm grouped into specific categories. Reflectance was determined for plants treated with acifluorfen, bentazon, clethodim, imazapic, paraquat, or 2,4-DB at 3 and 24 h and 3 and 6 d after application. Reflectance following herbicide applications was significant at bands 470-500 nm, 500-590 nm, 800 nm, and UMIR, at 3 and 24h after treatment and for bands 470-500 nm, 500-590 nm, 680-700 nm, 800 nm, and UMIR at 3 and 6d after treatment. Differences in reflectance were observed when comparing herbicides with different sites of action, especially when measurements were taken 3 and 6 d after treatment. These results indicate that there is potential for multi-spectral imaging to be used to discriminate among herbicides with different sites of action.

Introduction

Peanut *(Arachis hypogea L.)* is an economically important crop in North Carolina and the southern region of the United States. Managing peanut and making decisions to implement production and pest management practices can be challenging. While the possibility of using remote sensing technology with hyperspectral or multispectral imaging to follow crop stress has been addressed in some crops, research with peanut is limited. Research from a number of agricultural studies indicates that reflectance of crops is influenced at certain wavelengths by a variety of environmental factors. Some of these include stress due to weeds, water, and nutrient deficiencies or excesses [13-18,31]. For example, plant leaves with higher nitrogen (N) content have greater spectral reflectance in certain blue (450-500 nm) and near infrared (NIR) wavebands (700-940 nm) [30]. Taylor et al. [33] demonstrated that spectral measurements in the red (650-700 nm), green (500-550 nm), and NIR ranges could be used to estimate forage yield in bermudagrass [*Cynodon dactylon (L.)* Pers.]. Other research has focused on crops infested with fungal pathogens, where the red, green, and NIR bands were used to help characterize biophysical features of diseased and healthy plants [1,36].

Chlorophyll concentration, which is an indicator of nutritional stress, photosynthetic capacity, and senescence can be detected using red-shoulder (800 nm) or red-edge (690-750 nm) reflectance [4]. This region characterizes the boundary between dominance by the strong absorption of red light by chlorophyll and the high scattered radiation in the leaf mesophyll. Fluorescence occurs when red and far-red light is emitted from green plants in response to excess stimulation by photosynthetically active radiation. Changes in chlorophyll function often precede changes in chlorophyll content so that it is possible to observe changes in the red-edge reflectance before chlorosis may be visually observed in the leaves [32]. The red-edge region has also been tested as an index of plant stress [32]. Nutter and Littrell [25] examined peanut defoliation caused by late leaf spot *Cercosporidium personatum* (Berk. & M. A. Curtis), using the red-shoulder region of the spectrum. They reported significant reflective differences between the healthy green foliage and the diseased foliage. Aquino et al. [2] found that canopy reflectance, also at the 800 nm band in peanut affected by *C. personatum* decreased as disease severity and defoliation increased throughout the season. Spectral reflectance has been used to examine specific changes of leaf reflectance due to nutrient deficiencies. Masoni et al. [19] used spectral properties from barley *(Hordeum vulgare L.)*, wheat (Triticum aestivum L.), and sunflower *(Helianthus annuus L.)* leaves to detect iron, sulfur, magnesium, and manganese deficiencies. Graeff et al. [8] reported that the wavelength ranges of 380-390 nm, 430-780 nm, 516-780 nm, and 540-600 nm provided good prediction of nitrogen, phosphorus, magnesium, and iron status of corn *(Zea mays L.)* because reflectance changed when levels of the nutrients were low. Spectral measurements were also useful for establishing critical levels for manganese, zinc, iron, and copper under controlled conditions. However, they reported that iron and zinc deficiencies were not discriminated very well.

Nitrogen content has been studied frequently with respect to spectral properties of crops. Bands also in the green region of the visible spectrum have also been reported to be relatively important in determining turf quality in selected turf grass species under drought stress [11]. In studies of sorghum [*Sorghum bicolor (L.)* Moench], Zhao et al. [37] reported that nitrogen-deficit stress mainly increased leaf reflectance at 555 nm and 715 nm and caused a red-edge shift to shorter wavelength. Similar research conducted in corn found that the ratio of light reflectance between 550 and 600 nm to light reflectance between 800 and 900 nm

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also provided sensitive detection of nitrogen stress [5]. In an effort to maximize nitrogen application in winter wheat *(Triticum aestivum L.)*, Flowers et al. [7] investigated the relationship of tiller density to spectral indices and individual NIR, green, red, and blue bands, and reported that tiller density was consistently positively correlated with the NIR region. Osborne et al. [26] reported that the green waveband and normalized difference greenness vegetation index had the greatest ability to estimate grain yield in the presence of varying nitrogen and/or drought stresses. Spectral measures sensitive specifically to water stress have been developed and are capable of separating drought stressed leaves from nitrogen stressed and control leaves [27,28].

In soybean [*Glycine max (L.)* Merr.], herbicide and herbicide rate had a significant effect on normalized differential vegetation indices (NDVI) values derived from a multispectral ground-based radiometer in four of six site-years [35]. In addition, Nelson and Renner [21] reported an increase in the red to far-red light reflectance ratio in soybean treated with lactofen when compared with glyphosate and bentazon. Reflectance has also been shown to vary with leaf disease in some crops [20,29]. Malthus and Madeira [18] were able to correlate specific wavelengths with increased incidence of chocolate spot (Botrytis fabae Sard.) infection in field bean (Vicia faba L.). Newton et al. [22] reported that reflectance measurements could be used to aid in accurate estimation of disease and yield response to fungicide in barley *(Hordeum vulgare L.)*. However, cultivar and developmental stage had a large influence on measurements. Nutter et al. [24] used a handheld multispectral radiometer to compare visual and spectral assessments of foliar diseases as a means of measuring fungicide efficacy in peanut. In all but one case, they were able to determine that percent reflectance increased linearly as disease incidence and severity decreased. Reflectance measurements were correlated more closely with pod yield than the traditional visual assessments leading the researchers to suggest that reflectance measurements from peanut canopies can provide more accurate means to evaluate fungicide efficacy. Certain nutrients reveal a unique spectral signature and remote sensing can be used to help detect these deficiencies [6,13,23]. Serrano et al. [30] determined that nitrogen fertilization promoted significant increases in plant growth and, to a lesser extent, in radiation use efficiency. To successfully implement remote sensing technology, reflectance signatures for a variety of stresses are necessary along with accurate ground checking to define signatures and accompanying peanut injury from foliar applied herbicides. Therefore, the objective of this research was to develop signatures at various intervals after herbicide representing six sites of action were applied using a portable spectroradiometer with an internal light source for individual leaves.

Materials and Methods

Leaf reflectance in the form of radiant energy was taken as close to solar noon as possible, with a cloudless sky, using a portable spectroradiometer (ASD "FieldSpec Pro", Analytical Spectral Devices, Inc., Bolder, CO), and an ASD Leaf Clip (Analytical Spectral Devices, Inc., Bolder, CO). Leaf measurements were taken using an internal light source found within the leaf clip. Five individual leaves per plot were measured nondestructively, for five consecutive readings, for a total of fifty reflectance measurements per plot. Individual bandwidths from 350 to 2500 nm were averaged for each leaf reading, and those readings were then averaged to get five individual readings at each bandwidth for an individual plot. Bandwidths were combined into eleven bandwidth groupings based on their location in the spectrum (350-399, 400-449, 470-500, 500-590, 590-700, 700-760, 800, 950-999, 1000-1049, 1550-1750, and 2000-2400 nm) for analysis [3] (Table 1). Because broad bands (from 10 to 70 nm in width) in the visible (400-760 nm) and near-infrared (NIR) (800-1049 nm) regions of the spectrum have been demonstrated to be optimum for estimating crop biophysical information [35], bands within this region were used for analysis, as were bands in the mid infrared (MIR) (1550-1750 nm) and upper middle infrared (UMIR) (2000-2400 nm). Portions of the spectrum coincide with water absorption bands [mid-infrared (1350-1450 nm) and far-infrared (1800-1950 nm)], which obscure reflectance measurements, and were not used for this analysis [10]. Bandwidths were subjected to statistical analyses based on the bandwidth groupings. The only spectral index used was the NDVI, which was calculated as:

$$\left[NDVI = \left(near\ infrared - red\right) / \left(near\ infrared + red\right)\right]$$

The NDVI, a number between -1 and +1, quantifies the relative difference between the near infrared reflectance 'peak' and red reflectance 'dip' in the spectral signature. The experimental design was randomized complete block in all experiments. Pooled data were subjected to analysis of variance (SAS Systems Software, Cary, NC). Means were separated using Fisher's Protected LSD test at $p < 0.05$. The experiment was conducted in North Carolina at the Peanut Belt Research Station located near Lewiston-Woodville in two separate fields during 2005. Soil was a Norfolk sandy loam. Plot size was two rows spaced 91cm apart by 6m. The peanut cultivar Gregory was seeded in conventionally prepared seedbeds to establish an in-row density of 13 plants/m.

Table 1: Corresponding wavelength characterization for hyperspectral bandwidths used to compare canopy reflectance.

Bandwidth nm	Spectrum characterization[a]
350-399	Ultraviolet
400-449	Violet
470-499	Blue
500-590	Green
590-680	Yellow/Orange
680-700	Red
700-760	Red-Edge
800-1099	Near Infrared (NIR)
1550-1750	Mid Infrared (MIR)
2000-2400	Upper Mid Infrared (UMIR)

Treatments consisted of postemergence application of acifluorfen at 0.44 kg ai/ha, bentazon at 1.12 kg ai/ha, clethodim at 0.14 kg ai/ha, imazapic at 70 g ai/ha, paraquat at 0.14 kg ai/ha, and 2,4-DB at 0.28 kg ai/ha applied to peanut with a diameter of 30 cm in late June. These herbicides represent six differing mechanisms of action [12]. A nonionic surfactant (Induce, Helena Chemical Corp., Memphis, TN) at 0.25% (v/v) was applied with acifluorfen, bentazon, imazapic, and paraquat. A crop oil concentrate (Agri-Dex, Helena Chemical Corp., Memphis, TN) at 1.0% (v/v) was applied with clethodim. Adjuvant was not applied with 2,4-DB. Herbicides were applied using a CO_2-pressurized backpack sprayer calibrated to deliver 140L/ha using regular flat fan nozzles (Spraying Systems Co., Wheaton, IL). Reflectance was recorded 3 and 24h after treatment (HAT) and 3 and 6 d after treatment (DAT) using the ASD Leaf Clip assembly described previously.

Results and Discussion

Significant differences in reflectance were noted at bands 470-500 nm, 500-590 nm, 800 nm, and UMIR when recorded 3 and 24 HAT (Table 2). Reflectance differed when 2,4-DB was applied for bands 470-500, 500-590, and 800 nm when compared with reflectance following application of all other herbicides (Table 3). Although 2,4-DB can affect peanut foliage within several hours after application, the effect is transient and is generally not observed several days after application. In the UMIR region, paraquat and bentazon displayed the highest reflectance. Paraquat affects the membrane integrity of sensitive plants, and higher reflectance in the infrared range following application of paraquat may have resulted from membrane damage and adverse effects on cellular integrity [9].

Table 2: Analyses of variance for reflectance on peanut treated with acifluorfen, bentazon, clethodim imazapic, or paraquat, or 2,4-DB at 3 and 24 h and 3 and 6 days after herbicide application during 2005[a,b].

Bandwidth groups nm	Timing after Herbicide Application			
	3 h	24 h	3 d	6 d
	p-value			
350-399	0.2736	0.2257	0.0941	0.2355
400-450	0.2601	0.2164	0.2115	0.2489
470-500	0.0001	0.0001	0.0001	0.0001
500-590	0.0001	0.0001	0.0001	0.0309
680-700	0.0463	0.0623	0.0208	0.0011
700-760	0.1726	0.1295	0.1941	0.25
800	0.0022	0.0301	0.01	0.0065
950-999	0.3483	0.1923	0.7091	0.1543
1000-1049	0.1603	0.3971	0.231	0.6124
MIR	0.103	0.396	0.0001	0.0725
UMIR	0.0001	0.0001	0.522	0.024
NDVI	0.4133	0.0524	0.0641	0.2251

[a]Abbreviations: MIR, mid infrared; UMIR, upper mid infrared; NDVI, normalized

difference vegetation index.
[b*]indicates significance when comparing means within a bandwidth category at $p < 0.05$. Data are pooled over two experiments.

Table 3: Differences in magnitude of reflectance for peanut leaves treated with acifluorfen, bentazon, clethodim imazapic, paraquat, or 2,4-DB at 3 hours after application for experiments during 2005[a,b].

Herbicide	Reflectance			
	Bandwidth category (nm)			
	470-500	500-590	800	UMIR
	relative units			
No herbicide	2.1 b	15.1 b	6.8 d	49.5 c
Acifluorfen	2.0 b	12.4 b	11.0 bc	49.0 c
Bentazon	2.0 b	14.8 b	13.6 b	51.7 ab
Clethodim	2.2 b	14.2 b	6.3 d	49.0 c
Imazapic	2.2 b	14.9 b	6.0 d	50.2 bc
Paraquat	2.3 b	14.1 b	10.8 c	53.1 a
2,4-DB	5.9 a	24.4 a	24.2 a	43.5 d

[a] Abbreviations: UMIR, upper mid infrared.
[b] Means within a bandwidth category followed by the same letter are not significantly different according to Fisher's Protected LSD test at $p < 0.05$. Data are pooled over two experiments.

Reflectance 24 HAT was higher following application of acifluorfen, imazapic, and 2,4-DB in the 470-500 nm range (Table 4). At 500-590 nm, reflectance was different following application of acifluorfen, imapic, and paraquat to the no-herbicide control and application of bentazon, clethodim, and 2,4-DB. In the infrared range, paraquat had the highest reflectance, and reflectance following 2,4-DB was lower than reflectance following application of the other herbicides. In the UMIR range, reflectance of only acifluorfen was higher than the other treatments. Throughout the 24h period, reflectance following clethodim and the no-herbicide control was similar. Peanut is able to metabolize clethodim rapidly into non-phototoxic metabolites that prevent injury with no effects on cellular function and most likely would result in no difference in reflectance [34]. Additionally, seldom does clethodim visually injury peanut and other dicotyledonous crops. It was not surprising that reflectance following application of acifluorfen, bentazon, and paraquat differed because visible damage to leaves was expressed within several hours after application [9,12].

Table 4: Differences in magnitude of reflectance for peanut leaves treated with acifluorfen, bentazon, clethodim imazapic, paraquat, or 2,4-DB at 24 hours after application for experiments during 2005[a,b].

Herbicide	Reflectance			
	Bandwidth category (nm)			
	470-500	500-590	800	UMIR
	relative units			
No herbicide	2.1 b	15.1 b	6.8 d	49.5 c
Acifluorfen	2.0 b	12.4 b	11.0 bc	49.0 c
Bentazon	2.0 b	14.8 b	13.6 b	51.7 ab
Clethodim	2.2 b	14.2 b	6.3 d	49.0 c
Imazapic	2.2 b	14.9 b	6.0 d	50.2 bc
Paraquat	2.3 b	14.1 b	10.8 c	53.1 a
2,4-DB	5.9 a	24.4 a	24.2 a	43.5 d

[a] Abbreviations: UMIR, upper mid infrared.
[b] Means within a bandwidth category followed by the same letter are not significantly different according to Fisher's Protected LSD test at $p < 0.05$. Data are pooled over experiments.

When reflectance was determined 3 and 6 DAT, differences in reflection were noted at bandwidth categories 470-500 nm, 500-590 nm, 680-700 nm, 700-760 nm, and 800 nm and UMIR (Table 2). When recorded 3 DAT, reflectance differed for bands 470-500 nm when 2,4-DB was applied compared to all other herbicides (Table 5). At the bandwidth category 500-590 nm, reflectance following imazapic did not differ from reflectance following 2,4-DB, and at 680-700 nm, reflectance following acifluorfen and imazapic did not differ from 2,4-DB. Reflectance from leaves following application of clethodim was not different from the no-herbicide control except at 800 nm. At this bandwidth, reflectance following application of acifluorfen and imazapic was different from the reflectance of following bentazon and 2,4-DB. However, reflectance following application of bentazon, clethodim, and 2,4-DB did not differ from the no-herbicide control. In the UMIR region, leaf reflectance was highest in plants following the application of bentazon and imazapic (Table 5). Visually, the plants treated with bentazon were somewhat chlorotic, and this likely affected reflectance in the leaves.

Table 5: Differences in magnitude of reflectance for peanut leaves treated with 2,4-DB, acifluorfen, bentazon, clethodim imazapic, or paraquat at 3 days after application for experiments during 2005[a,b].

Herbicide	Reflectance				
	Bandwidth category (nm)				
	470-500	500-590	680-700	800	UMIR
	Relative Units				
No herbicide	1.1 c	9.7 c	22.2 c	32.0 bc	46.8 cd
Acifluorfen	3.1 b	11.9 b	31.8 a	34.9 a	55.1 bc
Bentazon	2.6 b	8.3 d	18.8 d	29.7 c	62.5 a
Clethodim	1.5 c	9.4 c	24.0 c	33.4 a	45.0 d
Imazapic	3.0 b	12.7 ab	33.4 a	32.8 ab	58.8 ab
Paraquat	2.7 b	11.6 b	28.3 b	30.4 bc	49.9 c
2,4-DB	4.3 a	13.4 a	32.1 a	29.6 c	51.8 c

[a] Abbreviations: UMIR, upper mid infrared.
[b] Means within a bandwidth category followed by the same letter are not significantly different according to Fisher's Protected LSD test at $p < 0.05$. Data are pooled over two experiments.

Reflectance 6 DAT application was significant for the bandwidth categories 470-500, 500-590, 680-700, and 800 nm and UMIR. The highest reflectance was noted following bentazon, imazapic, and 2,4-DB in the 470-500 nm range (Table 6). Reflectance was highest at 500-590 nm following acifluorfen and 2,4-DB. Reflectance was highest at 500-590 nm. At 680-700 nm, reflectance was higher after applications of 2,4-DB compared to the other herbicides. Reflectance following application of acifluorfen, bentazon, clethodim, and paraquat did not differ from the no-herbicide control. Reflectance of leaves following application of bentazon and imazapic was highest at 800 nm, and reflectance of leaves following application of 2,4-DB was highest at UMIR range.

Table 6: Differences in magnitude of reflectance for peanut leaves treated with acifluorfen, bentazon, clethodim imazapic, paraquat, or 2,4-DB at 6 days after application for experiments during 2005[a,b].

Herbicide	Reflectance				
	Bandwidth category (nm)				
	470-500	500-590	680-700	800	UMIR
	relative units				
No herbicide	1.1 c	9.7 c	22.2 c	32.0 bc	46.8 cd
Acifluorfen	3.1 b	11.9 b	31.8 a	34.9 a	55.1 bc
Bentazon	2.6 b	8.3 d	18.8 d	29.7 c	62.5 a
Clethodim	1.5 c	9.4 c	24.0 c	33.4 a	45.0 d
Imazapic	3.0 b	12.7 ab	33.4 a	32.8 ab	58.8 ab
Paraquat	2.7 b	11.6 b	28.3 b	30.4 bc	49.9 c
2,4-DB	4.3 a	13.4 a	32.1 a	29.6 c	51.8 c

[a] Abbreviations: UMIR, upper mid infrared.
[b] Means within a bandwidth category followed by the same letter are not significantly different according to Fisher's Protected LSD test at $p < 0.05$. Data are pooled over two experiments.

The objective of this research was to develop multi-spectral signatures at various intervals after herbicide application for herbicides representing six sites of action. There

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was a consistent trend for significance in reflectance among herbicides across recording dates for bandwidths 470-500 nm, 500-590 nm, and 800 nm. However, when comparing the magnitude of difference in reflectance, variation was noted among herbicides. Although inconsistent responses suggest that developing individual signatures for herbicides may prove difficult, there is potential for multi-spectral imaging to be used to differentiate injury from herbicides with different sites of action.

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