

DETERMINATION OF CROP INJURY FROM AERIAL APPLICATION OF GLYPHOSATE USING VEGETATION INDICES AND GEOSTATISTICS

B. V. Ortiz

*Agronomy and Soils Department
Auburn University
Auburn, Alabama*

S. J. Thomson, Y. Huang, K. Reddy

*Crop Production Systems Research Unit
USDA-ARS-CPSRU
Stoneville, MS*

W. Ding

*Agronomy College
Northeast Agricultural University
Harbin, Heilongjiang, China*

H. Stone

*Agronomy and Soils Department
Auburn University
Auburn, Alabama*

ABSTRACT

Injury to crops caused by off-target drift of glyphosate can seriously reduce growth and yield, and is of great concern to farmers and aerial applicators. Determining an indirect method for assessing the levels and extent of crop injury could support management decisions. The objectives of this study were to evaluate multiple vegetation indices (VIs) as surrogate variables for glyphosate injury identification and to evaluate the combined use of Geostatistical methods and the VIs to assess the level and extent of crop injury. The experiment evaluated glyphosate injury between the cotton and corn crops. Cotton and corn were planted on July 23, 2009 in eight row strips spaced 102-cm apart and 80 m long with four replications. A single aerial application of glyphosate was made on August 12, 2009 using an Air Tractor 402B airplane equipped with fifty-four CP-09 spray nozzles. Multispectral images were collected from the same airplane using a MS 4100 camera at 1, 7, 14 and 21 days after the glyphosate application. On the same days as the image collections, plant damage data including visual injury ratings, 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 selected within each experimental unit. Seven VIs, calculated from the images, were entered along with the plant damage data into a canonical correlation analysis (CCA). Semivariograms were computed for each vegetation index/crop and replication. The range of spatial correlation derived from the semivariograms was used to evaluate differences in the extent of injury between replications and crops/replications. The results suggest that vegetation indices, especially the Chlorophyll Vegetation Index (CVI), can be used as surrogate for glyphosate injury identification, and the range of spatial correlation indicated the extent of crop damage.

Keywords: Geostatistics, Glyphosate, Semivariogram, Remote Sensing, Vegetation Indices

INTRODUCTION

Managing aerial herbicide applications is the key to minimizing off-target drift of pesticides that could cause crop damage, deposit harmful residues on edible crops, and contaminate water supplies. Spray drift of glyphosate, one of the most common non-selective herbicides used in row crop production, is of concern due to post application consequences such as inhibition of growth, chlorosis at the newest growing points, necrosis throughout the entire plant within 1 to 2 weeks after application, and yield reduction (Henry et al., 2004).

Crops often affected by off-target glyphosate drift including corn (Buehring et al., 2007; Brown et al., 2009), soybean (Bellaloui et al., 2008), and rice (Ellis et al., 2003) have been the target for evaluation of several methods for identifying injury or damage. Rowland (2000) found stand height as one of the best parameters to identify the degree of glyphosate damage in corn. Raw remote sensing data and derived vegetation indices, commonly used to indirectly assess differences in growth and chlorophyll content of several crops (Zarco-Tejada et al. 2005; Gitelson et al. 2003, Zhang et al. 2009), have been also used to determine the extent and location of herbicide injury. Henry et al. (2004), comparing herbicide injury of soybean and corn, distinguished healthy and injury plants using hyperspectral reflectance and several vegetation indices such as the ratio vegetation index and the DINO12, a NDVI-like index comprising a region around 2,220 nm. Thelen et al. (2004) found significant differences between herbicide or herbicide rate by calculating NDVI from digital aerial images of soybean.

A challenge in detection of herbicide injury by remote sensing is the identification of technique used to enhance within-field spatial variability for detection of the level and extent of crop injury. Spectral band ratios described as vegetation indices have been used to facilitate image classification (Lu and Weng, 2007). Moreover, vegetation often displays some degree of spatial autocorrelation, sometimes at different scales of variation, observed on the images. These scales of spatial variation can be detected through the use of

semivariograms (Matheron, 1963). Within an image, an object's spectral properties (e.g., reflectance respect to the level of leaves' chlorophyll or amount of biomass) are more homogeneous compared with the surrounding features (Jupp et al, 1988a, 1988b). Therefore the range of spatial correlation could be used to identify specific features within an image. Factorial kriging, a geostatistical technique that allows filtering spatial components identified from nested variograms, has been used to extract from satellite images scale-dependent information of land characteristics related to topography, soil drainage, and land use (Meirvenne and Goovaerts, 2002). Rodgers and Oliver (2007) used factorial kriging to identify on a NDVI image the variation in landscape and to understand the processes controlling the physical properties of the soil and vegetation cover.

The objectives of this research were to determine whether vegetation indices derived from multispectral images could be used to identify crop injury by off-target glyphosate drift and to evaluate geostatistical procedures applied to the vegetation index images for evaluation of crop injury levels and extent of injury.

MATERIALS AND METHODS

Study field and experimental plan

Crop injury and biological responses of two row crops (cotton and corn) following glyphosate drift from an aerial application were evaluated in an experiment conducted during summer 2009. The study field was located at of the research farms of the U.S. Department of Agriculture-Agricultural Research Service in Stoneville, MS (33°26'N, 90°55'W). Cotton (non-GR cotton cultivar 'FM955LL') and corn (non-GR corn hybrid 'Pioneer 31P41') were planted on July 23, 2009 in eight row strips spaced 102-cm apart and 80 m long with four replications (Figure 1).

A single aerial application of glyphosate was made on August 12, 2009 when cotton was at two- to three-leaf stage and corn was at four-leaf stage. The glyphosate was applied using an Air Tractor 402B airplane equipped with fifty-four CP-09 spray nozzles (CP Products, Tempe, AZ) set at 5 degree deflection angle for this particular experiment. The aircraft and application system were adjusted to deliver the liquid at the rate of 46.8 L ha⁻¹ at a release height of 3.7 m and operating speed of 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, MO) applied at a rate of 866 g ae ha⁻¹. The airplane traveled west to east direction across the center of the study field perpendicular to the crop rows over a marked swath line (Fig. 1).

Biomass Measurements

Plant sampling locations by crop and replication were established downwind at 9, 12, 15, 20, 25, 35, and 45 m from the center of the spray swath (18.3 m size). One upwind sample location at 35.4 m from the upwind edge of the

18.3 m wide swath was included as a control (crops not exposed to glyphosate) for comparison of biological responses to drift.

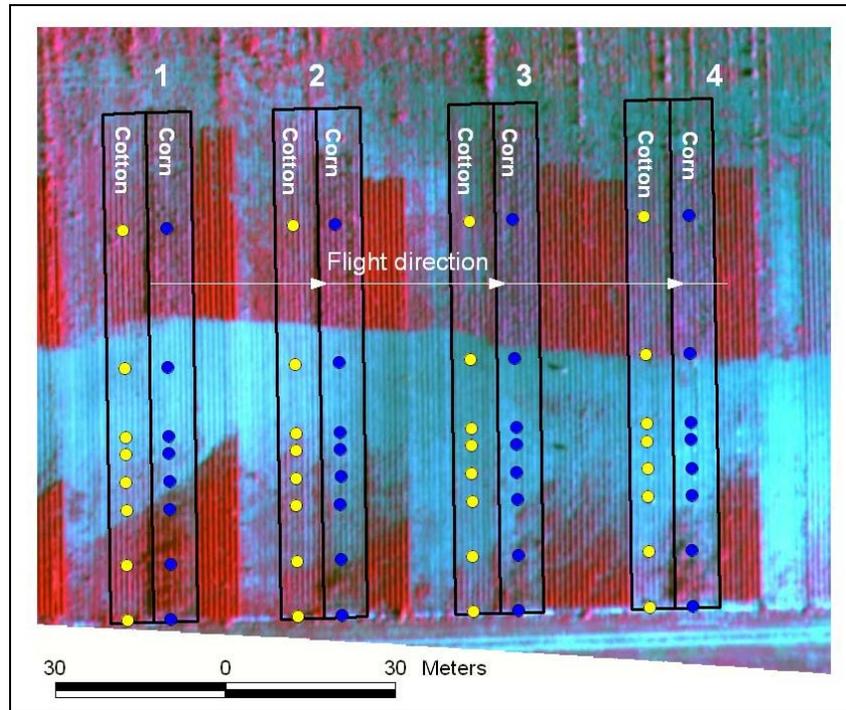


Fig. 1. Experimental layout for the spray test (plant sampling locations for cotton and corn are displayed with points)

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 9 m. For the 9 m sampling location, data were collected from the 18.3 m spray swath. The sampling location at 9 m downwind represented the highest exposure to glyphosate, while the 35.4 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 to 100, with 100 representing total plant mortality and 0 representing no injury. Plant height values resulted 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).

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 camera is a multi-spectral 3-CCD (Charge - Coupled Device) color/color infrared (CIR) digital camera and provides a digital imaging quality of 1920 (horizontal) x 1080 (vertical) pixel array per sensor and wide field of view of 60 degrees with 14 mm, f/2.8 lens. The camera captures imagery in four spectral bands: blue (460 nm - 45 nm bandwidth), green (540 nm - 40 nm bandwidth), red (660 nm - 40 nm bandwidth), and near infrared- NIR (800 nm - 65 nm bandwidth), however for this experiment the camera was configured to produce CIR images (red, green and NIR bands). Multispectral images with a spatial resolution of 11 x 20 cm/pixel were collected 1, 7, 14 and 21 days after the glyphosate application (DAA). For this study, only results from the image collected 21 DAA are presented because it should represent the highest extent of injury one can expect from the set of three images.

Data Processing and Statistical Analysis

Subsets of the 21 DAA image corresponding to each crop and replication were extracted and individually analyzed (4 individual images per crop – 8 images total). Seven vegetation indices (VIs) as different band ratio combinations were calculated from the images (Table 1). Each vegetation index (VI) data was rescaled to unit variance by dividing each pixel value by the VI standard deviation (e.g., NDVI pixel $_{ij}$ / 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). Each omnidirectional semivariogram was computed for 80 lags with 0.56 m lag distance using the usual computing equation (Webster and Oliver, 2001). The best semivariogram 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 vegetation index values at each plant sampling location (Kerry and Oliver, 2003) using TerraSeer STIS software (Avruskin et al. 2004).

Canonical correlation analyses (CCA) by crop were conducted to identify the vegetation indices strongly related to the on-ground measured glyphosate crop damage. CCA assesses the relationship between a linear combination of a set of Y variables (on-ground measured plant damage variables) and a linear combination of a set of X variables (vegetation indices). Through this method it is possible to create independent pairs of new variables, where each component of the canonical variable pair 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 variable d_i . Variables having a high contribution to the canonical variable d_i are those that exhibit large loadings.

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

Vegetation Index (VI)	Formula	Reference
Normalized Difference Vegetation Index (NDVI)	$(\text{NIR}-\text{Red})/(\text{NIR}+\text{Red})$	Rouse et al. (1974), Tucker (1979)
Green Normalized Difference Vegetation Index (GNDVI)	$(\text{NIR}-\text{Green})/(\text{NIR}+\text{Green})$	Gitelson et al. (1996)
Simple Ratio Index (NIR/R)	NIR/Red	Jordan (1969)
NIR/G	NIR/Green	Jordan (1969)
Chlorophyll Vegetation Index (CVI)	$(\text{NIR}/\text{Green}) * (\text{Red}/\text{Green})$	Vincini et al. (2008)
Modified Simple Ratio (MSR)	$(\text{NIR}/\text{Red} - 1) / ((\text{NIR}/\text{Red})^{1/2} + 1)$	Chen (1996)
Infrared Percentage Vegetation Index (IPVI)	$\text{NIR} / (\text{NIR} + \text{Red})$	Crippen (1990)

Assessment of the extent of glyphosate drift injury on each crop was determined by analyzing each range of spatial correlation derived from the standardized semivariograms calculated for each index/replication. The semivariogram standardization allowed a more reliable comparison between ranges. Comparison of the level and extent of injury between cotton and corn was performed by analyzing the range of spatial variability and frequency distribution of the residuals values (pixel value – mean) calculated from the vegetation indices selected through the canonical correlation analysis.

RESULTS

Canonical correlation analysis

For each crop, the CCA between the on-ground measured plant damage data and vegetation indices calculated from the image collected 21 DAA, resulted in four pairs of canonical variables (Table 2). The correlation ($r = 0.90$) between the first pair of canonical variables for the cotton crop was significant while for the second and third pairs even though higher (0.54 and 0.43 respectively) were not significant. For the corn crop, the correlation for the first and second pair of canonical variables was significant ($P < 0.05$) with correlation coefficients of 0.86 and 0.70 respectively. The significant and strong canonical correlation between on ground-measured plant damage variables and remotely-sensed vegetation indices

validates the hypothesis that vegetation indices can be used for assessment of degree of crop injury caused by Glyphosate drift as well as the extent of damage.

For cotton, the discussion of results herein from the CAA is focused on the correlation between the first significant pair of canonical variables that explained 84% of the variability between the plant damage data and the vegetation reflectance (data not shown). The largest correlation in the plant damage variable was for dry matter and injury data and the lowest correlation was for chlorophyll. The variability on the vegetation reflectance canonical variable was strongly correlated with the indices CVI ($r = 0.77$), NIR/G ($r = 0.76$) and GNDVI ($r = 0.64$) respectively. Contrasting with cotton, two significant canonical variables from the CAA for corn data showed that plant damage was strongly correlated with the first canonical damage variable; not so much for the second canonical variable. The vegetation indices strongly correlated with the damage variable were also CVI, NIR/G and GNDVI with correlations of 0.86, 0.82, and 0.71, respectively. Although low correlation was observed for chlorophyll and injury variable with the second plant damage canonical variable, this correlation might also be explained by the variability of the NDVI, NIR/R, MSR, and IPVI index values. The agreement between CVI, NIR/G and GNDVI indices, especially CVI, explained most of the variability in ground-measured plant damage for cotton and corn. This provides strong evidence of the potential for remote assessment of glyphosate plant damage.

Geostatistical Analyses

The ranges of spatial correlation summarized the average extent of crop injury which changed by replication, with the highest damage area observed on replication 3 for both crops (Tables 3 and 4). There were no differences between the ranges of spatial correlation calculated for each vegetation index within each replication, which indicated that there were not differences between vegetation indices for assessing the extent of crop injury. The ranges of spatial correlation indicated that the overall average extent of glyphosate injury at 21 DAA was within a range of 34 to 36 m for both crops. The comparison of ranges between crops by replication showed low differences in the extent of damage/injury between the cotton and corn crops.

Differences between the average range (data from all vegetation indices) for cotton and corn were observed for replication 2 and slightly for replications 3 and 4. The extent of damage was higher for corn for replications 2 and 3 with ranges of 34.6 m and 43.6 m compared with cotton with ranges of 29.9 m and 41.1 m, respectively (Tables 3 and 4). A more reliable comparison of the levels and extent of damage was possible when the residuals values (pixel value i_j – average pixel values) from a single vegetation index (CVI) were analyzed.

Table 2. Canonical correlation between plant damage data and seven vegetation indices calculated from cotton and corn images.

Canonical component	Cotton			Corn			
	Eigen value	CC†	Pr > F	Eigen value	CC†	Pr > F	
1	3.89	0.90	0.0018	2.92	0.86	0.0002	
2	0.41	0.54	0.6178	0.97	0.70	0.0593	
3	0.23	0.43	0.7114	0.49	0.58	0.3309	
4	0.09	0.28	0.7209	0.05	0.23	0.8567	
Wilks' Lambda		0.002			0.0002		
Correlations between the plant damage variables and their canonical variables							
Plant damage	Damage 1	Damage 2	Damage 3	Damage 1	Damage 2	Damage 3	Damage 4
Injury	0.90	0.16	0.39	0.86	-0.27	0.33	0.27
Chlorophyll	-0.22	-0.20	-0.23	-0.94	0.28	0.18	-0.01
Dry Matter	-0.97	-0.07	0.18	-0.93	-0.16	-0.34	0.02
Height	-0.85	-0.52	-0.07	0.96	0.04	-0.02	-0.26
Correlations between the VIs and the canonical variables of the plant damage variables							
VIs	Damage 1	Damage 2	Damage 3	Damage 1	Damage 2	Damage 3	Damage 4
NDVI	-0.47	-0.31	-0.16	-0.44	0.33	-0.08	0.01
GNDVI	0.64	-0.01	-0.07	0.71	0.21	0.00	-0.08
NIR/R	-0.38	-0.30	-0.17	-0.38	0.39	-0.17	0.00
NIR/G	0.76	0.07	0.00	0.82	0.10	0.04	-0.04
CVI	0.77	0.12	0.12	0.86	-0.03	0.02	-0.01
MSR	-0.26	0.25	-0.18	-0.54	0.32	-0.17	0.04
IPVI	0.07	-0.23	-0.17	0.01	0.48	-0.04	0.00

† Canonical Correlation

Table 3. Range of spatial correlation of different vegetation index images collected at 21 days after glyphosate application – Cotton

VI	Rep 1		Rep 2		Rep3		Rep4		Average range
	Range	Model	Range	Model	Range	Model	Range	Model	
NDVI	36.4	Cubic	30.7	Spherical	39.2	Spherical	32.6	Spherical	34.8
GNDVI	31.6	Spherical	30.1	Spherical	41.4	Spherical	31.1	Spherical	33.5
NIR/R	36.5	Cubic	29.0	Spherical	39.7	Spherical	31.9	Spherical	34.3
NIR/G	38.6	Cubic	30.3	Spherical	43.7	Spherical	31.9	Spherical	36.1
CVI	39.4	Cubic	30.8	Spherical	41.9	Spherical	30.8	Spherical	35.7
MSR	36.0	Cubic	29.1	Spherical	40.6	Spherical	33.3	Spherical	34.8
IPVI	36.8	Cubic	29.5	Spherical	40.9	Spherical	32.7	Spherical	35.0
Average	36.5		29.9		41.1		32.1		

Table 4. Range of spatial correlation of different vegetation index images collected at 21 days after glyphosate application – Corn

VIs	Rep 1		Rep 2		Rep3		Rep4		Average range
	Range	Model	Range	Model	Range	Model	Range	Model	
NDVI	37.3	Cubic	36.8	Cubic	39.9	Spherical	29.9	Spherical	36.0
GNDVI	35.7	Cubic	36.3	Cubic	38.5	Spherical	28.3	Spherical	34.7
NIR/R	35.9	Cubic	31.5	Spherical	46.1	Cubic	30.0	Spherical	35.9
NIR/G	37.1	Cubic	38.3	Cubic	44.1	Cubic	28.4	Spherical	37.0
CVI	38.7	Cubic	39.0	Cubic	42.9	Cubic	33.5	Cubic	38.5
MSR	36.0	Cubic	29.8	Spherical	46.6	Cubic	30.2	Spherical	35.6
IPVI	37.7	Cubic	30.6	Spherical	47.3	Cubic	34.7	Cubic	37.6
Average	36.9		34.6		43.6		30.7		

The range of spatial correlation of CVI residuals for cotton and corn was similar for replications 1 and 4; however, a higher range was observed for corn on replications 2 (39.1 m) and 3(43.3 m) compared with cotton on the same replications, 35.3 m and 40.7 m, respectively (Table 5). The higher ranges observed for corn are an indication of a larger damaged area compared to the cotton crop. Negative CVI residual values on a map indicate areas where either the growth or chlorophyll of plants is less than the overall average conditions; therefore, differences in the percentage of negative residuals between cotton and corn might be indicative of a higher level of damage. Two class intervals with negative residuals values derived from frequency histograms were analyzed for both cotton and corn. A higher percentage of negative values < - 1.32 for corn respect to cotton was observed on replications 2, 3, and 4. These differences were represented by a 3, 9, 6 and 17% more negative pixels (-3.13 to -1.32 interval) for corn than cotton on the replications 1, 2, 3, and 4 respectively (Table 6). This result is evidence of higher glyphosate susceptibility of corn compared to cotton.

Table 5. Ranges of spatial correlation for CVI-Mean (damage assessment).

Replication ID	Crop	Range
1	Cotton	38.5
	Corn	39.7
2	Cotton	35.3
	Corn	39.1
3	Cotton	40.7
	Corn	43.3
4	Cotton	34.7
	Corn	32.8

Table 6. Percentage of pixels in the interval of frequency with negative values (areas of damage).

Interval of frequency	% of pixels representing damage areas							
	Rep 1		Rep 2		Rep3		Rep4	
	Cotton	Corn	Cotton	Corn	Cotton	Corn	Cotton	Corn
-3.13 - -1.32	32	33	20	35	32	34	35	41
-1.32 - 0.54	35	35	50	28	26	31	24	23

CONCLUSIONS

The results from this study showed that VIs were correlated with the ground-based measurement of plant damage. From the VIs evaluated, CVI, NIR/G and GNDVI indices, especially CVI, explained most of the variability in ground-measured plant damage on cotton and corn, which provided evidence of the potential for remote assessment of glyphosate plant damage. The ranges of spatial correlation calculated for each vegetation index summarized the average extent of crop injury, which changed by replication. Although there were not significant differences between vegetation indices for assessing the extent of injury for cotton or corn, comparison of the level and extent of damage between both crops was based on the analysis of the CVI data. Higher CVI ranges observed for corn indicated a larger damage area compared to the cotton crop. The comparison of frequency distribution of the CVI residuals for cotton and corn indicated that corn was more susceptible to glyphosate injury than cotton. These results provide evidence of the potential for remote sensing images, collected from a low-altitude aerial platform, to indirectly assess the effects of glyphosate drift from aerial application in cotton and corn.

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