

## Glyphosate Effect on Shikimate, Nitrate Reductase Activity, Yield, and Seed Composition in Corn

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When glyphosate is applied to glyphosate-resistant (GR) crops, drift to nonglyphosate-resistant (non-GR) crops may cause significant injury and reduce yields. Tools are needed to quantify injury and predict crop losses. In this study, glyphosate drift was simulated by direct application at 12.5% of the recommended label rate to non-GR corn (*Zea mays* L.) at 3 or 6 weeks after planting (WAP) during two field seasons in the Mississippi delta region of the southeastern USA. Visual plant injury, shikimate accumulation, nitrate reductase activity, leaf nitrogen, yield, and seed composition were evaluated. Effects were also evaluated in GR corn and GR corn with stacked glufosinate-resistant gene at the recommended label rate at 3 and 6 WAP. Glyphosate at 105 g ae/ha was applied once at 3 or 6 weeks after planting to non-GR corn. Glyphosate at 840 (lower label limit) or 1260 (upper label limit) g ae/ha was applied twice at 3 and 6 WAP to transgenic corn. Glyphosate caused injury (45–55%) and increased shikimate levels (24–86%) in non-GR compared to nontreated corn. In non-GR corn, glyphosate drift did not affect starch content but increased seed protein 8–21% while reducing leaf nitrogen reductase activity 46–64%, leaf nitrogen 7–16%, grain yield 49–54%, and seed oil 18–23%. In GR and GR stacked with glufosinate-resistant corn, glyphosate applied at label rates did not affect corn yield, leaf and seed nitrogen, or seed composition (protein, oil, and starch content). Yet, nitrate reductase activity was reduced 5–19% with glyphosate at 840 + 840 g/ha rate and 8–42% with glyphosate at 1260 + 1260 g/ha rate in both GR and GR stacked corn. These results demonstrate the potential for severe yield loss in non-GR corn exposed to glyphosate drift.

**KEYWORDS:** Corn; drift injury; glyphosate; nitrate reductase; seed nitrogen; shikimate; transgenic corn

### INTRODUCTION

Efficacy of glyphosate on a wide spectrum of weeds, simplicity and flexibility in application, lower herbicide cost, and freedom to rotate crops have encouraged US farmers to plant more areas with glyphosate-resistant (GR) crops each year (1, 2), particularly for soybean [*Glycine max* (L.) Merr.], cotton (*Gossypium hirsutum* L.), and corn. In 2009, 91% of the soybean, 71% of the cotton, and 68% of the corn hectareage in the US was planted with GR cultivars/hybrids (3). Despite the widespread adoption of GR crops, considerable area is planted with non-GR cultivars/hybrids. Twelve years after the introduction of GR corn, about 32% of the corn area is still planted with non-GR (conventional) corn hybrids, with many growers planting GR corn as insurance against glyphosate drift damage.

Glyphosate application frequency has increased with the adoption of GR crops, and the application window has widened because of differences in planting dates among GR crops. Glyphosate drift complaints from ground or aerial applications are common in the Mississippi Delta. In 2008, 56 cases of herbicide drift onto nontarget crops were reported in Mississippi, an increase of 21 cases from 2007 (Campbell, J. Mississippi Department of Agriculture

and Commerce. Personal communication, 2009). Rice, *Oryza sativa* L. (38%), wheat, *Triticum aestivum* L. (18%), soybean (9%), cotton (5%), and other crops (30%) were the nontarget crops impacted by herbicide drift in 2008. Fifty-eight percent of the 56 Mississippi drift complaints were due to glyphosate ground and aerial applications (Campbell, J. Mississippi Department of Agriculture and Commerce. Personal communication, 2009). Simulated glyphosate drift injury has been reported in corn (4–6), soybean (7, 8), rice (6, 9), and peanut, *Arachis hypogaea* L. (10). Although drift rates appear to be sublethal, the injury can be severe in sensitive crops, such as corn, and could reduce crop yield, particularly if drift occurs during a sensitive growth stage (4–6).

Glyphosate inhibits the biosynthesis of aromatic amino acids, which leads to several metabolic disturbances, including the arrest of protein synthesis and the deregulation of the shikimate pathway, leading to general metabolic disruption and plant death. Nonlethal physiological and metabolic disturbances due to glyphosate exposure were also observed in GR soybean (11, 12), non-GR corn (4), rice (9), and sunflower, *Helianthus annuus* L. (13). Glyphosate has been known to reduce ferric reductase activity in sunflower (13) and GR and non-GR soybean (14), alter uptake and translocation of micronutrients in GR soybean (15, 16), reduce nitrate reductase activity in GR (17) and non-GR soybean (7, 18), increase antioxidant enzymes (19), and alter GR soybean seed

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composition (protein, oil, and fatty acids) (17). In corn, glyphosate drift at 100 and 200 g/ha caused 11 to 61% injury, decreased plant height by 19 to 45%, and reduced yield by 9 to 56% (5).

Nitrate reductase is a key enzyme for nitrogen assimilation in plants. Glyphosate has been shown to reduce levels of nitrate reductase activity in GR and non-GR soybean, but the effect of glyphosate on nitrogen assimilation, nitrate reductase activity, and seed composition in non-GR and GR corn is unknown. The objectives of this study were to (1) determine the effects of glyphosate drift on visual injury, shikimate accumulation, nitrate reductase activity, leaf nitrogen, yield, and seed composition in non-GR corn, and (2) determine the effects of glyphosate at label rates on nitrate reductase activity and seed composition in both GR and GR stacked with glufosinate-resistant trait corn.

## MATERIALS AND METHODS

**General Experimental Conditions.** A 2-yr field study was conducted during 2008 and 2009 at the USDA-ARS Crop Production Systems Research farm, Stoneville, MS, under an irrigated environment. The soil was a Dundee silt loam (fine-silty, mixed, active, thermic Typic Endoqualf) with pH 6.7, 1.1% organic matter, a cation exchange capacity of 15 cmol/kg, and soil textural fractions of 26% sand, 55% silt, and 19% clay. The experimental area was under GR cotton production in 2007. Seedbed preparation consisted of disking, subsoiling, disking, and bedding in the fall of the previous year. The experimental area was treated with paraquat at 1.1 kg ai/ha 1 week prior to corn planting to kill the existing vegetation. Prior to planting, the raised beds were smoothed as needed. Corn was planted in 102-cm wide rows using a MaxEmerge 2 planter (Deere and Co., Moline, IL) at 75,000 seeds/ha on April 16, 2008 and April 1, 2009. S-Metolachlor at 1.12 kg ai/ha plus pendimethalin at 1.12 kg ai/ha were applied preemergence to the entire experimental area immediately after planting. The commercial formulation of the potassium salt of glyphosate (Roundup Weathermax, Monsanto Agricultural Co., St. Louis, MO) was applied broadcast over-the-top to corn with no additional adjuvant. Herbicides were applied with a tractor-mounted sprayer with TeeJet 8004 standard flat spray nozzles (TeeJet Spraying Systems Co., Wheaton, IL), delivering 187 L/ha water at 179 kPa. All plots including glyphosate-treated ones were hand weeded periodically throughout the season to keep them weed free. Fertilizer application and insect control programs were standard for corn (20), and the crop was irrigated on an as-needed basis each year. Specifically, nitrogen was applied at 202 kg/ha in the form of urea-ammonium nitrate solution within 3 wk of planting corn.

**Nonglyphosate-Resistant Corn.** A non-GR corn hybrid, Pioneer 31P41 was planted in both years. A single application of glyphosate at 105 g ae/ha was applied at 3 (early POST, EPOST) or 6 (late POST, LPOST) weeks after planting (WAP) corn. At 3 WAP, corn was in the two- to four-leaf stage, and at 6 WAP, corn was in the six- to eight-leaf stage. In Mississippi, corn is typically planted in March–April followed by soybean and cotton in April–May. As a result, corn at early growth stage has greater potential for drift exposure from glyphosate applications made prior to planting soybean and cotton. Application timings of 3 and 6 WAP were selected to reflect the early growth stages of corn. Glyphosate rate of 105 g/ha was selected to represent 12.5% of the recommended rate (840 g/ha) to simulate herbicide drift. Other researchers have used 1 to 20% of the recommended rate in simulated drift studies (5–7).

**Glyphosate and Glufosinate-Resistant Corn.** Glyphosate-resistant corn hybrid, Pioneer 31G97RR2, and GR stacked with glufosinate-resistant corn hybrid, Pioneer 31G71RR2LL, were planted in two separate experiments to assess nitrate reductase activity and seed composition responses to label rates of glyphosate. Two applications of glyphosate at 840 + 840 (lower label limit) or 1260 + 1260 (upper label limit) g ae/ha (21) were applied broadcast over-the-top at 3 (EPOST) and 6 (LPOST) WAP.

**In Vivo Nitrate Reductase Assay.** Six to eight youngest fully expanded leaves (with collars) were sampled randomly from the middle two rows, immediately transported to the laboratory, and assayed for nitrate reductase activity (NRA). NRA was measured on the basis of indigenous nitrate using the methods described previously (7, 22). Briefly, about 0.3 g of tissue was placed in 10 mL of potassium phosphate buffer (19.171 g/L of  $K_2HPO_4 \cdot 3H_2O$  + 2.177 g/L of  $KH_2PO_4$  + 10 mL of 1%

(v/v) 1-propanol) at a concentration of 100 mM, pH 7.5, in the flask. The incubation solution was vacuum filtered for 1 min, and the flask and contents were flushed with nitrogen gas for 30 s and then incubated at 30 °C. Samples of 0.5 mL were taken at regular intervals (0, 60, 120, 180, and 300 min) for nitrite determination. Then, 5 mL of deionized water and reagents of 1.0 mL of 1% (w/v) sulfanilamide in 10% v/v HCl and 1.0 mL of *N*-naphthyl-(1)-ethylenediamine dihydrochloride (0.1%) were added to the samples. After 30 min, the samples were read at 540 nm using a Beckman Coulter DU 800 spectrophotometer. The concentration of nitrite was calculated from a standard calibration curve using  $KNO_2$ . NRA was measured in each treatment at 3 d EPOST, 3 d LPOST, 16 d LPOST, and 32 d LPOST. The sampling dates were selected to measure the response immediately following glyphosate application through silking stage.

**Leaf and Seed Total Nitrogen.** The youngest fully expanded leaves from 10 plants were sampled randomly from each plot at 3 d EPOST, 3 d LPOST, 16 d LPOST, and 32 d LPOST. Corn grain was sampled from each plot at harvest. The leaf (48 h) and grain (96 h) samples were oven-dried at 60 °C and finely ground. Samples were redried the night before nitrogen analysis to remove any moisture that may have been absorbed prior to analysis. Total nitrogen was determined from duplicate samples (10–15 mg) using a Flash EA 112 elemental analyzer (CE Elantech, Lakewood, NJ). Nitrogen was expressed as percent of leaf and seed dry weight.

**Shikimate Assay with Leaf Discs.** Shikimate was determined using a spectrophotometric method following the protocols described previously with modifications (23, 24). One disc (6 mm diam) per leaf was excised using a standard paper hole-punch adjacent to the midrib from the youngest fully expanded leaves that were collected as described above for leaf nitrogen determination. Leaf discs were placed in screw-top 7-mL plastic vials and stored in a freezer until analyzed (about 4 to 6 wk). One milliliter of 0.25 M HCl was added to each vial, vortexed to ensure that leaf discs were in solution, and incubated at room temperature for 90 min. A 25- $\mu$ L aliquot of solution in duplicate was placed in a well of a 96-well microtiter plate containing 100  $\mu$ L of 0.25% periodic acid/0.25% meta-periodate solution and incubated for 60 min at room temperature. A 100- $\mu$ L aliquot of 0.6 M sodium hydroxide/0.22 M sodium sulfite solution was added to each well and the absorbance read at 380 nm using a microplate reader (Synergy HT microplate reader, BIO-TEK Instruments, Inc., Winooski, VT). Shikimate was determined using a standard curve generated from known concentrations of shikimate and expressed in ng shikimate/mL HCl solution.

**Corn Injury and Yield.** Corn injury was visually estimated on a scale of 0 (no corn injury) to 100% (corn death). Injury was estimated at 7 d EPOST and 7, 14, and 21 d LPOST. Corn from all four rows in each plot was harvested on September 9, 2008 and August 26, 2009 using a combine, and grain yield was adjusted to 15% moisture.

**Seed Protein, Oil, and Starch.** Corn grain from each treatment was analyzed for protein, oil, and starch content in 2008 and 2009 using a near-infrared reflectance diode array feed analyzer (Perten, Spring Field, IL). Calibrations were developed using Perten Thermo Galactic Grams PLS IQ using 240 corn samples for protein and oil, and 143 samples for starch as reference samples. The reference samples were analyzed on the basis of AOAC methods for protein, oil, and starch. Protein levels were estimated by measuring total nitrogen using the Kjeldahl method (25). Protein was calculated from total nitrogen using Dumas,  $N \times 6.25$ . Oil was determined using the Soxhlet extraction method (26). Starch was determined by the enzymatic method using glucoamylase (27), where glucose, liberated by the action of the enzyme on starch, was measured using glucose oxidase. The near-infrared reflectance diode array feed analyzer proved to be able to predict results very close to the results from the reference method. The analysis was performed on the basis of percent dry matter (7, 17). Total seed protein production (kilograms per hectare) was calculated as the product of corn yield and seed protein concentration.

**Statistical Analysis.** The experiment was conducted in a randomized complete block design with four replications. Each treatment plot consisted of four rows spaced 102-cm apart and 15.2 m long. The data were subjected to analysis of variance using PROC GLM (Statistical Analysis Systems, Statistical Analysis Systems Institute, Cary, NC), and treatment means were separated at the 5% level of significance using Fisher's protected LSD test. Data were averaged across years because year by glyphosate treatment interactions were not significant.

**Table 1.** Glyphosate Simulated Drift Effect on Plant Injury and Shikimate Concentration in Nonglyphosate-Resistant Corn at Stoneville, MS, 2008 and 2009<sup>a</sup>

| glyphosate  |                         | corn injury (%) |           |            |            | shikimate (ng/mL) |           |            |            |
|-------------|-------------------------|-----------------|-----------|------------|------------|-------------------|-----------|------------|------------|
| rate (g/ha) | application timing, WAP | 7 d EPOST       | 7 d LPOST | 14 d LPOST | 21 d LPOST | 3 d EPOST         | 3 d LPOST | 16 d LPOST | 32 d LPOST |
| 0           |                         | 0 b             | 0 c       | 0 b        | 0 b        | 16.4 b            | 14.0 b    | 7.2 c      | 6.5 b      |
| 105         | 3                       | 55 a            | 71 a      | 45 a       | 45 a       | 24.4 a            | 26.0 a    | 11.9 a     | 8.1 a      |
| 105         | 6                       | nd              | 30 b      | 60 a       | 55 a       | nd                | 25.0 a    | 9.2 b      | 8.1 a      |

<sup>a</sup> Data is averaged across 2008 and 2009. Means within a column followed by the same letter are not significantly different at the 5% level as determined by Fisher's LSD test. EPOST, early postemergence applied at 3 wk after planting; LPOST, late postemergence applied at 6 wk after planting; nd, not determined; WAP, wk after planting corn.

**Table 2.** Glyphosate Simulated Drift Effect on Nitrogen Content and Nitrate Reductase Activity in the Youngest Fully Expanded Leaf in Nonglyphosate-Resistant corn at Stoneville, MS, 2008 and 2009<sup>a</sup>

| glyphosate  |                         | leaf nitrogen content (%) |           |            |                         | nitrate reductase activity ( $\mu\text{mol nitrite/g fwt/h}$ ) |           |            |            |
|-------------|-------------------------|---------------------------|-----------|------------|-------------------------|--|-----------|------------|------------|
| rate (g/ha) | application timing, WAP | 3 d EPOST                 | 3 d LPOST | 16 d LPOST | 32 d LPOST <sup>b</sup> | 3 d EPOST  | 3 d LPOST | 16 d LPOST | 32 d LPOST |
| 0           |                         | 4.07 a                    | 4.51 a    | 3.84 a     | 3.68 a                  | 5.86 a   | 5.99 a    | 7.27 a     | 7.62 a     |
| 105         | 3                       | 3.40 b                    | 4.18 b    | 3.71 a     | 3.42 a                  | 2.96 b   | 2.14 c    | 2.92 b     | 3.41 b     |
| 105         | 6                       | nd                        | 4.10 b    | 3.59 a     | 3.52 a                  | nd   | 3.09 b    | 3.37 b     | 4.13 b     |

<sup>a</sup> Data is averaged across 2008 and 2009. Means within a column followed by the same letter are not significantly different at the 5% level as determined by Fisher's LSD test. EPOST, early postemergence applied at 3 wk after planting; fwt, fresh weight; LPOST, late postemergence applied at 6 wk after planting; nd, not determined; WAP, wk after planting corn. <sup>b</sup> One year data from 2009.

## RESULTS AND DISCUSSION

**Nonglyphosate-Resistant Corn.** The visible injury symptoms such as chlorosis, necrosis, stunted growth, and plant death were apparent within 7 days after glyphosate application. At 7 d after application, 30 to 55% of corn was injured from glyphosate applied either at 3 or 6 WAP (**Table 1**). At 21 d LPOST, corn injury still remained at 45 to 55% with both application timings, and the corn never recovered completely from glyphosate injury over time. Corn plants continued to exhibit stunted growth throughout the season, producing small and deformed ears. These findings are similar to those reported in the literature. Glyphosate applied at drift rates (70 to 200 g/ha) to 4- to 6-leaf stage corn caused 3 to 80% injury at 14 d after treatment (5, 6). In soybean, however, glyphosate applied at drift rates (9 to 140 g/ha) caused injury, but the soybean was able to recover rapidly from herbicide injury without affecting yields negatively (7, 8).

In nontreated corn, shikimate was present at all four sampling dates; however, levels declined with growth stage from 16.4 ng/mL at 3 d EPOST to 6.5 ng/mL at 32 d LPOST (**Table 1**). Elevated levels of shikimate (24–86% increase) were observed in corn treated with glyphosate, regardless of application timing. Shikimate levels in glyphosate-treated corn ranged from 8.1 to 26 ng/mL. Overall, shikimate levels in treated corn were elevated at a greater proportion between 3 d EPOST and 16 d LPOST than at 32 d LPOST. However, at 32 d LPOST, shikimate levels in treated-corn were still higher than the levels in nontreated corn. Similarly, levels of shikimate in rice treated with 105 g/ha were higher than the levels in nontreated rice (9). Glyphosate causes many-fold increases in shikimate levels by blocking 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in sensitive plants, and elevated shikimate levels are used as an indicator of glyphosate effects on sensitive plants (4, 23, 28, 29).

Glyphosate effects on corn leaf nitrogen content were minimal, and significant reductions in leaf nitrogen concentration due to glyphosate application were only observed at early sampling dates (**Table 2**). Glyphosate caused about 16% reduction in nitrogen at 3 d EPOST and 7–9% reduction in nitrogen at 3 d LPOST. There was no difference in leaf nitrogen content between treated and nontreated corn at 16 and 32 d LPOST. The reduction in leaf nitrogen content at early sampling dates correlates with chlorosis of young leaves observed immediately following glyphosate application. Although corn growth was stunted, plant biomass increased with time, and new leaves were free of chlorosis. Leaf

nitrogen content was determined using the youngest fully expanded leaves at each sampling date. Similarly, glyphosate at 105 g/ha applied 3 WAP reduced shoot nitrogen content by 26% 2 wk after application, but glyphosate had no measurable effect on nitrogen content at subsequent sampling dates (7).

Glyphosate applications at either EPOST or LPOST inhibited NRA in non-GR corn, regardless of sampling date. Decreased NRA 3 d after EPOST and LPOST was accompanied with a decrease in leaf nitrogen content (**Table 2**). This indicates that the sensitivity of nitrate reductase enzyme in corn leaves to glyphosate at an early stage was manifested in reduced nitrogen assimilation. Although both EPOST and LPOST led to NRA reduction, LPOST did not decrease leaf N content beyond 3 d LPOST. This may have been due to (1) NRA not necessarily correlating with leaf N concentration but correlating with leaf nitrate concentration as the nitrate is the substrate for nitrate reductase enzyme. (2) NRA activity was only measured in newly developed leaves, and there may have been sufficient nitrate assimilated in other leaves that were translocated to support the nitrogen requirement. For example, imazethapyr, a branched amino acid synthesis inhibitor, reduced NRA and nitrate uptake by soybean roots (30). In cowpea, metobromuron applied at 125 g/ha increased nitrate and protein concentration in leaves but decreased NRA (31). When applied at 625 g/ha, metobromuron increased NRA throughout the growth period. Our results indicate that NRA may not correlate with leaf nitrogen under glyphosate exposure. The response of NRA and leaf N dynamics to glyphosate may depend on species, genotype, glyphosate application rate, and their interaction with the environment. Similarly, NRA reduction in GR soybean was previously reported (17). It was suggested that glyphosate influences nitrate (enzyme substrate) availability or de novo synthesis of the nitrate reductase enzyme and that this influence is greater at early stages (7, 17). In non-GR soybean, glyphosate reduced NRA mostly in newly developing leaves and roots, and the effect was indirect (18). Glyphosate is also known to reduce the uptake and translocation of iron resulting in impairment of iron nutrition, possibly due to the formation of a glyphosate–metal complex (16). Nitrate reductase whose activity depends on iron may have been compromised. In wheat, NRA was decreased significantly when iron was withdrawn from the culture media (32). Glyphosate is also known to inhibit ferric reductase activity in iron deficient sunflower roots (13).



**Table 3.** Glyphosate Simulated Drift Effect on Yield and Seed Composition in Nonglyphosate-Resistant Corn at Stoneville, MS, 2008 and 2009<sup>a</sup>

| glyphosate (g/ha) | application timing, WAP | corn yield (kg/ha) | seed N (%) | seed protein (%) | total protein (kg/ha) | seed oil (%) | seed starch (%) |
|-------------------|-------------------------|--------------------|------------|------------------|-----------------------|--------------|-----------------|
| 0                 |                         | 8240 a             | 1.59 ab    | 8.6 c            | 704 a                 | 3.8 a        | 73.4 a          |
| 105               | 3                       | 3820 b             | 1.52 b     | 9.2 b            | 348 c                 | 3.2 b        | 73.4 a          |
| 105               | 6                       | 4180 b             | 1.63 a     | 10.4 a           | 434 b                 | 2.9 b        | 72.8 a          |

<sup>a</sup> Data is averaged across 2008 and 2009. Means within a column followed by the same letter are not significantly different at the 5% level as determined by Fisher's LSD test. WAP, wk after planting corn.

**Table 4.** Effect of Glyphosate on Nitrate Reductase Activity in the Youngest Fully Expanded Leaf in Glyphosate-Resistant and Glyphosate-Resistant Stacked with Glufosinate-Resistant Corn, 2008 and 2009<sup>a</sup>

| glyphosate  |                         | nitrate reductase activity ( $\mu\text{mol nitrite/g fwt/h}$ ) |           |            |            |  |           |            |            |
|-------------|-------------------------|--|-----------|------------|------------|--|-----------|------------|------------|
| rate (g/ha) | application timing, WAP | glyphosate-resistant corn                                      |           |            |            | glyphosate- and glufosinate-resistant corn |           |            |            |
|             |                         | 3 d EPOST  | 3 d LPOST | 16 d LPOST | 32 d LPOST | 3 d EPOST                                  | 3 d LPOST | 16 d LPOST | 32 d LPOST |
| 0           |                         | 6.78 a   | 7.11 a    | 7.56 a     | 7.16 a     | 6.13 a                                     | 6.42 a    | 7.64 a     | 7.30 a     |
| 840 + 840   | 3 + 6                   | 5.52 b   | 6.34 b    | 6.28 b     | 6.37 a     | 5.40 b                                     | 5.24 b    | 7.24 a     | 6.91 a     |
| 1260 + 1260 | 3 + 6                   | 4.21 c   | 4.33 c    | 5.14 c     | 5.44 b     | 3.56 c                                     | 3.83 c    | 5.43 b     | 6.75 a     |

<sup>a</sup> Data is averaged across 2008 and 2009. Means within a column followed by the same letter are not significantly different at the 5% level as determined by Fisher's LSD test. EPOST, early postemergence applied at 3 wk after planting; fwt, fresh weight; LPOST, late postemergence applied at 6 wk after planting; WAP, wk after planting corn.

Glyphosate applied at 3 or 6 WAP reduced corn yield 49 to 54% compared to that of the nontreated plot (Table 3). Yield decrease was due to stunted plant growth, malformed ears, and reduced seed number and size. Similarly, Brown et al. (5) reported a corn yield loss of 49 to 56% when glyphosate was applied at 200 g/ha. Ellis et al. (6) noted a corn yield loss of 22 to 78% when glyphosate was applied at 35 to 140 g/ha. However, glyphosate applied at 105 g/ha to non-GR soybean had no effect on yield (7) indicating that soybean has the potential to recover from sublethal glyphosate drift rates.

Glyphosate applied at 3 and 6 WAP increased seed protein and had no effect on seed starch content, and the effect on seed nitrogen was not clear (Table 3). Elevated seed protein levels following glyphosate exposure have been reported for other species and may be the result of stress response (33) and decrease in seed size and seed number. In cowpea, pendimethalin increased leaf nitrate concentration and leaf protein (31) but did not influence seed protein. However, the herbicide metolbromuron increased leaf nitrate concentration, decreased NRA, but increased seed protein by 29% at 125 g/ha in 60D cultivar (31). Impaired translocation of vegetative protein to developing seed was indicated by the negative correlation between leaf crude protein and seed protein. The inverse relationship between protein and oil was reported in other species (34). Although the protein concentration was higher in glyphosate-treated non-GR corn, the total protein production (kg protein/ha) was higher in nontreated plants (Table 3).

**Glyphosate-Resistant and Glufosinate-Resistant corn.** Glyphosate applied sequentially at label rates (840 and 1260 g/ha) did not cause injury in either GR or GR stacked with glufosinate corn, as was expected (data not shown). Unlike in non-GR corn, shikimate levels were not elevated in glyphosate-treated plants compared to those of nontreated plants of both corn types (data not shown). Lack of a glyphosate effect on shikimate levels in both GR corn types indicates that the insensitive EPSPS utilized all of the shikimate that would have accumulated from inhibition of the native EPSPS. Similarly, shikimate levels in GR soybean and GR corn were unaffected by glyphosate in other studies (29, 35). Glyphosate applied at both rates had no effect on corn yield, leaf and seed nitrogen, and seed composition (protein, oil, and starch content) in either GR corn type when compared to that in nontreated plots (data not shown). Likewise, in GR soybean, application of glyphosate did not affect the levels of oil, ash, fiber,

carbohydrates, or protein as compared to that in non-GR soybean (36).

Nitrate reductase activity was reduced by glyphosate in both GR corn types at 3 d EPOST and 3, 16, and 32 d LPOST (Table 4). Nitrate reductase activity was reduced by 11–19% with glyphosate at 840 + 840 g/ha rate and 24–39% with glyphosate at 1260 + 1260 g/ha rate in GR corn. In GR stacked with glufosinate corn, nitrate reductase activity was reduced by 5–18% with glyphosate at 840 + 840 g/ha rate and 8–42% with glyphosate at 1260 + 1260 g/ha rate. NRA was less affected by glyphosate treatment in GR (24% decrease) and GR stacked with glufosinate-resistant corn (8% decrease) compared to that in non-GR corn (55% decrease), especially at 32 d LPOST. Although nitrate reductase was reduced up to 16 d after glyphosate treatment, there was no effect on leaf nitrogen indicating that sufficient nitrogen was assimilated to support the physiological need of the leaf sampled. Furthermore, no differences in leaf and seed nitrogen content were detected between glyphosate-treated and nontreated GR and GR stacked with glufosinate-resistant corn. No seed composition differences were detected between treated and nontreated GR corn, suggesting that corn may be less sensitive to seed composition alteration than other species.

In summary, simulated glyphosate drift (12.5% of label rate) injured non-GR corn and reduced yields by 49–54%. Corn appeared to be more sensitive than soybean. Although soybean was injured from glyphosate, soybean completely recovered from injury within 14 d after treatment, and glyphosate had no effect on soybean yield, seed nitrogen, protein, and oil content (7). Unlike corn, soybean has the potential to compensate after short duration of stress. In GR and GR stacked with glufosinate-resistant corn, glyphosate at label use rates had no effect on yield and seed composition. Glyphosate reduced nitrate reductase activity by 46–64% in non-GR corn and by 5–42% in GR and GR stacked with glufosinate-resistant trait corn. The fact that glyphosate reduced nitrate reductase activity in both GR and non-GR corn suggests a secondary effect of glyphosate on nitrate reductase. These results demonstrate that potential for severe yield loss in non-GR corn exposed to glyphosate spray drift.

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