# ASSESSING CROP INJURY CAUSED BY AERIALLY APPLIED GLYPHOSATE DRIFT USING SPRAY SAMPLING

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ABSTRACT. Crop injury caused by off-target drift of aerially applied glyphosate is of great concern to farmers and aerial applicators. An experiment was conducted in 2009 to determine the extent of injury due to near-field glyphosate drift from aerial application to glyphosate-sensitive cotton, corn, and soybean. The drift effects on different crops were characterized in a field planted in alternating blocks of these sensitive crops. Spray samplers were placed in the spray swath and downwind to quantify relative concentrations of the applied chemical. An Air Tractor 402B spray airplane equipped with fiftyfour CP-09 nozzles was flown down the center of the field, applying 866 g a.e. ha<sup>-1</sup> glyphosate (Roundup Weathermax) and rubidium chloride tracer at a 2.6 g ha<sup>-1</sup> spray rate. Relative concentrations of the tracer were quantified from downwind spray samplers by atomic absorption spectroscopy. Biological responses of the crops to the glyphosate drift were measured at weekly intervals, along with airborne multispectral imaging. Statistical analysis indicated that spray drift sampling was able to explain downwind crop injury, and physical responses could be estimated for evaluating crop injury caused by the drift of aerially applied glyphosate. Correlations between the relative concentration of the spray tracer and the crop biological responses identified that cotton was less sensitive to glyphosate drift than corn and soybean. Regression models for the injuries of cotton and soybean one and two weeks after field treatment and for the injury of corn one week after treatment with the percent applied glyphosate from the label rate were developed and evaluated with chlorophyll data. The cotton models for visual injury and plant height at one and two weeks after treatment were well validated with chlorophyll data (average of 1 for the ratio of estimated vs. measured chlorophyll, and low root mean squared deviations). However, in validation of the corn model, the ratio of estimated vs. measured chlorophyll deviated from 1. Compared with validation of the corn model, the validation of the soybean models showed less bias, with a value close to 1 for the ratio of estimated vs. measured chlorophyll. These results have established a method of characterizing crop injury caused by aerially applied glyphosate and can provide guideline data for use by farmers and aerial applicators.

**Keywords.** Aerial application, Crop injury, Glyphosate drift, Spray sampling.

erial application of crop production and protection materials is often employed in agriculture production for convenience and timeliness. However, off-target drift of these applied agents can be a potential source of environmental concern. When aerially applied pesticides drift off-target close to sensitive crops, exposed crops can exhibit characteristics of injury or damage.

The fate and transport of aerially applied sprays is a complex process involving a number of variables, including spray equipment, meteorological factors, application pa-

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rameters, tank mix characteristics, and crop canopy effects. Several projects characterizing the relationship between these variables were conducted in an effort to control and reduce off-target (Smith et al., 2000; Huang et al., 2009; Huang et al., 2010b).

Glyphosate, a foliar-applied, nonselective broadspectrum herbicide widely used for burndown and postharvest applications in glyphosate-resistant transgenic crops (James and Krattiger, 1996), has seen increased usage as the acreage of glyphosate-resistant (GR) crops has increased. As a result of the increased herbicide usage, the number of drift incidences increased 60% from 2007 to 2008 in Mississippi (Reddy et al., 2010a).

Glyphosate injury from simulated drift has been reported in corn, soybean, and rice (Buehring et al., 2007; Brown et al., 2009; Ellis et al., 2003; Bellaloui et al., 2006; Ellis and Griffin, 2002; Koger et al., 2005; Reddy et al., 2010a, 2010b). A number of field studies assessing the impact of drift from aerially applied glyphosate on individual sensitive plant species have been conducted Reddy et al. (2010b) reported that corn injury resulting from drift associated with aerially applied glyphosate is highest at the edge of the spray swath and decreases gradually with distance downwind. To evaluate crop injury, Huang et al. (2010c) demonstrated that NDVI (normalized difference vegetation index) obtained from aerially acquired multi-

spectral imaging could be used to document cotton injury occurring from drift associated with aerially applied glyphosate. Huang et al. (2010a) described that the downwind deposition of aerially applied glyphosate decreases with downwind distance from the edge of the spray swath, and the injury of cotton is decreased as well. Therefore, the deposition of applied glyphosate could be used to estimate and characterize the crop injury downwind of the spray swath.

This study examines the effect of glyphosate drift from aerial application on non-GR cotton (*Gossypium hirsutum*), corn (*Zea mays* L.), and soybean (*Glycine max*) by spray drift sampling, and models the relationship between the deposition of applied glyphosate and the crop injury downwind of the spray swath. The specific objectives of this study were to: (1) study the relationship between spray drift deposition and crop biological responses, and (2) develop and evaluate regression models for estimation of crop injury based on spray drift deposition.

## MATERIALS AND METHODS

### FIELD TEST LAYOUT

The USDA-ARS Crop Production Systems Research farm at Stoneville, Mississippi (33° 26′ N, 90° 55′ W) was used to conduct the aerial application experiment to determine injury and biological responses to glyphosate drift on non-GR cotton, corn, and soybean. For the experiment, non-GR cotton cultivar FM955LL at 100,000 seed ha<sup>-1</sup>, non-GR corn hybrid Pioneer 31P41 at 75,000 seed ha<sup>-1</sup>, and

non-GR soybean cultivar SO80120LL at 285,000 seed ha<sup>-1</sup> were planted on 23 July 2009. Each crop was planted in eight rows spaced 102 cm apart and 80 m long with four replications (fig. 1).

#### **EXPERIMENT OPERATION**

One aerial application of glyphosate was made on 12 August 2009 using an Air Tractor 402B airplane equipped with fifty-four CP-09 spray nozzles (CP Products, Tempe, Ariz.) set at a 5° deflection angle. The spray solution consisted of of Roundup Weathermax (Monsanto Co., St. Louis, Mo.) applied at a rate of 866 g a.e. ha<sup>-1</sup> with rubidium chloride (RbCl) at 2.6 g ha<sup>-1</sup> as a tracer. The aerial sprayer delivered the liquid at a rate of 46.8 L ha<sup>-1</sup> with a release height of 3.7 m. The effective swath width was 18.3 m at an operating speed of 225 km h<sup>-1</sup>.

The flight line followed the center of the field perpendicular to the crop rows (fig. 1), going from west to east. Weather conditions were recorded during the 4 s spray run. The average wind speed was 11.2 km h<sup>-1</sup> from the northeast, averaging 64° from true north. Average air temperature was 28.5°C and relative humidity was 72% as acquired during the spray run using a Kestrel 4500 weather tracker (Nielsen-Kellerman, Boothwyn, Pa.) mounted on a tripod.

At application, cotton was at two- to three-leaf stage, corn was at four-leaf stage, and soybean was at two- to three-trifoliolate leaf stage.

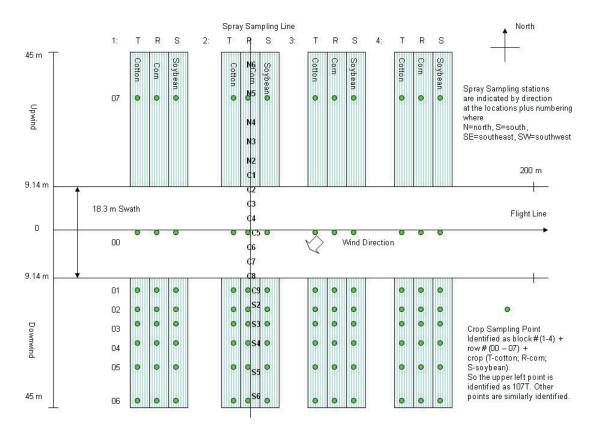


Figure 1. Field layout for the aerial glyphosate spray test.

726 Transactions of the ASABE

#### **SPRAY SAMPLING**

The downwind in-swath and drift sampling stations were set up and marked from north to south at 0 m (C5), 3 m (C6), 6 m (C7), 9 m (C8), 12 m (C9), 15 m (S2), 20 m (S3), 25 m (S4), 35 m (S5), and 45 m (S6) measured from the flight line downwind in the 18.3 m swath (fig. 1).

Mylar sampling sheets ( $130 \times 127$  mm) were placed at all downwind in-swath and drift sampling stations (C5 to C9, S2 to S6), and collected right after field treatment. Mylar sheets were processed by added 28.78 mL of 1% nitric acid to the sample bag and then agitated on a shaker for 20 min (10 min on each side) to ensure complete washing of the sheet. The nitric acid solution was also used as a calibration blank for the atomic absorption spectrometer (AAnalyst 600, Perkin-Elmer, Waltham, Mass.), which was then used to determine the concentration of RbCl tracer on each sheet.

### CROP SAMPLING AND MEASUREMENT

Crops were sampled at points parallel with the northsouth spray sampling line (fig. 1). The crop sampling stations were lined up in the west-east direction to establish four replications corresponding to the spray sampling stations C5, C9, S2, S3, S4, S5, S6, and N5. The repeated crop sampling stations were labeled 00, 01, 02, 03, 04, 05, 06, and 07, respectively, with replications 1, 2, 3, and 4. The crop sampling stations corresponding to N5 (the upwind sample station at 35 m measured from the flight line upwind in the 18.3 m swath) were included as a control (crops not exposed to glyphosate). At each crop sampling station, crop plants were visually inspected and collected at one, two, and three weeks after the field treatment. With laboratory measurement, the crop sampling process resulted in the following two groups of crop biological responses to the drift of aerially applied glyphosate:

## **Physical Responses**

Crop injury and plant height: Cotton (C), corn (R), and soybean (S) injury was estimated visually at each sampling location based on chlorosis, necrosis, stunted growth, and death. Crop injury was visually estimated on a scale of 0 (no injury) to 100 (plant death) at one, two, and three weeks after aerial application (1, 2, and 3 WAT). Plant height was determined on five randomly selected plants at 2 and 3 WAT.

**Shoot dry weight:** At each sampling location, ten plants were randomly excised at the soil surface and transported to the laboratory at 1, 2, and 3 WAT. The collected plants were oven dried (60°C, 72 h), and dry weights were recorded.

## Physiological Responses

Chlorophyll content: Chlorophyll was determined at 1, 2, and 3 WAT. The youngest fully expanded leaf from three randomly selected plants at each location was sampled. Chlorophyll was extracted with 10 mL dimethyl sulfoxide, and chlorophyll concentrations were determined spectrophotometrically, as described by Hiscox and Israelstam (1979).

**Shikimate content:** At each sampling location, ten plants were randomly excised at the soil surface and imme-

diately transported to the laboratory for assaying shikimate content at 1, 2, and 3 WAT. One 6 mm diameter disc per leaf was sampled from the youngest fully expanded leaf of the ten plants using a standard paper hole-punch adjacent to the midrib. Shikimate levels were determined spectrophotometrically following the protocols described by Reddy et al. (2010b).

The physical responses (excluding shoot dry weight) are direct observations of the crop injury, while the physiological responses are laboratory measurements of crop reaction to the sprayed herbicide. The crop physical responses were modeled with the downwind drift deposition of aerially sprayed glyphosate, and then the models were evaluated by determining the fitness of the measured and estimated physiological response variables.

#### STATISTICAL ANALYSIS

The correlation between the crop biological response variables, such as percent visual injury, crop height, shoot dry weight, chlorophyll, and shikimate, and the RbCl concentration was evaluated. The crop physical response variables were regressed with the percent applied glyphosate from the label rate to estimate crop injury. The percent applied glyphosate was converted from the relative concentration of RbCl by dividing the RbCl concentration on each Mylar sheet by the total RbCl mixed rate (2.6 g ha<sup>-1</sup>). The statistical analysis was conducted using PROC GLM in SAS (SAS Institute, Inc., Cary, N.C.).

## **RESULTS AND DISCUSSION**

Correlation coefficients between cotton, corn, and soybean biological response variables and RbCl concentration at 1, 2, and 3 WAT were calculated and analyzed. For cotton, the data indicate that the concentration of RbCl tracer is positively correlated with visual injury and shikimate and negatively correlated with plant height, shoot dry weight, and leaf chlorophyll content (fig. 2). For cotton physical responses, the correlation of RbCl was strong but decreased gradually with time at 1, 2, and 3 WAT with visual injury (0.89 for 1 WAT, 0.75 for 2 WAT, and 0.69 for 3 WAT), plant height (-0.78 for 2 WAT and -0.66 for 3 WAT), and shoot dry weight (-0.86 for 1 WAT, -0.70 for 2 WAT, and -0.67 for 2 WAT, and -0.68 for 2 WAT, and -0.79 for

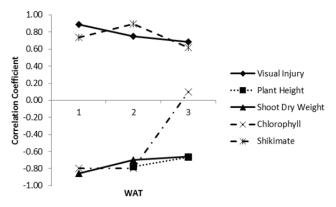


Figure 2. Correlation coefficients between RbCl and cotton biological responses.

55(3): 725-731 727

0.66 for 3 WAT). The shape of the relationship changed with time so that greater visible injury occurred at lower concentrations of RbCl inferring glyphosate.

highest positive correlation with RbCl at 2 WAT (0.89) compared with the correlations at 1 WAT (0.74) and 3 WAT (0.62). This indicates that, at 2 WAT, the drift of aerially applied glyphosate had a strong impact on cotton and is consistent with the observation that, at 3 WAT, the plant began to recover. Chlorophyll had strong negative correlations with RbCl at 1 WAT (-0.80) and 2 WAT (-0.80) and no correlation at 3 WAT (0.10). This also indicates that spray drift had a strong impact on cotton during the first two weeks after glyphosate application; however, by 3 WAT, plant color returned. The physiological responses (shikimate and chlorophyll) indicated that the physical responses (visual injury and plant height) could be used effectively to determine the injury of cotton caused by the drift of aerially applied glyphosate at 1 and 2 WAT.

In the corn plots, the RbCl concentration was positively correlated with visual injury; negatively correlated with plant height, shoot dry weight, and leaf chlorophyll content; and alternatively correlated with shikimate (fig. 3). For crop physical responses, the correlation of RbCl was initially strong but decreased gradually with time with visual injury (0.72 for 1 WAT, 0.65 for 2 WAT, and 0.50 for 3 WAT), plant height (-0.70 for 2 WAT and -0.53 for 3 WAT), and shoot dry weight (-0.73 for 1 WAT, -0.63 for 2 WAT, and -0.59 for 3 WAT), indicating that the impact of glyphosate drift on corn was also transient.

For corn physiological responses, shikimate had a significantly positive correlation with RbCl at 1 WAT (0.81) and then reversed to a significantly negative correlation at 2 WAT (-0.85) and 3 WAT (-0.70). The significantly negative correlations were because, at 2 and 3 WAT, the corn plants near the aerial spray swath had died and the shikimate levels determined from the plants became lower than normal or close to zero. This indicates that serious injury of corn in the first week after field treatment could be well characterized by the drift of the aerially applied glyphosate. This also indicates that corn was more sensitive to the glyphosate drift than cotton. Chlorophyll exhibited a rapid change in corn, with a steep reduction of correlation with RbCl from 1 WAT (-0.80) and 2 WAT (-0.74) to 3 WAT

ry, but plant height not available at 1 WAT) could be used For cotton physiological responses, shikimate had the effectively to determine the injury of corn caused by the drift of aerially applied glyphosate at 1 WAT. In the soybean plots, concentration of RbCl was positively correlated with visual injury; negatively correlated

with plant height, shoot dry weight, and leaf chlorophyll content; and alternatively correlated with shikimate (fig. 4). For crop physical responses, the correlation of RbCl was strong but decreased with time at 1 and 2 WAT, leveled off at 3 WAT with visual injury (0.81 for 1 WAT, 0.76 for 2 WAT, and 0.77 for 3 WAT) and shoot dry weight (-0.71 for 1 WAT, -0.69 for 2 WAT, and -0.73 for 3 WAT), and decreased with plant height (-0.91 for 2 WAT and -0.78 for 3 WAT). As with cotton, the shape of the relationship changed with time so that greater visible injury occurred at lower concentrations of RbCl. However, unlike cotton, the shape of the RbCl vs. injury curve changed abruptly at 2 WAT and essentially leveled off at 3 WAT.

(-0.35). The physiological indicators (shikimate and chlo-

rophyll) also indicated that physical responses (visual inju-

For soybean physiological responses, shikimate had a significantly positive correlation with RbCl at 1 WAT (0.93) and then dropped significantly at 2 WAT (0.12) and 3 WAT (-0.30). The negative correlation at 3 WAT was caused by the fact that the soybean plants near the aerial spray swath had died, and the shikimate levels determined from the plants became lower than normal or close to zero. As with the RbCl vs. injury correlation, this indicates that serious injury of soybean happened in the first two weeks after field treatment. Soybean was less sensitive to the glyphosate drift than corn but more sensitive than cotton. Chlorophyll showed a gradually decreased correlation with RbCl from 1 WAT (-0.96) and 2 WAT (-0.95) to 3 WAT (-0.78). The indication of the physiological responses (shikimate and chlorophyll) shows that the physical responses (visual injury and plant height) could be used effectively to determine injury to soybean caused by the drift of aerially applied glyphosate at 1 and 2 WAT.

Based on the above analysis, regression equations were built with the percent applied glyphosate for visual injury and plant height of cotton at 1 and 2 WAT, visual injury of corn at 1 WAT, and visual injury and plant height of sovbean at 1 and 2 WAT, as shown in table 1. These equations could be used to assess cotton, corn, and soybean injury

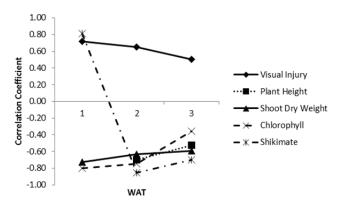


Figure 3. Correlation coefficients between RbCl and corn biological

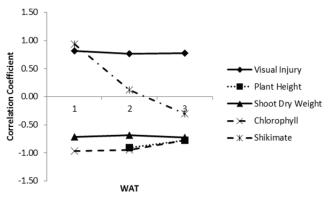


Figure 4. Correlation coefficients between RbCl and soybean biologi-

Table 1. Regression equations of visual injury and plant height (y) with percent applied glyphosate (x).

Crop	Visual Injury at 1 WAT	Plant Height at 2 WAT
Cotton	y = 16.293x + 21.779	y = -2.63767x + 22.268
	$(R^2 = 0.7879)$	$(R^2 = 0.6029)$
Corn	y = 19.90066x + 42.568	NA
	$(R^2 = 0.5148)$	
Soybean	y = 19.09662x + 40.054	y = -9.73206x + 37.185
-	$(R^2 = 0.6612)$	$(R^2 = 0.8222)$

caused by glyphosate drift simply based on crop physical responses (visual injury and plant height).

The crop injury and plant height were visually observed and measured from the spray center line (00) downwind toward the border of the field (06), as shown in figure 1. The chlorophyll and shikimate levels also showed physiological reactions to the glyphosate drift from the spray center line (00) downwind toward the border of the field (06). Table 2 shows the crop injury ranges characterized by mean values of visual injury, plant height, chlorophyll level, and shikimate level at 1 WAT and 2 WAT compared to no glyphosate exposure control (upwind 35.4 m sampling station N5; fig. 1).

The 1 WAT data indicate that the injury in cotton was visually observed from the spray center line (00T) downwind toward the border of the field (06T), injury in corn was visually observed from the spray center line (00R) to the field downwind (05R), and injury in soybean was visually observed from the spray center line (00S) to the field downwind (06S). However, chlorophyll showed physiological reactions to the glyphosate drift from the spray center line (00) to positions 03T, 03R, and 01S for cotton, corn, and soybean, respectively, and shikimate levels showed physiological reactions to the glyphosate drift from the spray center line (00) to positions 03T, 03R, and 04S for cotton, corn, and soybean, respectively.

The 2 WAT data indicate that the downwind distance of visual injury in cotton was reduced to position 05T from the spray center line (00T), and the distance of visual injury in soybean was reduced to position 04S from the spray center line (00S). The chlorophyll data showed that the downwind distance of physiological reaction to the glyphosate drift was reduced to position 01T and stayed at 01S from the spray center line (00) for cotton and soybean, respectively. The shikimate levels showed that the downwind distance of physiological reaction to the glyphosate drift was reduced to position 02T from the spray center line (00) and to position 01S for cotton and soybean, respectively. Differences between crop visual inspection and physiological indication of crop injury could have caused some error in the models for estimation of crop injury based on sprayed glyphosate.

To evaluate the models in table 1, regressions of chloro-

Table 2. Crop injury range in spray downwind distance.

		Visual	Plant	Chlorophyll	Shikimate
Crop	WAT	Injury	Height	Level	Level
Cotton	1	00T to 06T	NA	00T to 03T	00T to 03T
	2	00T to 05T	00T to 06T	01T	00T to 02T
Corn	1	00R to 05R	NA	00R to 03R	00R to 03R
Soybean	1	00S to 06S	NA	00S to 01S	00S to 04S
	2	00S to 04S	00S to 03S	00S to 01S	01S

Table 3. Regression equations of chlorophyll (y) with percent applied glyphosate (x).

Crop	Chlorophyll at 1 WAT	Chlorophyll at 2 WAT
Cotton	y = -0.11021x + 1.0883	y = -0.07805x + 1.3882
	$(R^2 = 0.6375)$	$(R^2 = 0.6398)$
Corn	y = -0.36407x + 1.0833	NA
	$(R^2 = 0.6427)$	
Soybean	y = -0.34906x + 1.5318	y = -0.40095x + 1.742
-	$(R^2 = 0.9311)$	$(R^2 = 0.9053)$

phyll with the percent applied glyphosate were developed for cotton, corn, and soybean, as shown in table 3. For the evaluation, the percent applied glyphosate was estimated inversely with the models of visual injury and plant height. The estimated values of percent applied glyphosate were then fed into the models for chlorophyll to estimate the values of chlorophyll level to validate the models for visual injury and plant height. Figure 5 shows the estimated versus measured chlorophyll. For cotton, on average, the ratio of estimated vs. measured chlorophyll for the visual injury model at 1 WAT was 1.00, with  $R^2 = 0.64$  and RMSD (root mean squared deviation) = 0.09 mg g<sup>-1</sup>, and the ratio of estimated vs. measured chlorophyll for the plant height model at 2 WAT was 1.001, with  $R^2 = 0.65$  and RMSD = 0.07 mg g<sup>-1</sup>. Therefore, the two cotton models in table 1 are acceptable for estimating cotton injury based on aerially sprayed glyphosate deposition at 1 and 2 WAT.

In corn, on average, the ratio of estimated vs. measured chlorophyll for the visual injury model at 1 WAT was 0.82, with  $R^2 = 0.93$  and RMSD = 0.16 mg  $g^{-1}$ . Therefore, the corn model in table 1 is also acceptable for estimating corn injury based on aerially sprayed glyphosate deposition at 1 WAT. For soybean, on average, the ratio of estimated vs. measured chlorophyll for the visual injury model at 1 WAT was 1.03, with  $R^2 = 0.73$  and RMSD = 0.25 mg  $g^{-1}$ , and the ratio of estimated vs. measured chlorophyll for the plant height model at 2 WAT was 0.99, with  $R^2 = 0.88$  and RMSD = 0.17 mg  $g^{-1}$ . Therefore, the two soybean models in table 1 are acceptable for estimating soybean injury based on aerially sprayed glyphosate deposition at 1 and 2 WAT.

## **SUMMARY AND CONCLUSIONS**

This research studied correlations between the downwind drift of aerially applied glyphosate at a labeled usage rate of 866 g a.e. ha<sup>-1</sup> and biological responses in cotton, corn, and soybean. Models of visual injury and plant height with the percent applied glyphosate were developed and tested for assessing injury of non-GR cotton, corn, and soybean caused by the drift of aerially applied glyphosate at the labeled usage rate. The cotton models at 1 WAT with visual injury and at 2 WAT with plant height were well validated with chlorophyll data (value closest to 1 for the ratio of estimated vs. measured chlorophyll, and low RMSD). The validation of the corn model indicated a high R<sup>2</sup> value, but the ratio of estimated vs. measured chlorophyll deviated from 1. Compared with the corn model, the validation of the soybean models showed less bias, with a value close to 1 for the ratio of estimated vs. measured chlorophyll. At

55(3): 725-731

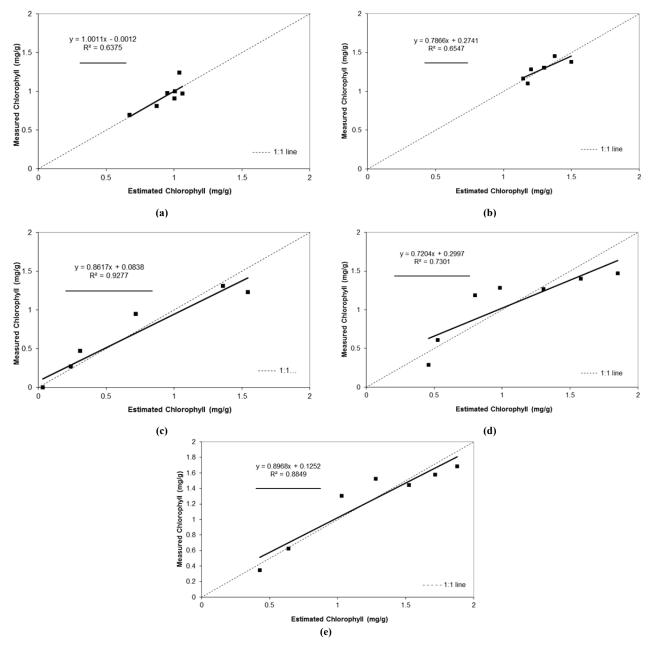


Figure 5. Measured vs. estimated chlorophyll (a) cotton at 1 WAT with visual injury, (b) cotton at 2 WAT with plant height, (c) corn at 1 WAT with visual injury, (d) soybean at 1 WAT with visual injury, and (e) soybean at 2 WAT with plant height.

2 WAT, the cotton model for plant height had a better fit than the soybean model (value close to 1 for the ratio of estimated vs. measured chlorophyll, and lower RMSD), although the soybean model had a higher  $R^2$  value.

Based on analysis of the results, the following conclusions can be drawn:

- Impact of glyphosate drift on cotton, corn, and soybean was observable but decreased with time in three weeks due to plant death (especially in corn and soybean), and cotton showed regrowth.
- The glyphosate drift had a strong impact on cotton in the first two weeks after treatment; however, cotton started to recover from injury at 3 WAT.
- Corn was the most sensitive to the glyphosateinduced injury and showed extensive injury at

1 WAT.

- Soybean was less sensitive to the glyphosate drift than corn and more sensitive than cotton, and soybean injury was clearly observable at 1 WAT and 2 WAT.
- Crop physical responses (visual injury and plant height) can be directly used to model the injury of cotton caused by the drift of aerially applied glyphosate at 1 and 2 WAT, the injury of corn at 1 WAT, and the injury of soybean at 1 and 2 WAT. In turn, the models can be evaluated by a physiological response such as chlorophyll.
- The regression models for cotton visual injury at 1 WAT and plant height at 2 WAT can be used to estimate cotton injury caused by off-target drift based

730 Transactions of the ASABE

on the percentage of applied glyphosate. The regression model for corn visual injury at 1 WAT can be used to estimate corn injury caused by the off-target drift based on the percentage of applied glyphosate. The regression models for soybean visual injury at 1 WAT and plant height at 2 WAT can be used to estimate soybean injury caused by off-target drift based on the percentage of applied glyphosate.

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