Computers and Electronics in Agriculture 77 (2011) 204–213

Contents lists available at ScienceDirect

Computers and Electronics in Agriculture

journal homepage: www.elsevier.com/locate/compag

Determination of differences in crop injury from aerial application of glyphosate using vegetation indices

B.V. Ortiz [a,*], S.J. Thomson [b], Y. Huang [b], K.N. Reddy [a], W. Ding [c]

[a] Agronomy and Soils Department, Auburn University, AL, United States
[b] United States Department of Agriculture, Agricultural Research Service, Crop Production Systems Research Unit, Stoneville, MS, United States
[c] Agronomy College, Northeast Agricultural University, Harbin, Heilongjiang, China

ARTICLE INFO

Article history:
Received 8 September 2010
Received in revised form 11 April 2011
Accepted 10 May 2011

Keywords:
Canonical correlation analysis
Glyphosate
Semivariogram
Remote sensing
Vegetation Indices

ABSTRACT

Crop injury caused by off-target drift of herbicide can seriously reduce growth and yield and is of great concern to farmers and aerial applicators. Farmers can benefit from identifying an indirect method for assessing the level of crop injury. This study evaluates the combined use of statistical methods and vegetation indices (VIs) derived from multispectral images to assess the level of crop injury. An experiment was conducted in 2009 to determine glyphosate injury differences among the cotton, corn, and soybean crops. The crops were planted in eight rows spaced 102 cm apart and 80 m long with four replications. Seven VIs were calculated from multispectral images collected at 7 and 21 days after the glyphosate application (DAA). At each image collection date, visual injury estimates were assessed and data were collected for plant height, chlorophyll content, and shoot dry weight. From the seven VIs evaluated as surrogate for glyphosate injury identification using a canonical correlation analysis (CCA), the Chlorophyll Vegetation Index (CVI) showed the highest correlation with field-measured plant injury data. CVI image values were subtracted from the CVI average values of the non-injured area to generate CVI residual images (CVI_{res}). Frequency distribution histograms of CVI_{res} image values were calculated to assess the level of injury between crops. These data suggested that injury increased from 7DAA to 21DAA with corn exhibiting higher severity of injury than cotton or soybean, while only moderate injury was observed for cotton. The techniques evaluated in this study are promising for estimating the level of glyphosate herbicide drift, which can be used to make appropriate management decisions considering crop proximity.

1. Introduction

Properly managing aerial herbicide applications is key to minimizing off-target drift that can cause crop injury. The increased use of glyphosate, a non-selective herbicide, as a burndown herbicide for no-till soybean and post applications in glyphosate-resistant transgenic crops elevates the risk for injury. Frequent claims received by insurance companies are related to herbicide-induced injury; however, farmers face difficulty assessing the percentage of acres impacted by drift and the degree of injury. Pringnitz (1999) found that between 1998 and 1999 in Iowa, insurance complaints related with herbicide off-target drift increased by 280%. In Mississippi, 145 cases of drift were reported in 2000 and 2001 (Henry et al., 2004).

Spray drift of glyphosate after foliar applications could have severe impact on crops if the drift dosage exceeds the target tolerance. Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase in the shikimate pathway resulting in depletion of aromatic amino acids essential for plant growth (Amrhein et al., 1980). Besides growth reduction, glyphosate injury may cause chlorosis at the newest growing points, necrosis throughout the plant within 1–2 weeks after application, yield reduction, or complete destruction of a susceptible crop (Henry et al., 2004).

Crops often affected by off-target drift of glyphosate including corn (Buehring et al., 2007; Brown et al., 2009), soybean (Bellaloui et al., 2006), and rice (Ellis et al., 2003) have been targets for several injury identification methods. Rowland (2000) found that stand height was the best parameter to identify the degree of glyphosate injury in corn. Remote sensing data and derived vegetation indices (VIs), which are mathematical transformations of spectral reflectance commonly used to indirectly assess differences in growth and chlorophyll content of several crops, disease severity, and nutrient and water deficiency have been also used to determine injury caused by herbicide drift. Comparing herbicide injury of soybean and corn, Henry et al. (2004) distinguished healthy and injured plants using hyperspectral data and several VIs. Thelen et al. (2004) found significant differences among herbicides and herbicide rates by calculating the Normalized Difference

* Corresponding author. Address: 204 Extension Hall – Auburn University, United States. Tel.: +1 334 844 5534; fax: +1 334 844 4586.
E-mail address: bortiz@auburn.edu (B.V. Ortiz).

0168-1699/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.compag.2011.05.004

Vegetation Index (NDVI) from digital aerial images of soybean. Tamhankar et al. (2002) investigated the effect of glyphosate (Roundup) and N,N′-dimethyl-4,4′-bipyridinium dichloride (Paraquat) on the spectral reflectance of corn and soybean as a first approach to develop an automated protocol for herbicide drift detection.

A challenge for detecting herbicide injury by remote sensing is to identify techniques to enhance detection of within-field spatial variability for determination of the level of crop injury. Therefore, in this study we adopted the use of VIs to verify the hypothesis that VIs can be used as surrogate data to identify differences in crop injury levels. To test this hypothesis, a canonical correlation analysis (CCA) was used to identify VIs best correlating with field-measured crop injury. CCA has been used extensively in soil and plant sciences (Noe and Barker, 1985; Dieleman et al., 2000; Martin et al., 2005). The CCA has the ability to analyze correlations between two groups of variables by assessing the correlation between the linear combinations of one group of variables with the linear combinations of the second group of variables (Gittins, 1985; Johnson and Wichern, 2002).

Objectives of this research were to determine if vegetation indices derived from multispectral images acquired with an airborne multispectral camera could identify crop injury by off-target drift of glyphosate and to investigate how the vegetation index images can be used for identification of crop injury levels.

2. Materials and methods

2.1. Study field and experimental plan

Crop injury and biological responses of three row crops (cotton, corn, and soybean) following glyphosate drift from an aerial application were evaluated in an experiment conducted during summer 2009 (Huang et al, 2010). The study field was located at the research farms of the US Department of Agriculture-Agricultural Research Service in Stoneville, Mississippi (33°26′ N, 90°55′ W). Cotton (non-glyphosate resistant (GR) cotton cultivar ‘FM955LL’-100,000 seed ha^{-1}), corn (non-GR corn hybrid ‘Pioneer 31P41’-75,000 seed ha^{-1}), and soybean (non-GR soybean cultivar ‘SO80120LL’-285,000 seed ha^{-1}) were planted on July 23, 2009 in four replications of 8-row by 80 m plots; each one spaced 102 cm (Fig. 1).

A single aerial application of glyphosate was made on August 12, 2009 when cotton was at two- to three-leaf stage, corn was at four-leaf stage, and soybean was at two- to three-trifoliolate leaf stage. The glyphosate was applied using an Air Tractor 402B airplane equipped with 54 CP-09 spray nozzles (CP Products, Tempe, Arizona) set to a 5° deflection angle. This is a standard setting but one that is slightly more prone to enhance the potential for off-target drift (Thomson, 2008). The aircraft and application system were adjusted to deliver the liquid at the rate of 46.8 L ha^{-1}. Spray release height was set at 3.7 m and ground speed was set to 225 km h^{-1} over an 18.3 m wide spray swath. The sprayed liquid was a glyphosate solution of Roundup Weathermax® (Monsanto Co., St. Louis, Missouri) applied at a rate of 866 g of active ingredient (a.i.) ha^{-1}. The airplane travelled in a west-to-east direction across the center of the study field perpendicular to the crop rows over a marked swath line (Fig. 1). On-site weather conditions were recorded during the 4 s flight. The average wind speed was 11.2 km h^{-1} from the northeast direction at an average of 64° from the North. Average air temperature was 28.5 °C and relative humidity was 72% as acquired during the spray run using a tripod mounted Kestrel 4500 weather tracker (Nielsen-Kellerman, Boothwyn, PA).

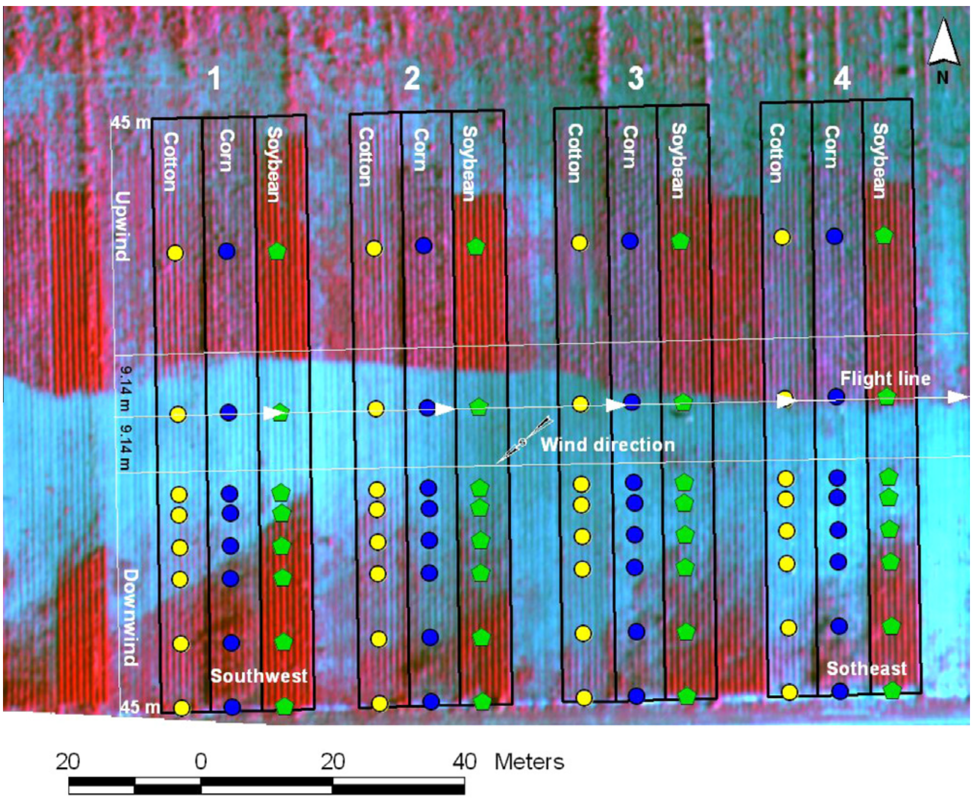

Fig. 1. Experimental layout for the spray test (On the false color composition of image collected 21 DAA, the dots denote plant sampling locations for cotton, corn, and soybean).

2.2. Biomass measurements

Plant sampling locations by crop and replication were established downwind at 0, 12, 15, 20, 25, 35, and 45 m from the center of the spray swath (18.3 m size) (Fig. 1). One upwind sample location at 35 m from the north edge of the 18.3 m spray swath was included as a control (crops not exposed to glyphosate) of crops' biological responses to drift (Fig. 1).

Data of percentage plant injury, plant height, chlorophyll content, and shoot dry weight were collected from all eight rows in a 0.5-m-wide band centered over the sampling location except at 0 m. For the 0 m downwind sampling location, data were collected from an area of eight rows by the 18.3 m spray swath. The sampling location at 0 m downwind represented the highest exposure to glyphosate, while the 35 m upwind sampling location represented no glyphosate exposure. Visual injury ratings were based on chlorosis, necrosis, stunted growth, and plant death and the rating scale was assigned on a scale of 0–100, with 100 representing total plant mortality and 0 representing no injury. Plant height values were determined from the average of five plants randomly selected within the sampling area at each location. Chlorophyll content was determined from three of the youngest fully expanded leaves from three randomly selected plants. Chlorophyll was extracted with 10 mL dimethyl sulfoxide and quantified spectrophotometrically (Hiscox and Israelstam, 1979). Shoot dry weight was calculated from ten plants selected from the sampling area, which were oven dried (60 °C, 72 h).

2.3. Aerial multispectral imaging and vegetation indices

Multispectral images were collected from the Air Tractor 402B airplane using a MS 4100 camera (Geospatial Systems, Inc., West Henrietta, New York). This multi-spectral camera uses three CCD (Charge Coupled Device) sensors, to acquire images in 3–5 spectral bands within the 400–1100 nm range of the electromagnetic spectrum and provides a digital imaging resolution of 1920 (horizontal) × 1080 (vertical) pixel array per sensor and 60° field of view when fitted with 14 mm, f/2.8 lens. The camera provides composite color images and individual color plane images that approximate Landsat Satellite Thematic Mapper bands (NASA, Washington, DC; USGS, Reston, Va.). The MS 4100 camera configures the digital output of image data with CameraLink standard or parallel digital data in either EIA-644 or RS-422 differential format. The camera works with the NI IMAQ PCI-1424/1428 frame-grabber (National Instruments, Austin, Texas). With the DTControl-FG (Geospatial Systems, Inc) software and the CameraLink configuration, the camera system acquires images from the frame-grabber directly from within the DTControl program. For this experiment, the camera was configured to acquire color infrared (CIR) images by using 3-bands: green (500 nm with 40 nm bandwidth), red (670 nm with 40 nm bandwidth), and near infrared (NIR) (800 nm with 60 nm bandwidth).

Multispectral images with a spatial resolution of 11 × 20 cm pixel size were collected 1, 7, 14, and 21 days after the glyphosate application (DAA). They were geo-corrected using data from USDA NAIP (National Agriculture Imagery Program) imagery, which are ortho-rectified, true color, 1 m resolution products. For this study, only results from the images collected 7DAA and 21DAA are presented because those should represent the lowest and highest extent of injury expected from the set of four images. The 1DAA image was not selected for this study mainly because: (1) biological and spectral changes in the crop are not always evident immediately after drift deposition of glyphosate, (2) spectral reflectance from the canopy might be confounded with reflectance from the soil making reliable discrimination of crop damage difficult, and (3) glyphosate injury is not visible within 1DAA.

Seven vegetation indices (VIs) were calculated from the digital values (DNs, 0–255) of the multispectral aerial images (Table 1). The transformation of image-based spectral reflectance into VIs is usually used to enhance features on an image and minimize differences in spectral response associated with shadow, atmosphere, canopy background, surface roughness, changes on illumination and soil background (Sullivan et al., 2004). For example, the Normalized Difference Vegetation Index (NDVI) is commonly used to assess chlorophyll and biomass vegetation differences. This index is based on the principle that healthy plants with high chlorophyll content absorb red light and reflect energy in the near infrared (NIR) which results in high NDVI values (Rouse et al. 1973; Tucker 1979). Conversely, as chlorophyll declines so does the plant health and the plant's ability to absorb red light and reflect NIR resulting in low NDVI values. The disadvantage of NDVI is that it saturates in a dense and multi-layered canopy and it does not show a linear correlation with leaf area index (LAI) (Baret and Guyot, 1991). The indices GVI, GNDVI, CVI, and MSR relate to leaf chlorophyll changes (Bronson et al., 2003; Vincini et al. 2008; Haboudane et al., 2004). RVI, and IPVI have exhibited good correlation with cotton biomass (Bronson et al., 2003), corn biomass and drought stress (Bahrun et al., 2003), soybean plant height (Batista and Rudorff, 1990), and grass biomass (Payero et al., 2004). Based on diverse uses of VIs and their responsiveness, seven VIs were investigated to determine the best relationship with field-measured glyphosate injury and to use them to determine the level of glyphosate injury.

Table 1
Vegetation indices evaluated for assessment of glyphosate injury on cotton, corn, and soybean.

Vegetation Index (VI)	Formula	Reference
Normalized Difference Vegetation Index (NDVI)	(NIR − Red)/(NIR + Red)	Rouse et al. (1973), Tucker (1979)
Green Normalized Difference Vegetation (GNDVI)	(NIR − Green)/(NIR + Green)	Gitelson et al. (1996)
Simple Ratio Index (RVI)	NIR/Red	Jordan (1969)
Green Vegetation Index (GVI)	NIR/Green	Bausch and Duke (1996)
Chlorophyll Vegetation Index (CVI)	(NIR/Green)∗(Red/Green)	Vincini et al. (2008)
Modified Simple Ratio (MSR)	$(NIR/Red - 1)/((NIR/Red) + 1)^{1/2}$	Chen (1996)
Infrared Percentage Vegetation Index (IPVI)	NIR/(NIR + Red)	Crippen (1990)

2.4. Data processing and statistical analysis

2.4.1. Standaridzed semivariograms of vegetation index images

Subsets of the 7DAA and 21DAA images corresponding to each crop and replication were extracted and analyzed individually (4 individual images per crop – 12 images total). Data from each vegetation index (VI) image were rescaled to unit variance by dividing each pixel value by the VI image standard deviation (e.g., NDVI pixel$_{i(column)j(row)}$/NDVI standard deviation). This procedure ensured that computations of experimental semivariograms calculated from each vegetation index/crop/replication were standardized to unit sill (Van Meirvenne and Goovaerts, 2002; Kerry and Oliver, 2008). The semivariogram, core of geostatistical analyses, has been used to describe spatial patterns in terms of the dissimilarity of observations as a function of the separation distance (Goovaerts, 1998) which include the spatial dependence in remotely sensed images (Atkinson and Lewis, 2000; Goodin and Henebry,1998).

Following exploratory data analyses, a total of 24 (3 crops × 4 replications × 2 dates) omnidirectional semivariograms per VI were computed for 80 lags with 0.56 m lag distance using the usual computing equation (Webster and Oliver, 2001). The best semi-variogram model for each variable was chosen based on the minimum residual sum of squares for the fit (Isaacs and Srivastava, 1989). Ordinary punctual kriging was used to estimate the VI values at each plant sampling location (Kerry and Oliver, 2003) using TerraSeer STIS software (Avruskin et al. 2004).

2.4.2. Canonical correlation analysis

Canonical correlation analysis (CCA) by crop and image collection date were conducted to identify the VIs strongly related to the field-measured glyphosate injury. CCA assesses the relationship between two sets of variables: Y variables (field-measured plant injury variables) and X variables (vegetation indices). Through this method it is possible to create a small number of new variables (pairs), where each component of the canonical variable pair, with the highest possible between-set correlations, is generated from the linear combination of the variables within each group of the original variables (Martin et al, 2005). The level of significance of the canonical correlation was assessed through the Wilkes-Lambda statistic. If $P < 0.05$, the pair of canonical variables was significantly associated by canonical correlation. The loadings, or correlations in the CCA, indicate the simple linear relationship between the original variables and the canonical variate. Variables having a high contribution to the canonical variate are those that exhibit large loadings to evaluate multivariate dependencies. Because one objective of this study is to identify VIs surrogate for crops glyphosate injury, an *interset* structure correlation (a measure of strength between the canonical variates of a measurement domain and the observed variables of the other domain), was considered. In this study, V_d was designated to represent a set of independent field-measured plant injury variables, and V_i was assigned to represent a set of dependent VIs.

2.4.3. Identification of the injury level

The results from the CCA indicated the vegetation indices best correlated with field-measured plant injury variables; then, those indices were used to identify the level of injury. For each index and crop/replication, residuals (*res*) values were obtained by calculating every VI image pixel value subtracted from pixel values of the upwind control area (average values within a circular area of 2.5 m radius from the upwind control sampling location). Frequency distribution histograms of residuals values were calculated to assess the level of injury between crops. Differences in percentage of pixels (positive or negative) within each frequency interval for each crop and image collection time were used to assess the progression of damage. A similar approach was implemented by Thelen et al. (2004) to distinguish herbicide rate effect on soybean. They calculated ΔNDVI, the difference between untreated and treated plots within an experimental block to detect treatment differences. Casadesús et al. (2007) used pixel values histograms to estimate areas associated with a specific feature on an image. They calculated the relative green area of digital images as the sum of frequencies of the histogram classes included in the Hue (Hue component from the color space intensity hue saturation) range from yellow (60°) to bluish green color (180°).

Analyses of variance, ANOVA, was conducted to evaluate pixel number differences by crop per each frequency interval and positive/negative frequency intervals by crop. Mean separation between variables was obtained by Tukey's and Waller-Duncan *K*-ratio significant difference tests ($P < 0.05$). A correlation analysis was conducted with data from sampling observations categorized by frequency interval of VIs images and field-measured injury variables. This analysis verified the strength of association between residual values and field-measured plant injury values.

3. Results and discussion

3.1. Canonical correlation analysis

For each crop, the CCA between the field-measured plant injury data and VIs calculated from the 7DAA and 21DAA images, resulted in two significant pairs ($P < 0.05$, Wilks' Lambda) of canonical variates (Table 2). At 7DAA, the canonical correlations between the first pair of canonical variates were 0.68, 0.84, and 0.89 for cotton, corn and soybean, respectively. Those between the second pair of canonical variates were 0.61–0.85 for the same crops, which suggest that glyphosate injury in crops could be assessed by VIs derived from multispectral aerial images. The data also showed that the standardized cumulative variance (SCV) was explained by their canonical variates; however, this varied by crop. The two significant canonical damage variates (V_d) explained 76%, 93%, and 83% of the total variance in field-measured plant injury data for cotton, corn, and soybean, respectively (Table 2). The canonical remote index variates (V_i) explained 36%, 75%, and 57% of the total variance in VIs for cotton, corn, and soybean, respectively.

At 21DAA, the SCV also varied by canonical variate and crop. The two significant canonical variates V_d explained 72%, 90%, and

Table 2
Canonical correlations, standardized cumulative variance and the canonical variates V_d and V_i generated through the CCA for Cotton, Corn and Soybean.

Sampling Date	Canonical variate	Cotton			Corn			Soybean		
		CC[a]		Pr > F	CC[a]		Pr > F	CC[a]		Pr > F
7DAA	1	0.678		0.0253	0.840		<0.0001	0.896		<0.0001
	2	0.611		0.0682	0.743		0.0091	0.846		<0.0001
	3	0.522		NS	0.466		NS	0.689		NS
	Wilks' Lambda		0.0253			<0.0001			<0.0001	
	SCV_d[b]		76			93			83	
	SCV_i[b]		36			75			57	
21DAA	1	0.892		0.0018	0.863		0.0002	0.908		<0.0001
	2	0.538		NS	0.702		0.0593	0.720		NS
	3	0.429		NS	0.576		NS	0.445		NS
	4	0.283		NS	0.228		NS	0.207		NS
	Wilks' Lambda		0.0018			0.0002			<0.0001	
	SCV_d[b]		72			90			93	
	SCV_i[b]		52			68			67	

V_d = set of independent field-measured plant injury variables.
V_i = set of dependent VIs.
[a] Canonical correlation.
[b] Standardized cumulative variance of the first two canonical variates ($P < 0.0001$).

93% of the total variance in field-measured plant injury variables for cotton, corn, and soybean, respectively. The total variance of the VIs explained by the canonical variate V_i was lower than the canonical variate V_d. The canonical variate V_i explained 52%, 68%, and 67% of the total variance of the VIs for cotton, corn, and soybean, respectively. The canonical correlations between the first pair of canonical variables were 0.89, 0.86, and 0.91 for cotton, corn, and soybean, respectively, and those between the second pair of canonical variables were 0.54–0.72 for the same crops. These values were similar to the ones obtained with the data collected 7DAA, except for cotton. These similar correlations also confirmed the relationship between field measured canopy variables and the VIs used as surrogate for crop injury.

3.2. Relationships between canonical variates and field-measured injury/VI

The highest correlation between canonical variates and the original variables, field-measured plant injury and VIs, was observed for the first pair of canonical variates (Table 2). Therefore, discussion of the results herein is focused on the *intraset* structure correlation coefficients, strength of the association between the original variable and the canonical variates, for the first pair of canonical variates. These correlation coefficients illustrate which original variables contribute most heavily to a canonical variate and the direction of the effect (Martin et al. 2005; Gittins, 1985). At 7DAA, the canonical variate Vd1 accounted for 24%, 52%, and 44% of the total variance of the field-measured plant injury variables for cotton, corn, and soybean, respectively (Table 3). Although there were considerable correlation differences between the field-measured plant injury variables and the canonical variate V_{d1}, percentage injury and dry matter exhibited the strongest correlations for all studied crops. The highest correlation was observed between V_{d1} and injury ($r = 0.93$) for cotton; dry matter ($r = -0.96$) and injury ($r = 0.79$) for corn; injury ($r = 0.99$) and dry matter ($r = -0.73$) for soybean. The *interset* correlation indicated that the strongest relationship between the canonical variate V_{d1} and the different VIs was observed for CVI and GVI; however, the degree of correlation varied by crop. For cotton, CVI ($r = 0.57$), GVI ($r = 0.52$), and GNDVI ($r = 0.48$) showed the best correlations with V_{d1}. The degree of correlation between CVI and V_{d1} increased to $r = 0.79$ for corn and $r = 0.78$ for soybean.

At 21DAA, the total variance percentage of the field-measured plant injury variables explained by the canonical variate V_{d1}

Table 3
Canonical correlations between the original variables and the canonical variate V_{di} generated through the CCA. Data collected seven days after the glyphosate application (7DAA).

Variable	Cotton		Corn		Soybean	
Correlation between the field-measured plant injury variables and the damage canonical variates						
	V_{d1}	V_{d2}	V_{d1}	V_{d2}	V_{d1}	V_{d2}
Injury	**0.93**	−0.36	**0.79**	**−0.59**	**0.99**	−0.15
Chlorophyll	−0.30	**0.91**	−0.73	0.55	−0.51	0.54
Dry matter	−0.03	**0.63**	**−0.96**	0.27	**−0.73**	**0.64**
SCV[a]	0.24	0.34	0.52	0.18	0.44	0.18
Correlation between the vegetation indices and the damage canonical variates[b]						
NDVI	−0.31	0.10	−0.10	0.58	−0.48	0.25
GNDVI	0.48	0.14	0.72	0.21	0.76	0.23
RVI	0.02	0.17	0.07	0.59	−0.33	**0.37**
GVI	0.52	0.10	**0.79**	0.11	**0.79**	0.02
CVI	**0.57**	0.05	**0.79**	−0.14	**0.78**	−0.17
MSR	−0.29	0.13	−0.19	**0.60**	−0.55	0.18
IPVI	0.18	0.14	0.40	0.46	0.13	0.55
SCV[a]	0.15	0.02	0.29	0.19	0.35	0.09

For each V_{di}, the highest correlation coefficient(s) is shown in bold format.
[a] Standardized cumulative variance.
[b] Interset structure correlation.

Table 4
nonical correlations between the original variables and the canonical variate V_{di} generated through the CCA. Data collected twenty one days after the glyphosate application (21DAA).

Variable	Cotton		Corn		Soybean	
Correlation between the field-measured plant injury variables and the damage canonical variates						
	V_{d1}	V_{d2}	V_{d1}	V_{d2}	V_{d1}	V_{d2}
Injury	**0.90**	−0.16	0.86	−0.27	**0.97**	0.12
Chlorophyll	−0.22	0.20	**−0.94**	0.28	−0.89	−0.28
Dry matter	**−0.97**	0.07	−0.93	0.04	−0.97	−0.01
Plant height	−0.85	**0.52**	**−0.96**	−0.16	**−0.98**	0.01
SCV[a]	0.63	0.09	0.85	0.04	0.91	0.02
Correlation between the vegetation indices and the damage canonical variates[b]						
NDVI	−0.47	**0.31**	−0.44	0.33	−0.72	0.04
GNDVI	0.64	0.01	0.71	0.21	0.77	−0.25
RVI	−0.38	0.30	−0.38	0.39	**−0.79**	0.08
GVI	**0.76**	−0.07	**0.82**	0.10	0.71	−0.11
CVI	**0.77**	−0.12	**0.86**	−0.03	**0.85**	−0.02
MSR	−0.26	−0.25	−0.54	0.32	−0.80	0.12
IPVI	0.07	0.23	0.01	0.48	−0.28	−0.19
SCV[a]	0.29	0.05	0.37	0.09	0.52	0.02

For each V_{di}, the highest correlation coefficient(s) is shown in bold format.
[a] Standardized cumulative variance.
[b] Interset structure correlation.

increased with respect to the analyses with the 7DAA data. The canonical variate V_{d1} accounted for 63%, 85%, and 91% of the total variance for cotton, corn, and soybean, respectively (Table 4). At this time of the growing season, most of the field-measured plant injury variables exhibited strong correlation with the canonical variate V_{d1}. For cotton, V_{d1} was an expression of dry matter ($r = -0.97$) and injury ($r = 0.90$). In a similar way, V_{d1} was an expression of plant height ($r = -0.96$) and chlorophyll ($r = -0.94$) for corn and plant height ($r = -0.98$) and injury ($r = -0.94$) for soybean. These strong correlations and high percentage of the total variance accounted for by the canonical variates V_{di} indicated that the VIs having strong correlation with V_{di}, especially V_{d1}, could be considered as surrogate for glyphosate plant injury.

Considerable differences existed in the strength of correlation of the VIs with the canonical variate V_{d1}; however, CVI, GVI, and GNDVI continued to exhibit the strongest correlation. CVI showed the strongest correlation for cotton ($r = 0.77$), corn ($r = 0.86$), and soybean ($r = 0.85$) from the set of VIs evaluated (Table 4). In contrast, IPVI exhibited the weakest correlation, 0.07, 0.01, and −0.28 for cotton, corn, and soybean, respectively. The strong correlation between CVI, GVI, and GNDVI and field-measured injury variables might be explained by the strong correlation of these indices with chlorophyll or leaf N content (Bronson et al., 2003; Vincini et al. 2008; Haboudane et al., 2004). The impact of glyphosate on crops has been documented as a reduction in chlorophyll content (Reddy et al., 2000; Zobiole et al., 2010), decrease in photosynthetic parameters like photosynthetic rate, transpiration and

Table 5
Correlation of chlorophyll content and percentage plant injury data with frequency intervals (injury classes) from CVI_{res} images.

Replication	Crop	*r* 7 DAA Chlorophyll	7 DAA Injury	21 DAA Chlorophyll	21 DAA Injury
1	Cotton	−0.21	0.60	−0.76	0.95
	Corn	−0.66	0.91	−0.86	0.74
	Soybean	−0.96	0.84	−0.92	0.94
2	Cotton	−0.29	0.45	−0.71	0.65
	Corn	−0.56	0.75	−0.77	0.61
	Soybean	−0.53	0.52	−0.88	0.82
3	Cotton	0.32	−0.58	−0.75	0.64
	Corn	−0.77	0.72	−0.86	0.89
	Soybean	−0.30	0.90	−0.80	0.87
4	Cotton	0.28	0.34	−0.14	0.33
	Corn	−0.34	0.42	−0.62	0.63
	Soybean	−0.97	0.57	−0.45	0.55

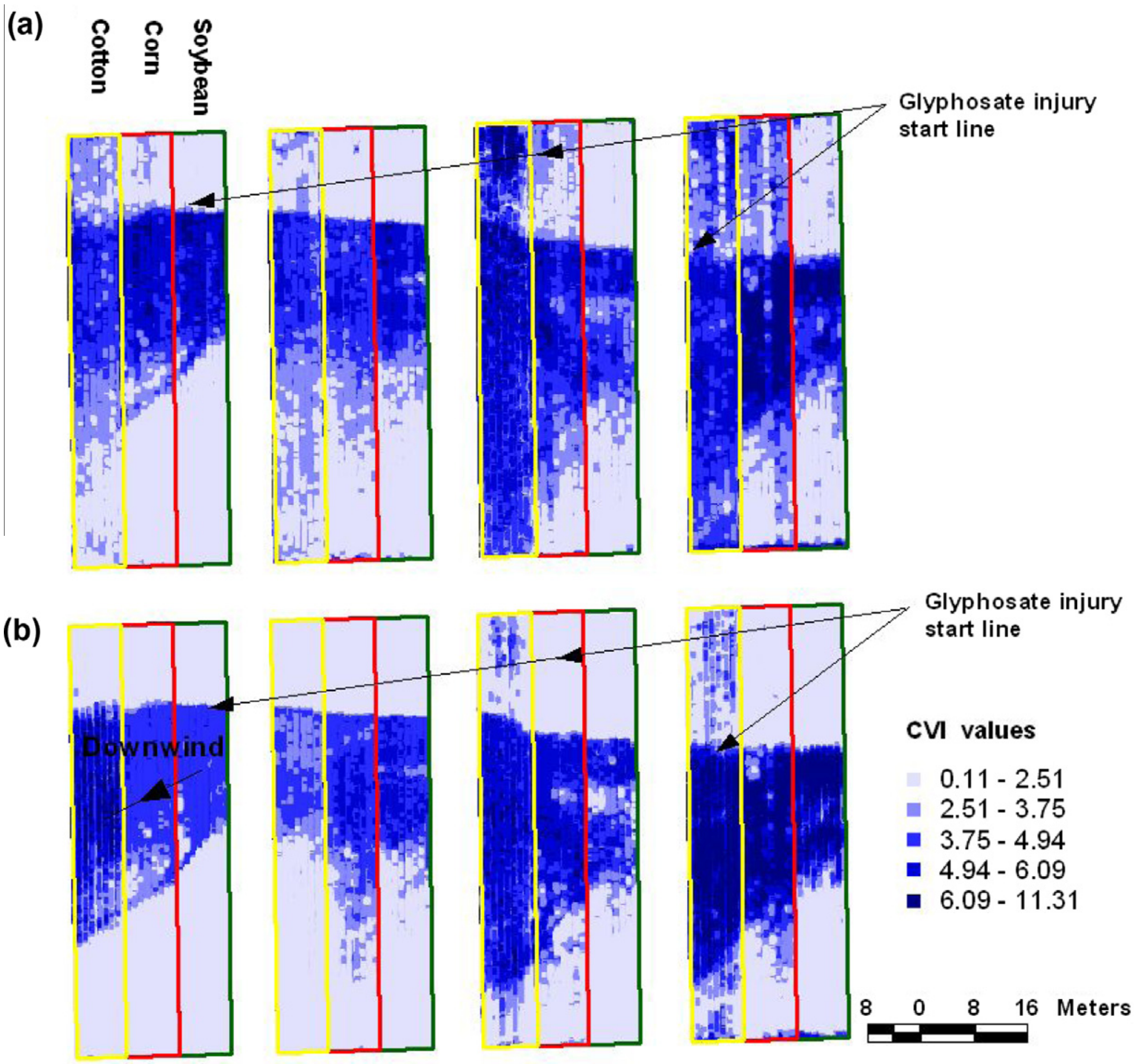

Fig. 2. CVI images over the experimental field. (a) CVI image collected 7DAA and (b) CVI image collected 21DAA. Dark blue on the images denote low biomass.

stomatal conductance (Zobiole et al., 2010), nitrate reductase activity (Bellaloui et al., 2008), decrease in nodule biomass, nitrogen fixation and accumulation (Reddy et al., 2000). These findings are evidence of glyphosate impact on chlorophyll, which was indirectly observed through the correlation of the field-measured plant injury variables by indices CVI, GVI, and GNDVI.

Although CVI, GVI, and GNDVI had similar weight in the CCA, the consistently strong correlation of CVI with the canonical variate V_{d1} is nonetheless as important as the enhanced sensitivity to leaf chlorophyll concentration and the reduced effect of LAI variation compared to the other two indices (Vincini et al., 2008). This feature is even more important when comparing changes in spectral reflectance from crops like cotton, corn, and soybean that have different canopy structure and LAI through the progression of growth stages.

3.3. Identification of injury magnitude

Based on the results from the CCA, CVI was selected as the vegetation index to assess the level of glyphosate injury to cotton, corn, and soybean. Unlike Vincini et al. (2008) who reported a positive relationship between CVI and chlorophyll content independently of a LAI value, a negative correlation was found for all the crops evaluated in this study at all the sampling dates (Table 5). On the CVI images, non-injured areas (upwind section from the study plots) exhibited lower CVI values than the injured areas (downwind) (Fig. 2).

Instead of using the raw CVI images values, CVI residual images (CVI_{res}) calculated by subtracting each CVI image pixel value from the average pixel value of the upwind control area (non-injured plants) were used to enhance and detect differences between the

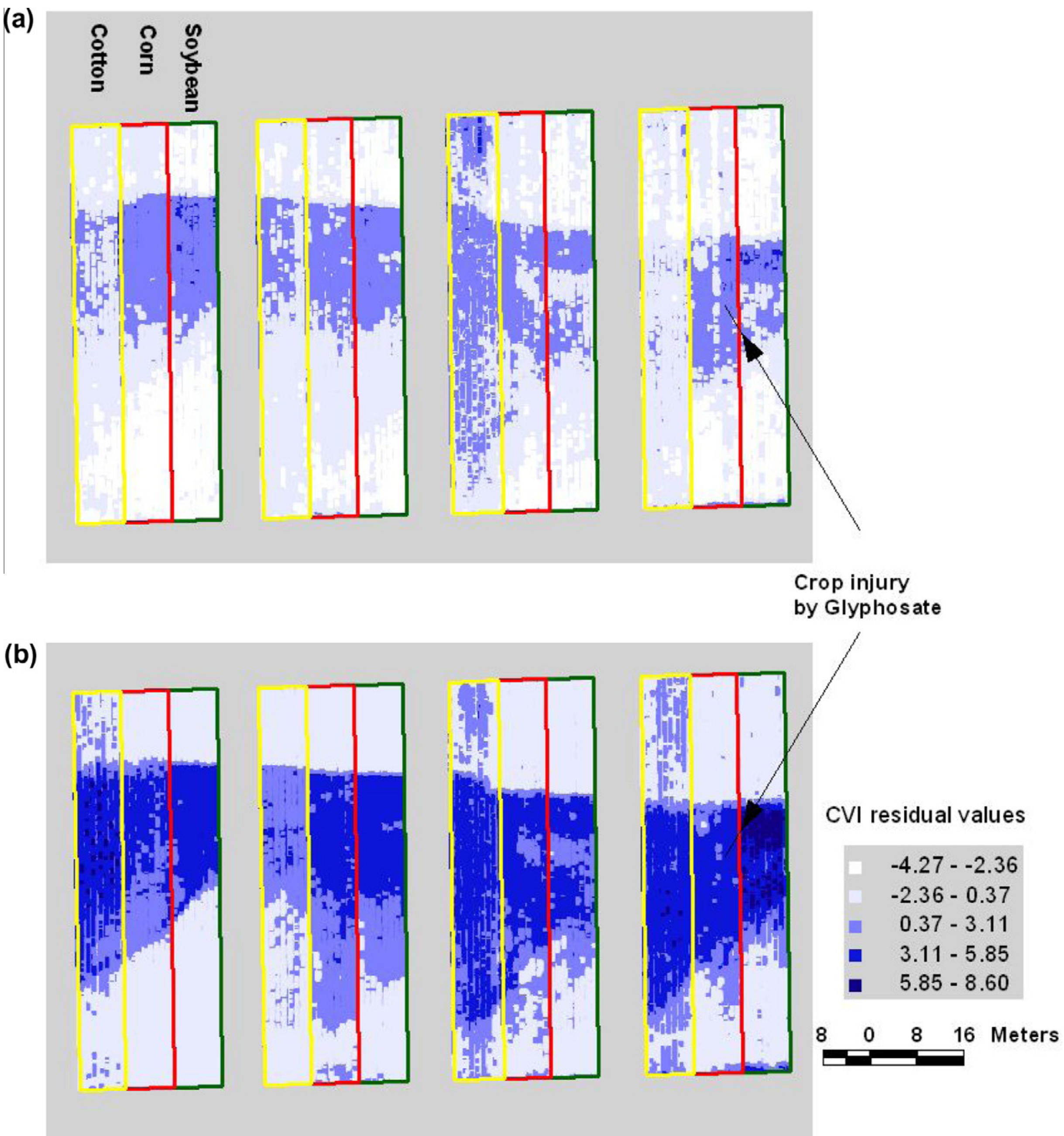

Fig. 3. CVI_{res} images over the experimental field. (a) CVI_{res} image collected 7DAA and (b) CVI_{res} image collected 21DAA. Dark blue areas on the images denote crop injury by glyphosate.

Table 6
Percentage of pixels for three frequency distribution intervals calculated from the CVI_{res} images.

Replication	Crop	7DDA % of pixels by frequency interval from CVI_{res} images			21DDA		
		1 <0.00	2 (0.00–3.11)	3 > = 3.11	1 <0.00	2 (0.00–3.11)	3 > = 3.11
1	Cotton	52	45	3	36	36	27
	Corn	49	33	18	43	24	33
	Soybean	43	31	25	43	24	33
2	Cotton	37	57	6	35	46	19
	Corn	32	53	15	20	42	38
	Soybean	29	48	23	19	43	38
3	Cotton	77	23	0	24	46	31
	Corn	42	57	1	22	38	39
	Soybean	32	53	15	32	37	31
4	Cotton	50	50	0	32	33	34
	Corn	46	44	10	38	25	37
	Soybean	35	44	21	46	27	27
Average by crop[a]	Cotton	54a	44a	2c	32a	40a	28b
	Corn	42ab	47a	11b	31a	32a	37a
	Soybean	35b	44a	21a	35a	33a	32ab
Average by frequency interval[b]	Cotton	55a	46a		32a	68b	
	Corn	44a	58b		31a	69b	
	Soybean	35a	65b		35a	65b	

Means followed by the same letter are not significantly different at $P \leqslant 0.05$.
[a] Means compared by crop per each frequency interval according to Waller-Duncan K-ratio t-test.
[b] Mean compared by frequency interval per crop according to Tukey's test.

injured and non-injured areas within the experimental plots and between crops (Fig. 3). Frequency distribution histograms of CVI_{res} image pixel values allowed assessment of glyphosate injury to each crop. Three-bin histograms were created, one frequency interval was comprised by negative pixel values and the other two intervals included only positive pixel values. The break between the two positive class intervals was chosen based on the CVI_{res} average value – 7DAA and 21 DAA images – of all sampling locations along the flight line/middle of spray swath (Fig. 1), which represented the highest exposure to glyphosate from which a severe injury was expected. Table 6 illustrates the percentage of pixels in the three frequency intervals or bins calculated from the CVI_{res} images for the cotton, corn, and soybean crops. Positive CVI_{res} values on an image, class 2 and 3, indicate areas where either the growth or chlorophyll content of plants is less than the non-injured areas (control locations); therefore, differences in the percentage of positive residuals between the crops might indicate a distinct degree of injury. Thelen et al. (2004) observed a decrease in NDVI values from digital aerial imagery with increasing herbicide (lactofen) rates on soybean. Following a similar approach, for this study a higher percentage of positive CVI_{res} values on the image respect to negative values might suggest large zones of glyphosate injury.

At 7DAA, significant differences between crops with respect to the percentage of pixels in the frequency interval three, $\geqslant 3.11$, were observed ($P = 0.0023$). A higher percentage of positive values, describing severe injury in this frequency interval, was observed for soybean and corn respect to cotton (Table 6). If frequency interval two classifies areas as moderate injury, then moderate injury was higher in corn and soybean than cotton. Overall, a higher percentage of negative pixel values (55% for frequency interval one) with respect to positive pixel values (46% for frequency interval two and three) was observed for cotton. In contrast, corn ($P = 0.025$) and soybean ($P = 0.0004$) showed a significantly higher percentage of positive pixel values than negative pixel values. This higher percentage of positive pixel values suggested that early in the season, soybean and corn exhibited more symptoms of injury than cotton.

At 21DAA, the percentage of positive values in the frequency interval three increased with respect to the 7DAA image indicating an increase in the injury level for all crops. As well as the interval three calculated from the 7DAA image, differences between crops with respect to the percentage of pixels was observed with corn exhibiting higher injury (37% positive pixels) than cotton or soybean (Table 6). Frequency interval two, moderate injury, showed a higher percentage of positive pixels for cotton than corn and soybean. Different from the 7DAA image, an increase in the percentage of positive pixels was observed at 21DAA for cotton and corn (from 16% to 48% increase in positive pixel values), which suggested an increase in injury extent.

A correlation analysis with data from sampling observations categorized by frequency interval of CVI_{res} images and percentage plant injury, field-measured injury variable with one of the highest loadings in the canonical correlation analysis, validated the hypothesis that positive CVI_{res} values in the images, frequency intervals two and three, indicated plant injury. Table 5 shows the correlation analysis for the data at 7DAA and 21DAA. The correlation differed among crops and replications, and between image collection dates. This might be due to different crop susceptibility to glyphosate, wind direction, growth stage at the moment of the glyphosate application and effects throughout the season. Despite these differences, the higher correlation between percentage injury data and the 21DAA-CVI_{res} image classified by three frequency intervals confirmed that higher percentage of positive pixels on the image corresponded to large areas or zones of crop injury from glyphosate.

The overall results showed that CVI_{res} derived from the CVI images were effective in separating injury levels between crops; however differences were observed between the 7DAA and 21DAA CVI_{res} images. This result is consistent with findings by Henry et al. (2004), who reported that at 4DAA and 7DAA only 77% classification accuracy was achieved from multiple vegetation index images used to distinguish injury on soybean and corn crops by two rates of glyphosate. The success in using CVI_{res} images to assess the level of glyphosate injury agreed with previous studies that used remote sensing imagery coupled with geospatial technologies to identify injury from herbicide drift. Thelen et al. (2004) distinguished herbicide rate effect on soybean using digital aerial imagery NDVI values. By calculating ΔNDVI, the difference between untreated and treated plots within an experimental block, they improved the ability to detect treatment differences over the average NDVI value for each treatment.

4. Conclusions

The results from the canonical correlation analysis showed that VIs derived from multispectral images were correlated with field-measured plant injury. The strong correlation between the first damage canonical variate and the field-measured plant injury variables and the high percentage of the total variance accounted by the damage canonical variates indicated that VIs having strong correlation with damage canonical variates, especially with the first damage canonical variate, could be considered as surrogate for glyphosate plant injury. From the VIs considered, CVI, GVI, and GNDVI indices and especially CVI, explained most of the variability in field-measured plant injury to cotton, corn, and soybean. This provided evidence of the potential for remote assessment of plant injury by application of glyphosate. The comparison of frequency distribution of the CVI_{res} derived from the CVI images, was an effective method to separate injury levels between crops. The higher percentage of positive pixels on the CVI_{res} images corresponded to large areas or zones of glyphosate injury. The data showed that the corn crop exhibited higher injury than cotton and soybean. These results provide evidence of the potential of remote sensing images collected from a low-altitude aerial platform, to indirectly assess the effects of glyphosate drift from aerial application to cotton, corn, and soybean. Future research will explore the use of indicator variogram to characterize the size of clusters of injured plants.

Disclaimer

Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by Auburn University or the US Department of Agriculture and does not imply approval of the product to the exclusion of others that may be available.

Acknowledgements

The authors would like to thank Phelesia Foster for her work in organizing the spray card labeling, card scanning, Mylar sampler preparation, and data processing. Thanks goes to Roger Bright for sampler setup and flight coordination, our pilot David Poythress for his precise flying and suggestions regarding the experiment, Linwood Roberts and Terry Newton for their attention to detail regarding requirements for late-season crop planting and management, Efren Ford and Earl Gordon for technical assistance in plant sampling and measurements, and Earl Franklin for his assistance in operating the trailer truck and airplane refueling system to assure successful flight. Thanks also to all the summer student workers for their hard work in placing and collecting samplers in hot weather. Thanks goes to Hunter Stone for his assistance with data processing.

References

Amrhein, N., Deus, B., Gehrke, P., Steinrucken, H.C., 1980. The site of the inhibition of the shikimate pathway by glyphosate. II. Interference of glyphosate with chorismate formation in vivo and in vitro. Plant Physiol. 66, 830–834.
Atkinson, P.M., Lewis, P., 2000. Geostatistical classification for remote sensing: an introduction. Comput. Geosci. 26, 361–371.
Avruskin, G., Jacquez, G., Meliker, J., Slotnick, M., Kaufmann, A., Nriagu, J., 2004. Visualization and exploratory analysis of epidemiologic data using a novel space time information system. Intl. J. Health Geogr. 3, 26.
Bahrun, A., Mogensen, V.O., Jensen, C.R., 2003. Water stress detection in field-grown maize by using spectral vegetation index. Commun. Soil Sci. Plant Anal. 34, 65–79.
Baret, F., Guyot, G., 1991. Potentials and limits of vegetation indices for LAI and APAR assessment. Remote Sens. Environ. 35, 161–173.
Batista, G.T., Rudorff, B.F.T., 1990. Spectral response of soybean by field radiometry. ISPRS J. Photogramm. Remote Sens. 45, 111–121.
Bausch, W.C., Duke, H.R., 1996. Remote sensing of plant nitrogen status in corn. Trans. ASAE. 39, 1869–1875.
Bellaloui, N., Reddy, K.N., Zablotowicz, R.M., Mengistu, A., 2006. Simulated glyphosate drift influences nitrate assimilation and nitrogen fixation in non-glyphosate-resistant soybean. J. Agric. Food. Chem. 54, 3357–3364.
Bellaloui, N., Zablotowicz, R.M., Reddy, K.N., Abel, C.A., 2008. Nitrogen metabolism and seed composition as influenced by glyphosate application in glyphosate-resistant soybean. J. Agric. Food. Chem. 56, 2765–2772.
Bronson, K.F., Chua, T.T., Booker, J.D., Keeling, J.W., Lascano, R.J., 2003. In-season nitrogen status sensing in irrigated cotton. Soil Sci. Soc. Am. J. 67, 1439–1448.
Brown, L.R., Robinson, D.E., Young, B.G., Loux, M.M., Johnson, W.G., Nurse, R.E., Swanton, C.J., Sikkema, P.H., 2009. Response of corn to simulated glyphosate drift followed by in-crop herbicides. Weed Technol. 23, 11–16.
Buehring, N.W., Massey, J.H., Reynolds, D.B., 2007. Shikimic acid accumulation in field-grown corn (*Zea mays*) following simulated glyphosate drift. J. Agric. Food. Chem. 55, 819–824.
Casadesús, J., Kaya, Y., Bort, J., Nachit, M.M., Araus, J.L., Amor, S., Ferrazzano, G., Maalouf, F., Maccaferri, M., Martos, V., Ouabbou, H., Villegas, D., 2007. Using vegetation indices derived from conventional digital cameras as selection criteria for wheat breeding in water-limited environments. Ann. Appl. Biol. 150, 227–236.
Chen, J., 1996. Evaluation of vegetation indices and modified simple ratio for boreal applications. Can. J. Remote Sens. 22, 229–242.
Crippen, R.E., 1990. Calculating the vegetation index faster. Remote Sens. Environ. 34, 71–73.
Dieleman, J.A., Mortensen, D.A., Buhler, D.D., Cambardella, C.A., Moorman, T.B., 2000. Identifying associations among site properties and weed species abundance. I. Multivariate analysis. Weed Sci. 48, 567–575.
Ellis, J.M., Griffin, J.L., Linscombe, S.D., Webster, E.P., 2003. Rice (*Oryza sativa*) and corn (*Zea mays*) response to simulated drift of glyphosate and glufosinate. Weed Technol. 17, 452–460.
Gitelson, A.A., Kaufman, Y.J., Merzlyak, M.N., 1996. Use of a green channel in remote sensing of global vegetation from EOS-MODIS. Remote Sens. Environ. 58, 289–298.
Gittins, R., 1985. Canonical Analysis: A Review with Applications in Ecology. Springer-Verlag, Berlin, pp. 13–36.
Goodin, D. G., Henebry, G. M. 1998. Variability of spectral reflectance and vegetation indices in tallgrass prairie: spatio-temporal analysis using semivariograms and close-range remote sensing. In: Proceedings of the IEEE international geoscience and remote sensing symposium (IGARSS'98), July, 1998, Seattle, Washington, 2, 825–827.
Goovaerts, P., 1998. Geostatistical tools for characterizing the spatial variability of microbiological and physico-chemical soil properties. Biol. Fertil. Soils 27, 315–334.
Haboudane, D., Miller, J.R., Pattey, E., Zarco-Tejada, P.J., Strachan, I.B., 2004. Hyperspectral vegetation indices and novel algorithms for predicting green LAI of crop canopies: modeling and validation in the context of precision agriculture. Remote Sens. Environ. 90, 337–352.
Henry, W.B., Shaw, D.R., Reddy, K.R., Bruce, L.M., Tamhankar, H.D., 2004. Remote sensing to detect herbicide drift on crops. Weed Technol. 18, 358–368.
Hiscox, J.D., Israelstam, G.F., 1979. A method for the extraction of chlorophyll from leaf tissues without maceration. Can. J. Bot. 57, 1332–1334.
Huang, Y., Thomson, S.J., Ortiz, B.V., Reddy, K.N., Ding, W., Zablotowicz, R.W., Bright, J.R., 2010. Airborne remote sensing assessment of the damage to cotton caused by spray drift from aerially applied glyphosate through spray deposition measurements. Biosyst. Eng. 107, 212–220.
Isaacs, E.H., Srivastava, M., 1989. An Introduction to Applied Geostatistics. Oxford University Press, New York.
Johnson, R.A., Wichern, D.W., 2002. Applied Multivariate Analysis, 5th ed. Prentice Hall, Upper Saddle River, New York.
Jordan, C.F., 1969. Derivation of leaf-area index from quality of light on the forest floor. Ecology 50, 663–666.
Kerry, R., Oliver, M.A., 2003. Co-kriging when soil and ancillary data are not co-located. In: Stafford, J.V., Werner, A. (Eds.), Precision Agriculture '03, Proceedings of the 4th European Conference on Precision Agriculture in Berlin, June 15–19, 2003. Wageningen Academic Publishers, Den Haag, The Netherlands, pp. 303–308.
Kerry, R., Oliver, M.A., 2008. Determining nugget:sill ratios of standardized variograms from aerial photographs to krige sparse soil data. Prec. Agric. 9, 33–56.
Martin, N.F., Bollero, G., Bullock, D.G., 2005. Association between field characteristics and soybean plant performance using canonical correlation analysis. Plant Soil 237, 39–55.
Noe, J.P., Barker, K.R., 1985. Relation of within-field spatial variation of plant-parasitic nematode population densities and edaphic factors. Phytopathol. 75, 247–252.
Payero, J.O., Neale, C.M.U., Wright, J.L., 2004. Comparison of eleven vegetation indices for estimating plant height of alfalfa and grass. Appl. Eng. Agric. 20, 385–393.

Pringnitz, B. 1999. Pesticide Drift: To Spray or Not to Spray? Iowa State University, Iowa State University Extension Pesticide Applicator Education Program. PCIC-99d.

Reddy, K.N., Hoagland, R.E., Zablotowicz, R.M., 2000. Effect of glyphosate on growth, chlorophyll, and nodulation in glyphosateresistant and susceptible soybean (*Glycine max*) varieties. J. New Seeds. 2, 37–52.

Rouse, J.W., Haas, R.H., Schell, J.A., Deering, D.W. 1973. Monitoring Vegetation Systems in the Great Plains with ERTS. Third ERTS Symposium, NASA SP-351 I: 309–317.

Rowland, C.D. 2000. Crop Tolerance to Non-target and Labeled Herbicide Applications. M.S. thesis. Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, MS.

Sullivan, D.G., Shaw, J.N., Mask, P., Rickman, D., Luvall, J., Wersinger, J.M., 2004. Evaluating corn (*Zea mays* L.) N variability via remote sensed data. Comm. Soil Plant Anal. 35, 2465–2483.

Tamhankar, H., Bruce, L.M., Henry, B., Shaw, D. 2002. Automated detection of herbicide drift effects on crops. In: Proceedings of the IEEE international geoscience and remote sensing symposium (IGARSS'02), June, 2002, Toronto, Canada, 5, 3023–3025.

Thelen, K.D., Kravchenko, A.N., Lee, C., 2004. Use of optical remote sensing for detecting herbicide injury in soybean. Weed Technol. 18, 292–297.

Thomson, S.J. 2008. Aerial application for control of soybean rust. Invited presentation for the Mississippi Agricultural Aviation Association. MAAA/SEAF 5th Annual Convention, Philadelphia MS, January 26, 2008.

Tucker, C.J., 1979. Red and photographic infrared linear combinations for monitoring vegetation. Remote Sens. Environ. 10, 23–32.

Van Meirvenne, M., Goovaerts, P., 2002. Accounting for spatial dependence in the processing of multi-temporal SAR images using factorial kriging. Int. J. Remote Sens. 23, 371–387.

Vincini, M., Frazzi, E., Alessio, P.D', 2008. A broad-band leaf chlorophyll vegetation index at the canopy scale. Prec. Agric. 9, 303–319.

Webster, R., Oliver, M.A., 2001. Geostatistics for Environmental Scientists. John Wiley and Sons Ltd., Chichester, England.

Zobiole, L.H.S., Oliveira Jr., R.S., Kremer, R.J., Constantin, J., Bonato, C.M., Muniz, A.S., 2010. Water use efficiency and photosynthesis of glyphosate-resistant soybean as affected by glyphosate. Pestic. Biochem. Physiol. 97, 182–193.