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Assessment of soybean injury from glyphosate using airborne multispectral remote sensing

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Abstract:

BACKGROUND: Glyphosate drift onto off-target sensitive crops can reduce growth and yield and is of great concern to growers and pesticide applicators. Detection of herbicide injury using biological responses is tedious, so more convenient and rapid detection methods are needed. The objective of this research was to determine the effects of glyphosate on biological responses of non-glyphosate-resistant (non-GR) soybean and to correlate vegetation indices (VIs) derived from aerial multispectral imagery.

RESULTS: Plant height, shoot dry weight and chlorophyll (CHL) content decreased gradually with increasing glyphosate rate, regardless of weeks after application (WAA). Accordingly, soybean yield decreased by 25% with increased rate from 0 to 0.866 kg AI ha^{-1}. Similarly to biological responses, the VIs derived from aerial imagery – normalized difference vegetation index, soil adjusted vegetation index, ratio vegetation index and green NDVI – also decreased gradually with increasing glyphosate rate, regardless of WAA.

CONCLUSION: The VIs were highly correlated with plant height and yield but poorly correlated with CHL, regardless of WAA. This indicated that indices could be used to determine soybean injury from glyphosate, as indicated by the difference in plant height, and to predict the yield reduction due to crop injury from glyphosate.

Keywords: soybean; glyphosate; crop injury; biological response; remote sensing; vegetation index

1 INTRODUCTION

Glyphosate [*N*-(phosphonomethyl) glycine] is the most commonly applied herbicide, either alone or with other herbicides, to manage a broad spectrum of weeds around the world. In the United States, the phenomenal adoption of genetically modified (GM) crops, which are resistant to glyphosate (GR), has led to an unprecedented increase in glyphosate usage. The United States, as the largest producer of soybean in the world (33% in 2011),[1] has seen an increase in the number of glyphosate applications per year on soybean. For example, in Mississippi the number of glyphosate applications increased from 1.2 in 1995 to 2.6 in 2006.[2] Increased use of glyphosate has raised concerns of drift injury to non-GR crops. Glyphosate drift onto non-target crops from ground or aerial applications is common in the Mississippi Delta region. In 2011, 55 of 71 cases of herbicide drift onto non-target crops reported in Mississippi were attributed to glyphosate (Campbell J, Mississippi Department of Agriculture and Commerce, private communication, 2010). Crops such as rice, corn, soybean and cotton were affected by herbicide drift in Mississippi. Glyphosate is a non-selective herbicide and is toxic to sensitive plant species. Simulated effects of glyphosate drift have been studied on soybean[3,4] and other crops such as corn,[5–8] rice[5,9] and peanut.[10] Furthermore, downwind drift deposition of glyphosate from aerial and ground applications has been investigated.[11–17] In previous studies, plant biological effects, such as visual injury, leaf chlorophyll (CHL), shikimate, plant height and shoot dry weight, of glyphosate drift arising from aerial application onto non-GR soybean and cotton have been investigated,[17] along with the same effects on corn.[16]

In general, spray deposit sampling and biological damage assessment are tedious and labor-intensive endeavors. Remote sensing technology has been widely used and developed in agriculture[18–20] and can provide a rapid, cost-effective method for assessing crop injury caused by glyphosate drift. Studies using a convenient agricultural aircraft platform for remote sensing have been documented for weed discrimination and CHL-a detection in catfish ponds,[21] and studies using thermal imagery have been reported for water stress, zonal soil suitability and irrigation system diagnostics.[22]

Henry *et al.*[23] indicated that multiple indices formulated from the band information extracted from hyperspectral reflectance could distinguish between healthy and injured soybean and corn plants to which herbicides, glyphosate and paraquat had been applied. Huang *et al.*[24] examined the effect of glyphosate drift from aerial application on non-GR cotton by spray drift sampling and aerial multispectral remote sensing. Ortiz *et al.*[25] systematically studied the effect of glyphosate drift from aerial application on non-GR soybean, cotton and corn, using vegetation indices generated from aerial multispectral remote sensing.

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Although, as described above, remote sensing can be used to assess plant injury as a function of droplet concentration on spray samplers or leaves, much work is still needed to establish the quantitative relationship between crop injury (biological responses) and herbicide dosage to assure the characterization of crop injury using remote sensing parameters as a surrogate of plant biological responses. The specific objectives of the present study were to focus on soybean in order (1) to determine the effect of glyphosate applied at experimental drift rates on plant height, plant dry weight, CHL and yield and (2) to relate spectral responses from aerial multispectral imagery to biological responses to quantify the level of soybean injury.

2 MATERIALS AND METHODS

2.1 Experimental field

A field experiment was conducted in 2011 on the farm of the Crop Production Systems Research Unit of the USDA Agricultural Research Service in Stoneville, Mississippi (90° 52.197′ W, 33° 26.701′ N). In the middle of the field, eight blocks were divided for soybean plants. The predominant soils planted in soybean were a clay soil of the Sharkey series (very fine, smectitic, thermic Chromic Epiaquerts) and Commerce sandy loam (fine silty, mixed, superactive, non-acid, thermic Fluvaquentic Endoaquepts). Commerce silty clay loam and Tunica clay series were also present in this field, but their relative areas represented only 4.6 and 6.4% of the total field area, based on information provided by the Web Soil Survey (WSS), USDA Natural Resources Conservation Service (http://websoilsurvey.sc.egov.usda.gov/). These soil types were not predominantly under soybean. Field preparation consisted of disking and bedding in the fall of the previous year. The experimental area was treated with paraquat at 1.1 kg AI ha^{-1} a week prior to crop planting to kill existing vegetation. Non-GR soybean (SO80120LL) was planted on 9 May 2011 at a rate of 285 000 seeds ha^{-1}. Pendimethalin at 2.34 kg AI ha^{-1} and *S*-metolachlor at 1.17 kg AI ha^{-1} were applied to the entire experimental area immediately after soybean planting to provide early-season weed control. Herbicides were applied with a tractor-mounted sprayer with Tee Jet 4003 standard flat-spray nozzles delivering 140 L ha^{-1} of water at 193 kPa.

2.2 Experimental design and glyphosate application

The experiment was conducted in a randomized complete block design with four replications (Fig. 1). Each plot consisted of eight rows spaced 97 cm apart and 24.4 m long. A single application of glyphosate at five rates – 0.1*X*, 0.2*X*, 0.4*X*, 0.5*X* and 1.0*X* (1*X* = 0.866 kg AI ha^{-1} represents the recommended use rate for GR soybean) – was made on 8 June 2011. A non-treated plot was included as control. The commercial formulation of potassium salt of glyphosate (Roundup WeatherMax®; Monsanto Co., St Louis, MO) was used with no additional adjuvant. Glyphosate was applied using a four-row hooded spray boom mounted on a tractor equipped with Tee Jet 4003 standard flat-spray nozzles delivering 140 L ha^{-1} water at 193 kPa. At application, the soybean was at the 4–5-trifoliolate-leaf stage.

2.3 Field measurements

The relative soil texture of the field was characterized using a VERIS 3100 soil electrical conductivity (EC) measurement system (VERIS Technologies Inc., Salina, KS). Data sets were obtained for the top 30 cm and 91 cm of soil. Soybean plot locations relative to VERIS soil texture measurements for 0–30 cm (corresponding to the predominant soybean rooting depth) are illustrated in Fig. 2. Darker areas represent finer soil texture.

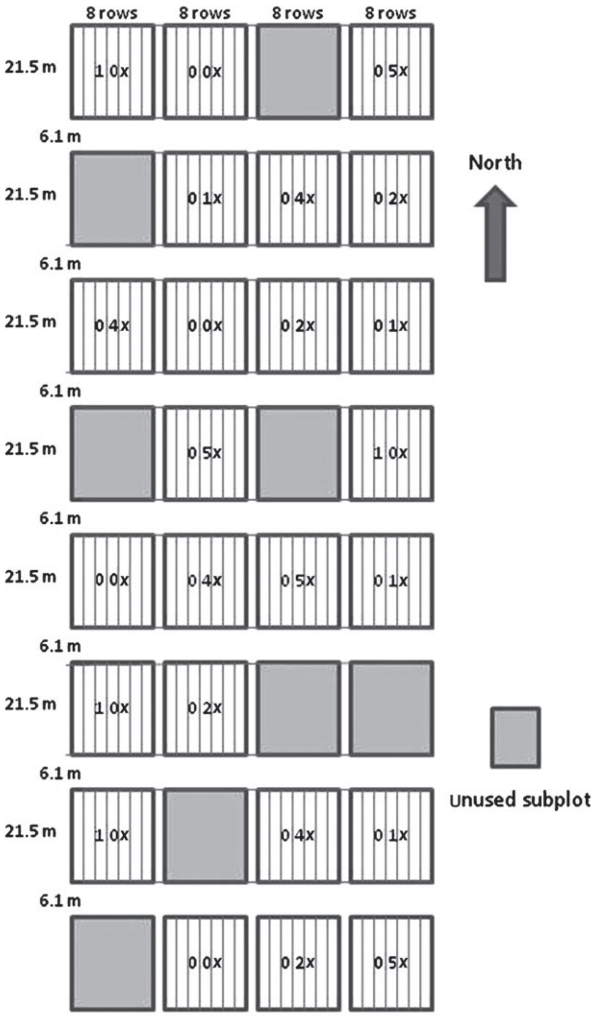

Figure 1. Experimental plots and treatments.

At 1, 2 and 3 weeks after application (WAA), each treated and non-treated control plot was sampled to measure biological responses: plant height, shoot dry weight and CHL. Soybean height was recorded on five randomly selected plants in each plot. Ten soybean plants from each plot were excised at the soil surface and oven dried at 60 °C for 72 h, after which dry weights were recorded. In each plot, the youngest fully expanded leaf from three plants was sampled for CHL determination. CHL was extracted with 10 mL of dimethyl sulfoxide and quantified using a spectrophotometer (UV160U; Shimadzu Corp., Kyoto, Japan) by a method previously described.[26]

At harvest, the yield of each plot was measured using the Ag Leader yield monitor and processed using SMS software (Ag Leader Technology, Ames, IA). All data were expressed as percentage reduction compared with the no-glyphosate-treated control.

2.4 Aerial multispectral imaging

Simultaneously with field measurements, multispectral images were collected from an Air Tractor 402B fixed-wing agricultural aircraft (Air Tractor, Inc., Olney, TX) using a MS 4100 camera (Geospatial Systems, Inc., West Henrietta, NY). This camera uses three

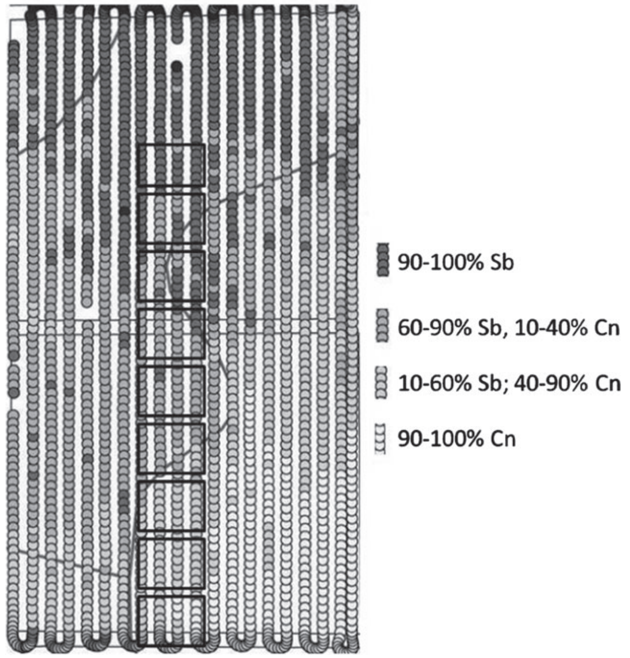

Figure 2. Soybean plots relative to VERIS readings. Darker areas signify finer soil texture.

charge coupled device sensors to acquire images in three spectral bands within the 400–1100 nm range of the electromagnetic spectrum to switch between the RGB and color infrared (CIR) mode, and in the present experiment provided a digital imaging resolution of 1920 (horizontal) × 1080 (vertical) pixel array per sensor with a 60° field of view using a 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) and configures digital output of image data with CameraLink standard or parallel digital data in either EIA-644 or RS-422 differential format. The camera was interfaced with the National Instruments IMAQ PCI-1428 frame grabber (National Instruments, Austin, Texas) connected to a Magma PCI conversion interface for use with a notebook computer. For this experiment, the camera was configured to acquire CIR images by using three 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). To assure the camera was pointed nadir during flight, the camera was shimmed backward 10° to compensate for the in-flight difference in vertical angle. The lens was calibrated for optimal focus and exposure for aerial operation.

Flights for aerial imaging were conducted at 1 and 3 WAA. The flight line was from south to north, and the flight altitude was 365 m. The latter was approximated from the aircraft's Global Positioning System (GPS) guidance system using an offset for inaccuracy in elevation noted at ground level using the Wide Area Augmentation System (WAAS)-corrected GPS signal. At this altitude and camera resolution, 20 cm horizontal ground spatial resolution and 40 cm vertical ground spatial resolution were obtained.

To convert the digital numbers of the CIR images to percentage reflectance, an IRR 180 radiometer (TerraVerde Technologies, Inc., Stillwater, OK) was used to record solar irradiance in the field to normalize images. This radiometer was set in the field on the same day as image acquisition, and signals were automatically recorded at a preset interval. After imaging was completed, the solar irradiance data were uploaded to the computer, and Image Correction Center software (TerraVerde Technologies, Inc.) in which the camera calibration parameters were stored was used to filter out anomalies caused by clouds. At the same time, the software read in the images to convert the digital numbers (DNs) of the images to the radiance images based on the camera calibration. Then, the radiance images were divided by the processed solar irradiance data to calculate true reflectance images. In this way, the original DN images were normalized to produce the percentage reflectance images without the requirement of standard reflectance panels.

2.5 Vegetation indices

Each image provided data in three original bands (green, red and NIR) from which vegetation indices were derived to demonstrate the relative vigor of the soybean plants in response to the glyphosate application in the early growth stage with soil background present. The vegetation indices (VIs) used in the study were as follows:

1 The normalized difference vegetation index (NDVI),[27] which was used for visualizing crop canopy vigor:

$$\mathrm{NDVI} = \frac{\mathrm{NIR} - \mathrm{Red}}{\mathrm{NIR} + \mathrm{Red}} \tag{1}$$

2 The soil adjusted vegetation index (SAVI),[28] which was used for considering the impact of soil when the canopy was not fully developed:

$$\mathrm{SAVI} = \frac{\mathrm{NIR} - \mathrm{Red}}{\mathrm{NIR} + \mathrm{Red} + L}(1 + L) \tag{2}$$

where L is a constant that is empirically determined to minimize VI sensitivity to soil background reflectance variation. If L is zero, SAVI equals NDVI. For intermediate vegetation cover ranges, L is typically around 0.5, which was assumed for the present study. For dense coverage, the L value would be lower, such as 0.25.

3 The ratio vegetation index (RVI),[29] which was used to verify the characterization of NDVI:

$$\mathrm{RVI} = \frac{\mathrm{NIR}}{\mathrm{Red}} \tag{3}$$

Note that the SR is not bounded, and its values can increase far beyond 1.

4 The green NDVI (GNDVI),[30] which was used to be more sensitive to CHL-a of the crop leaves:

$$\mathrm{GNDVI} = \frac{\mathrm{NIR} - \mathrm{Green}}{\mathrm{NIR} + \mathrm{Green}} \tag{4}$$

2.6 Statistical analysis

Results were analyzed using both PROC GLM and PROC Mixed in SAS 9.2 (Statistical Analysis System; SAS Institute, Cary, NC). The linear model was shown to provide the best fit to the data in GLM over the log-transformed data. The model determined yield as a

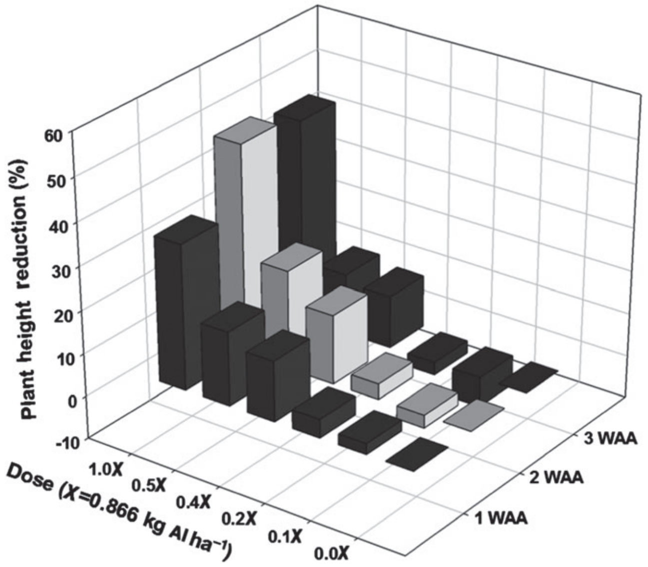

Figure 3. Percentage reduction in plant height at 1, 2 and 3 WAA in response to multiple glyphosate application rates.

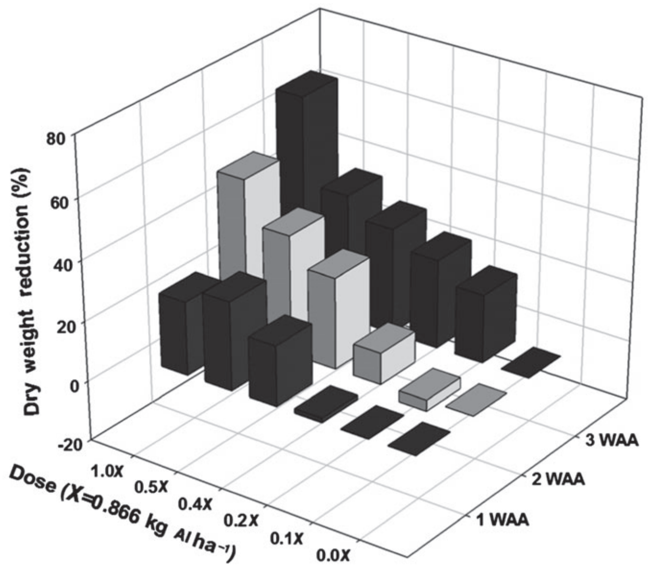

Figure 4. Percentage reduction in plant dry weight at 1, 2 and 3 WAA in response to multiple glyphosate application rates.

function of application rate and relative soil texture; the latter was determined by VERIS readings in each plot. Least-squares means (LSMEANS) were determined using PROC Mixed to model yield at each dose, holding soil texture at constant values. In this way, yield response could be quantified over a range of artificially set soil textures representing extremes and several points in between. The percentage reduction in biological response parameters at 1, 2 and 3 WAA were calculated relative to the control to determine the effects of glyphosate dose on biological responses to soybean injury. Similarly, the percentage reduction in the VIs at 1 and 3 WAA was calculated to compare the trend of the VIs with the biological responses. The VIs were regressed against biological response parameters and yield. Fisher's pairwise least significant difference (LSD) procedure was used for pairwise comparisons of dose effect on yield at the 0.01 level of significance.

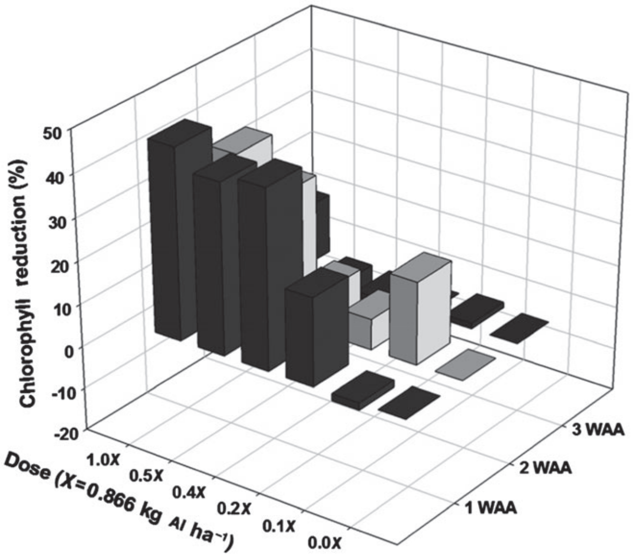

Figure 5. Percentage reduction in plant chlorophyll level at 1, 2 and 3 WAA in response to multiple glyphosate application rates.

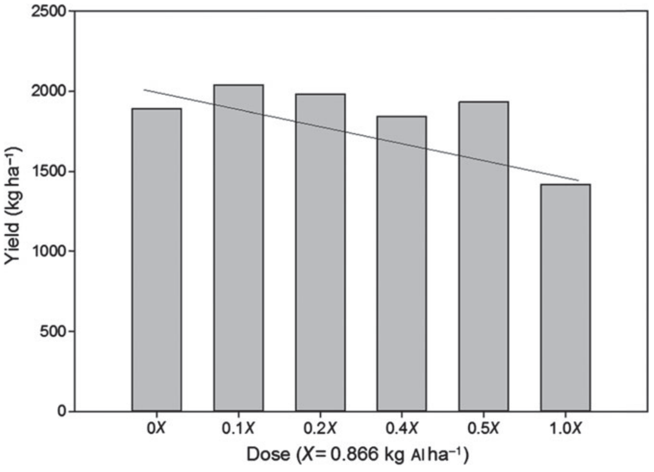

Figure 6. Soybean yield in response to multiple glyphosate application rates.

3 RESULTS AND DISCUSSION

3.1 Biological responses and yield

Reduction in plant height versus the control was apparent early on for dosages of 0.4*X* and higher, at 1 WAA (Fig. 3). The trend stayed relatively level to 3 WAA. Height differences were not pronounced at the 0.2*X* dose and below all the way to 3 WAA (Fig. 4). Reduction in plant dry weight was negligible at the 0.2*X* dose and below at 1 WAA, but was reduced by about 20% at the lowest dose (0.1*X*) at 3 WAA. At 1 WAA, 0.4–1.0*X* rates exhibited similar percentage reductions in dry weight; only until 2 WAA was there any response with the 0.2*X* dose (Fig. 4). Most of the reduction in plant CHL occurred at 1 WAA with the 0.4*X* dosage and higher (Fig. 5), but there was a slight reduction in CHL at the 0.2*X* dose at 1 WAA, as expected. Interestingly, yield response did not follow a clear trend with increasing dosage (Fig. 6), and the only significant reduction in yield occurred at the 1.0*X* dosage. Soybean had severe injury at the 1.0*X* rate, resulting in about 25% reduction in yield. Soybean yields were not affected at rates of 0.5*X* and lower. Other researchers have reported that there

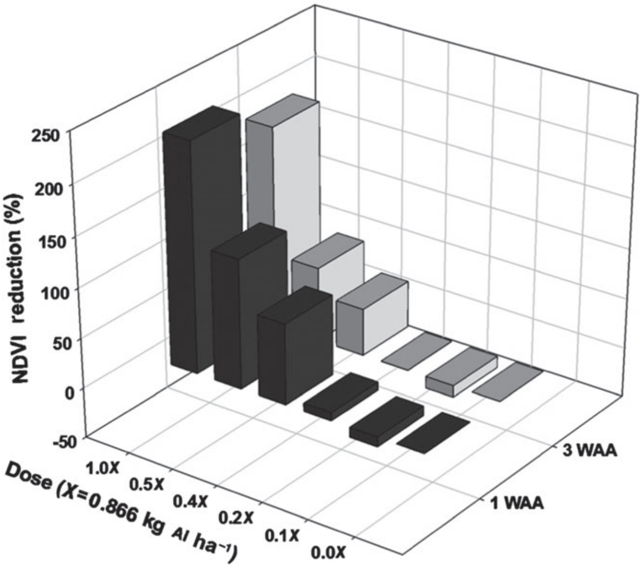

Figure 7. Percentage reduction in NDVI at 1, 2 and 3 WAA in response to multiple glyphosate application rates.

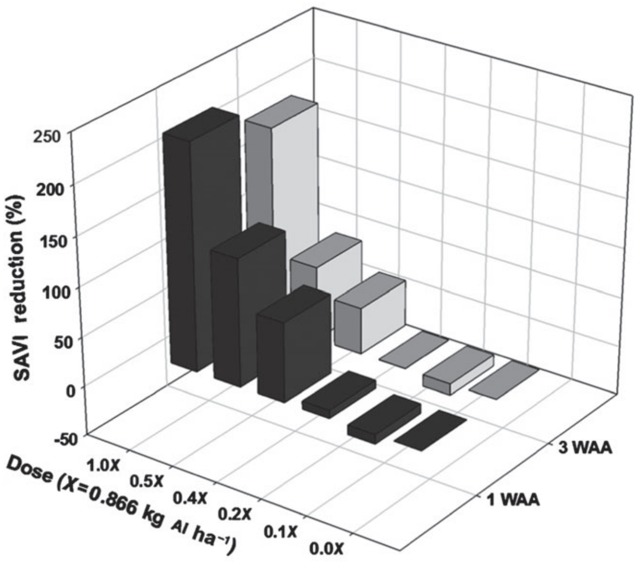

Figure 8. Percentage reduction in SAVI at 1, 2 and 3 WAA in response to multiple glyphosate application rates.

was no yield reduction in non-GR soybean at glyphosate rates of 105–140 g AI ha^{-1} (about 0.14*X*).[3,4] It has been documented that the glyphosate G_{50} rate (the rate required to cause a 50% reduction in plant growth) of non-GR soybean is 250 g AI ha^{-1} (about 0.29*X*).[31] Furthermore, glyphosate at 280 and 560 g AI ha^{-1} (about 0.32*X* and 0.65*X* respectively) reduced non-GR soybean shoot dry weight by 32 and 67% respectively at 4 WAA.[32] In the present study, absence of yield reduction at rates of 0.5*X* and below may have been due to cultivar differences. The cultivar used in this study was a glufosinate-resistant cultivar, as opposed to conventional (non-transgenic) cultivars used in other research.

Soil effect on yield was not significant at the 0.05 level ($P = 0.0613$). This can be further broken down by modelling yield estimates using least-squares means. Mean yields showed a trend consistent with Fig. 6 (data pooled across soil texture) when

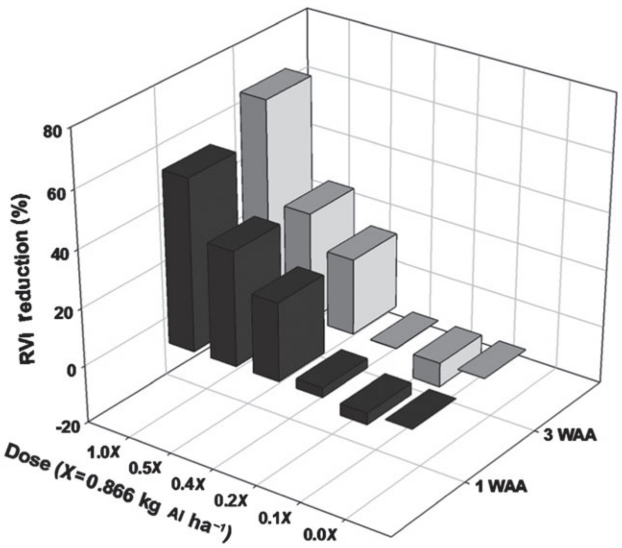

Figure 9. Percentage reduction in RVI at 1, 2 and 3 WAA in response to multiple glyphosate application rates.

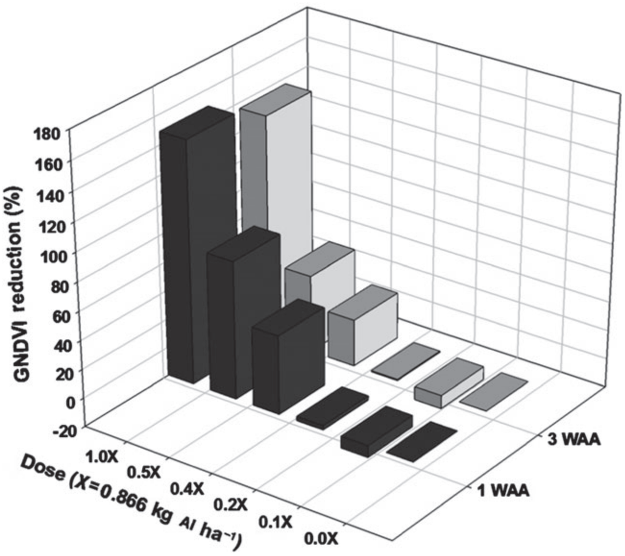

Figure 10. Percentage reduction in GNDVI at 1, 2 and 3 WAA in response to multiple glyphosate application rates.

soil texture was artificially set to a value matching a fine-medium textured soil. This trend still held true when set to the coarser textured soil (south end of Fig. 2), with the highest yield being at the 0.1*X* dose in both cases.

3.2 VIs and correlation with biological response and yield

As expected, the VIs that indicate vigor (NDVI and SAVI) showed clearly decreasing values with increase in dosage, but the only noticeable response appeared at doses of 0.4*X* and above. At 1 WAA there was only an 8% decrease in NDVI at 0.2*X*, rising to over 100% decrease at 0.4*X* (Fig. 7). SAVI showed a similar trend (Fig. 8). The RVI showed a similar trend, although the percentage reductions in this index were not as great overall (Fig. 9). The GNDVI showed an overall percentage reduction that was intermediate between the NDVI/SAVI and RVI (Fig. 10), indicating that the NDVI (and its variant SAVI) were the most sensitive indices.

Table 1. Regression of VIs with biological responses and yield at 1 and 3 WAA

	NDVI (x)	SAVI (x)	RVI (x)	GNDVI (x)
1 WAA	$PH = 18.41x + 23.34$ ($R^2 = 0.82$; $P < 0.0001$) $DW = 2.48x + 3.72$ ($R^2 = 0.20$; $P = 0.0278$) $CHL = 0.51x + 0.50$ ($R^2 = 0.28$; $P = 0.0076$) $YD = 987x + 1800$ ($R^2 = 0.43$; $P = 0.0005$)	$PH = 12.3x + 23.34$ ($R^2 = 0.82$; $P < 0.0001$) $DW = 1.66x + 3.72$ ($R^2 = 0.20$; $P = 0.0278$) $CHL = 0.34x + 0.50$ ($R^2 = 0.28$; $P = 0.0076$) $YD = 660x + 1800$ ($R^2 = 0.43$; $P = 0.0005$)	$PH = 7.96x + 14.89$ ($R^2 = 0.79$; $P < 0.0001$) $DW = 1.15x + 2.48$ ($R^2 = 0.22$; $P = 0.0203$) $CHL = 0.21x + 0.27$ ($R^2 = 0.25$; $P = 0.0135$) $YD = 399x + 1379$ ($R^2 = 0.36$; $P = 0.0021$)	$PH = 23.82x + 22$ ($R^2 = 0.81$; $P < 0.0001$) $DW = 3.18x + 3.53$ ($R^2 = 0.19$; $P = 0.0308$) $CHL = 0.67x + 0.46$ ($R^2 = 0.28$; $P = 0.0074$) $YD = 1270x + 1727$ ($R^2 = 0.42$; $P = 0.0007$)
3 WAA	$PH = 46.64x + 44.17$ ($R^2 = 0.89$; $P < 0.0001$) $DW = 12.75x + 7.82$ ($R^2 = 0.55$; $P < 0.0001$) $CHL = 0.11x + 0.70$ ($R^2 = 0.08$; $P = 0.1719$) $YD = 877x + 1720$ ($R^2 = 0.58$; $P < 0.0001$)	$PH = 31.16x + 44.17$ ($R^2 = 0.89$; $P < 0.0001$) $DW = 8.52x + 7.82$ ($R^2 = 0.55$; $P < 0.0001$) $CHL = 0.07x + 0.70$ ($R^2 = 0.08$; $P = 0.1719$) $YD = 586x + 1720$ ($R^2 = 0.58$; $P < 0.0001$)	$PH = 17.27x + 25.09$ ($R^2 = 0.83$; $P < 0.0001$) $DW = 5.20x + 1.89$ ($R^2 = 0.62$; $P < 0.0001$) $CHL = 0.03x + 0.68$ ($R^2 = 0.04$; $P = 0.3664$) $YD = 308x + 1387$ ($R^2 = 0.49$; $P = 0.0001$)	$PH = 61.21x + 39.68$ ($R^2 = 0.85$; $P < 0.0001$) $DW = 16.46x + 6.64$ ($R^2 = 0.51$; $P < 0.0001$) $CHL = 0.17x + 0.69$ ($R^2 = 0.13$; $P = 0.1245$) $YD = 1142x + 1637$ ($R^2 = 0.55$; $P < 0.0001$)

[a] PH = plant height; DW = dry weight; CHL = chlorophyll; YD = yield.

VIs regressed well with most plant biological response parameters except for dry weight at 1 WAA and CHL at 1 and 3 WAA, as illustrated in Table 1. There was considerable scatter in the plant dry weight (DW) and CHL data at 1 WAA, shown by way of example for NDVI (Fig. 11), but there appeared to be a definite CHL response. At 1 WAA, the soybean was in the 5–6-trifoliolate-leaf stage. The plant leaf dry weight (of dead and green shoots) did not vary much owing to the limited plant growth within 1 WAA, although the VIs varied proportionally to the degree of vegetation in each plot as affected by glyphosate rate. At the same time, the chlorophyll content of soybean leaves in response to glyphosate exhibited a fairly consistent trend of decrease from highest to lowest with increased rate from 0.0*X* to 1.0*X*. At 3 WAA, the correlation between plant dry weight and glyphosate rate improved considerably, with a high level of significance ($P = 0.0001$) (Fig. 12 and Table 1), mainly owing to abundant growth of plants in non-treated plots (0.0*X*) and survivor plants in glyphosate-treated plots, especially at lower rates. However, the converse was observed in CHL response, as

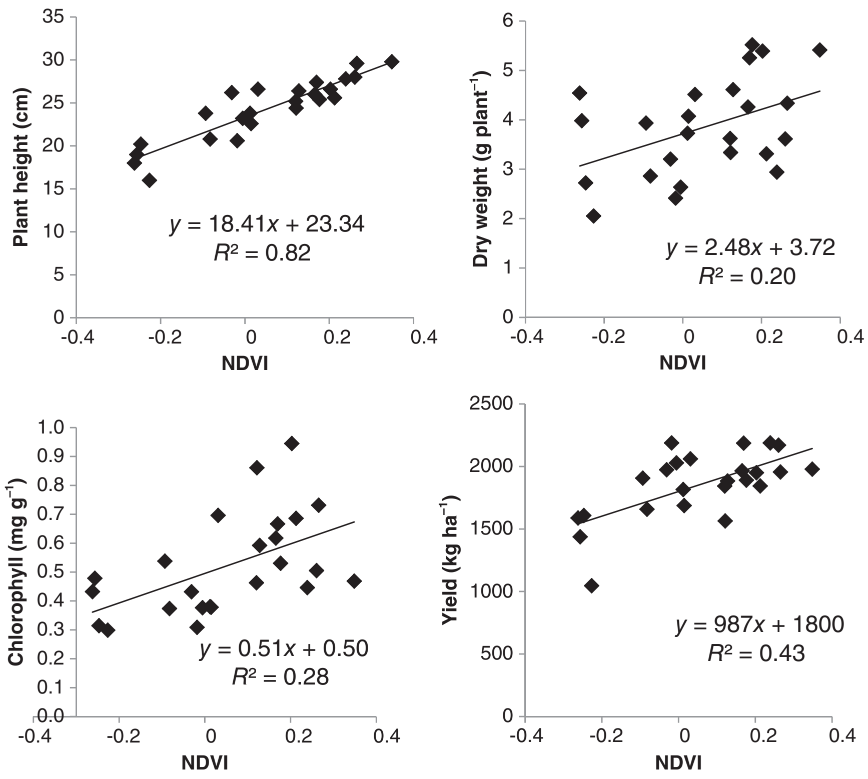

Figure 11. Regression of NDVI with biological responses and yield at 1 WAA.

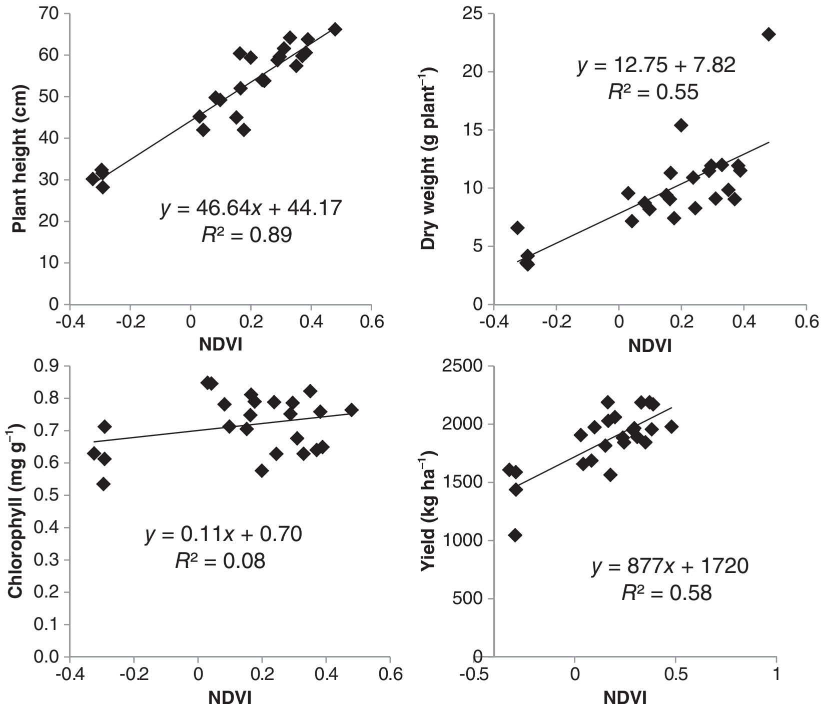

Figure 12. Regression of NDVI with biological responses and yield at 3 WAA.

surviving plants recovered from glyphosate injury and the chlorophyll content was restored almost to the levels of plants in non-treated plots. GNDVI (which uses spectral response in the green band as opposed to the red band in the case of NDVI) showed slightly better correlation than the other VIs with CHL at 3 WAA, but the response was still poor. In the plots, especially with a high glyphosate rate, although some plants were dead, surviving plants had a strong green pigment because they recovered by regaining CHL to normal levels, resulting in an insignificant variation in CHL, regardless of dose. The change in CHL was less relevant than the VIs. Plant height as an indicator of vigor, which was measured directly in the field, correlated very well with VIs both at 1 WAA and at 3 WAA ($P = 0.0001$), consistently. Yield response for all indices was quite good as early as at 1 WAA, and was further improved at 3 WAA. These responses were highly significant as well ($P = 0.0005$).

The correlation of many spectral indices with biological responses and yield using a multispectral imaging system operated from agricultural aircraft proved to be very successful. To the present authors' knowledge, these have been the only exhaustive studies documenting plant injury using this convenient remote sensing platform. Agricultural aircraft are used in the field for spraying in the Midsouth and many other areas of the United States, so it stands to reason that remote sensing runs could supplement spraying activity or be performed in stand-alone fashion. Different dosages of herbicide can be applied in a controlled way to assess the capabilities of the imaging systems to infer potential reduction in yield and possible recovery from varying degrees of injury.

4 CONCLUSIONS AND FUTURE NEEDS

The results of the research make it possible to conclude that yield as a function of dosage was predicted well both at 1 and at 3 WAA across all VIs. Yield was significantly less only at the highest dosage level (1.0*X*) because of strong regrowth of plants after the herbicide effect had gone away on surviving plants. Although not statistically different to other high-yielding responses, overall yield was highest at the 0.1*X* dose. This provides evidence for possible growth stimulation in response to a low glyphosate dose. The effect of soil texture on yield was not significant at the 5% level. Differences in CHL level as a function of dosage could not be detected using VIs, regardless of WAA. The poor correlation between VIs and CHL at 3 WAA was because the surviving plants in high-dose-treated plots recovered from the injury by regaining the CHL to the normal levels so that the overall variation in the CHL level across all doses was much smaller than the variations in the VIs. Differences in dry weight as a function of dosage could be detected reliably at 3 WAA, with RVI showing the best response. Differences in plant height, which was measured directly in the field, as a function of dosage could be detected reliably across all VIs. The VIs were well correlated with plant height and yield, but poorly with CHL, regardless of WAA. This showed that indices could be used to determine soybean injury from glyphosate, as indicated by plant height, and the yield reduction could also be predicted.

The experimental methods will be refined to formulate a protocol for plant sampling in plots treated at different glyphosate doses. The protocol should direct the sampling process to estimate the biomass available in the plots. This is very important for reducing the error in correlation between VIs and biological responses, especially leaf dry weight and CHL concentration. In order to verify the estimation of the biomass from plant sampling, correlation with the estimation from aerial CIR imagery can be performed.

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