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Effects of Glyphosate Application on Seed Iron and Root Ferric (III) Reductase in Soybean Cultivars

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Previous research demonstrated that plant nutrient assimilation was reduced by glyphosate (Gly). A 2 year field experiment investigated the effects of Gly at drift rate (12.5% of commercial use rate) on Fe concentrations in leaves and seeds of Gly-sensitive (GS) soybean, and a greenhouse experiment evaluated Gly effects on Fe assimilation using root *in vivo* ferric reductase activity (FRA) in two GS and one Gly-resistant (GR) soybean cultivars. Field studies showed that Gly drift rates resulted in a significant decrease in the Fe concentration in seeds and leaves compared to the nontreated plants. In greenhouse studies, leaf Fe and FRA were inhibited in GS cultivars Hutcheson and DP 5110 and the GR cultivar AG 4604RR and leaf Fe was positively correlated with root FRA ($p < 0.0001$). These results indicate that Gly can interfere with Fe assimilation in both GS and GR soybean. Understanding the implication of Gly on Fe nutrition in soybean seed would help soybean agronomists and breeders seeking to improve seed mineral nutrition qualities.

KEYWORDS: Glyphosate; iron nutrition; ferric reductase enzyme; ferric reductase activity; glyphosate-resistant soybean; glyphosate-sensitive soybean

INTRODUCTION

Soybean is a major crop in the world, and soybean seed quality is determined by its protein, oil, and mineral contents. Iron (Fe) is an essential nutrient for plant growth and development, and soybean seed Fe is affected by genotype and environment (*1*). Iron deficiency is an important nutritional concern in animal and human nutrition and can cause severe health complications in humans, especially in developing countries (*2*). Fe deficiency in humans can be attributed to the consumption of food crops containing low levels of Fe as a result of various soils and genetic factors (*3*). Recently, it was shown that Fe deficiency had been increasingly observed in cropping systems with frequent glyphosate (Gly) applications (*3*). The possible interpretation was that Gly interferes with root uptake of Fe by inhibiting ferric reductase in roots required for Fe acquisition by dicot and nongrass species (*3*).

A total of 13 years after the commercialization of Gly-resistant (GR) soybean cultivars, about 8% of the soybean areas in 2008 was still planted with conventional Gly-sensitive (GS) cultivars in the United States (*4*). The areas planted with GS soybean cultivars may increase in the coming years because the cost of GR seed is becoming expensive and GR weeds are becoming more prevalent. In 2008, about 63% of corn and 68% of cotton areas were planted with GR cultivars in the United States (*4*). As a result, the frequency of Gly use has increased rapidly with the adoption of GR crops. Herbicide drift can occur when herbicides are applied under windy or environmental conditions that favor volatilization and redeposition (*5*). Aerial applications are not uncommon and invariably increase the potential for spray drift to off-target crops. Off-target movement of herbicides can range from 0.01 to 10% of the applied rate (*6*). Previous research has shown that the simulated drift of 0.8−12.5% of the usage rate of 1.12 kg ai/ha Gly had injured GS soybean without affecting the yield (*7*).

The physiological and metabolic disturbances of Gly were observed in GR soybean (*8*, *9*), GS corn (*10*), GS rice (*11*), and GS sunflower (*3*, *12*). Recently, Gly application was found to reduce ferric reductase activity (FRA) in GS sunflower (*Helianthus annuus* L., cv. TR-3080) (*3*), alter uptake and translocation of micronutrients, such as Fe, Mn, and Zn (*13*), and reduce nitrate reductase activity (NRA) in GS (*14*) and GR (*15*). It was shown that Gly at 1, 3 and 6% of the recommended rate reduced FRA in GS sunflower (*H. annuus*) roots under Fe deficiency and 1.89 mM Gly resulted in about 50% inhibition of FRA within 6 h and complete inhibition within 24 h after the treatment (*3*). The observation that Gly decreased FRA was attributed to impairment of soil Fe uptake, resulting from the Fe−Gly complexes formed in soil, reduction of foliar Fe absorption by leaves, and translocation within the plant (*3*, *16*). For example, it was shown that Gly decreased Fe, Mn, and Zn concentrations in plant tissues of GR soybean, especially at low nutrient supply (*13*, *17*). The decrease in nutrient uptake, tissue concentration, and translocation was due to the formation of Gly−cation complexes in plant tissue (*18*). The decrease in nutrient uptake and content and complex formation was reported to have occurred during

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Gly foliar application, Gly drift to nontarget pants (*18*), Gly residue in soil, or root exudates of treated weeds (*18*, *19*). The most common negative effects of Gly were reported by Neumann et al. (*20*) using target and nontarget plants [soybean (*Glycine max* L., cv. BRSMG68; Nidera A8000 RR) and sunflower (*H. annuus* L., cv. TR 6149 SA)]: (1) increased sensitivity to plant diseases, associated with the low Mn and Fe nutritional status, (2) induced nematode infections, (3) inhibited root growth, possibly induced by Gly interactions with the calcium metabolism, (4) reduced honey production because of limited synthesis of flavonoids as flower pigments, and (5) reduced biological nitrogen fixation (*21*, *22*). The frequency of *Fusarium* spp. on roots increased after application of Gly or Gly plus conventional herbicides compared to the conventional herbicide alone (*23*).

Because the effects of Gly on the Fe concentration in seed and on FRA in GS soybean is still not known and the mechanisms controlling Gly action and their expression under field conditions are still not understood (*13*), the current experiments were conducted. The specific objectives of this study were to investigate the effect of Gly at drift rates on Fe assimilation using root *in vivo* FRA and Fe concentration in leaves and seed in soybean cultivars.

MATERIALS AND METHODS

Field Experiment and Sampling. A field study was conducted in 2005 and 2006 at the United States Department of Agriculture−Agricultural Research Service (USDA-ARS) Southern Weed Science Research Unit Farm, Stoneville, MS. The soil was a Dundee silt loam (fine-silty, mixed, thermic Aeric Ochraqualf) with pH 6.8, 1.2% organic matter, and a cation-exchange capacity of 21 cmol/kg. Soil textural fractions were 23% sand, 51% silt, and 26% clay. The Fe concentration in soil ranged between 7.4 and 9.4 g/kg, and this range is within the sufficiency level. The experimental area was tilled with a disk harrow followed by a field cultivator in the fall of the preceding year. The experimental area was under GR soybean production in 2004. Soybean cultivars were selected on the basis of their availability in the market. GS soybean cultivar "Delta Pine 4748STS" was planted on April 18, 2005, and "DP 5110STS" was planted on April 13, 2006, at a rate of 355 000 seeds/ha. Metolachlor at 2.30 kg ai/ha plus flumetsulam at 0.07 kg ai/ha plus paraquat at 1.12 kg ai/ha were applied to the entire experimental area immediately after planting. Paraquat was applied to kill existing vegetation, and metolachlor and flumetsulam were applied to provide residual weed control. A single application of Gly at 12.5% of use rate of 0.84 kg ae/ha was applied at 3 (V2, first trifoliate), 6 (V7, sixth trifoliate), or 8 (R2, flower at the node immediately below the uppermost node with a completely unrolled leaf) (*24*) weeks after planting (WAP) soybean to simulate Gly drift. For comparison purposes, a no Gly control was included. Herbicide treatments were applied with a tractor-mounted sprayer with TeeJet 8004 standard flat spray tips delivering 187 L of water/ha at 179 kPa. The commercial formulation of Gly was used with no additional adjuvant (Roundup Weathermax, Monsanto Agricultural Co., St. Louis, MO). To prevent Gly drift to non-Gly-treated soybean, corn borders were used. Soybean was grown nonirrigated for at least 5 WAP and was irrigated thereafter as needed because of late-season dry weather. Each treatment consisted of four soybean rows spaced 102 cm apart and 12.2 m long in 2005 and eight rows spaced 51 cm apart and 10.7 m long in 2006. Selection of row spacing in each year was based on the availability of the type of planter. All plots including Gly treated were hand-weeded periodically throughout the season to keep weed-free. Soybean was harvested from each plot using a combine on Sept 6, 2005 and Sept 5, 2006, and the grain yield was adjusted to 13% moisture.

For Fe determination, 15 fully expanded leaves were randomly sampled from the middle two rows of each plot approximately 4, 5, 7, 8, 9, and 10 WAP. These sampling dates correspond to about 1 and 2 weeks following each Gly application. At harvest, about 200 soybean pods were randomly hand-harvested from the middle two rows. Leaf and seed samples were oven-dried at 60 °C and ground using a Perten Laboratory Mill 3600, Huddinge, Sweden, and analyzed for Fe concentrations as described in the following sections.

Greenhouse Experiment and Sampling. Because the Fe concentration in leaves and seed was lower under Gly application in the field, we hypothesized that this decrease may had been due to a reduction in Fe assimilation, as indicated in other species (e.g., GS sunflower) (*3*). Therefore, a greenhouse experiment was conducted twice to investigate the relationship between the Fe concentration in leaves and FRA as affected by Gly in soybean. Two GS cultivars ("Hutcheson" and "DP 5110STS") and a GR cultivar ("AG 4604RR") were included in the study. Five soybean seeds were planted in a 15 cm diameter plastic pot containing Bosket sandy loam soil. After emergence, soybean plants were thinned to two uniform plants per pot. The greenhouse was maintained at 28/22 °C (±3 °C) day/night temperature with natural light. The daily photosynthetic photon flux density was about 460−1900 μmol m^{-2} s^{-1} measured by a quantum meter (Spectrum Technology, Inc., Plainfield, IL). The range of light intensity reflects a cloudy or sunny day, respectively. A single application of 12.5% of use rate of 0.84 kg ae/ha was applied at 18 days after planting (DAP) (V2, first trifoliate) (*24*) soybean to stimulate Gly drift. A full commercial rate of single application of 0.84 kg ae/ha was applied to GR cultivar ("AG 4604RR"). Fully expanded leaves, young leaves, and roots were sampled at 20 DAP [2 days after treatments (DAT)] and 23 DAP (5 DAT) for Fe measurement in leaves and FRA in roots.

Iron Concentration Measurements in Leaves and Seed. The concentration of Fe in leaves was measured after acid wet digestion, extraction, and reaction of the reduced ferrous Fe with 1,10-phenanthroline (*25*, *26*). Briefly, 2 g of dried ground leaves were acid-digested (*27*). The acids were removed by volatilization, and the soluble constituents were dissolved in 2 M HCl. Fe standard solutions were prepared in 0.4 M HCl, ranging from 0.0 to 4 μg/mL Fe. A phenanthroline solution of 0.25% (m/v) was prepared in 25% (v/v) ethanol. The quinol solution (1%, m/v) reagent was prepared on the day of use. About 4 mL of aliquot of digested sample was added into a 25 mL volumetric flask. The aliquot was diluted to 5 mL using 0.4 M HCl. The quinol solution (1 mL) was added and mixed. Then, 3 mL of phenanthroline solution and 5 mL of trisodium citrate solution (8%, m/v) were added. The solution was diluted to 25 mL and stood for 4 h. Samples were read at an absorbance of 510 nm using a Beckman Coulter DU 800 spectrophotometer (Fullerton, CA). Fe in seed and soil was analyzed at the Soil, Plant, and Water Laboratory, University of Georgia. Fe in seed was determined by digesting 0.5 g of ground seed in HNO_3 in a microwave digestion system. Fe in soil was determined in 5 g of soil in 20 mL Mehlich 1 solution. Fe in seed and soil was determined using an inductively coupled plasma (ICP) spectrometer.

Root FRA. Root *in vivo* FRA was determined on intact roots using a bathophenanthroline disulfonic acid (BPDS) method (*28*). Briefly, roots were gently immersed in the below medium solution to remove adhering soil that may have microorganisms reducing the Fe^{3+}. Then, intact roots were transferred to 50 mL of the following solution: 1.5 mM KNO_3, 1 mM $Ca(NO_3)_2$, 3.75 mM $NH_4H_2PO_4$, 0.25 mM $MgSO_4$, 25 μM $CaCl_2$, 25 μM H_3BO_3, 2 μM $MnSO_4$, 2 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 0.5 μM H_2MoO_4, and 0.1 μM $NiSO_4$, with 100 μM Fe^{III}−ethylenediaminetatraacetic acid (EDTA) and 250 μM BPDS for 2 h, after which the reading at 535 nm was conducted using a Beckman Coulter DU 800 spectrophotometer (Fullerton, CA). The assay was conducted under dark conditions and incubated in the above solution at 24 °C for 2 h. An aliquot of the solution that had no roots during the assay was used as a blank.

Statistical and Experimental Design. Treatments were arranged in a randomized complete block design with four replications. Data represent mean values from two independent experiments, each of which had four replicates. The data were subjected to analysis of variance using Proc general linear model (GLM) (*29*). Means were separated by Fisher's least significant difference (LSD) test at the 5% level of significance. Correlation was conducted using Proc Corr statement using SAS. For correlation, data were pooled and combined for all treatments in each cultivar, and $p \leq 5\%$ was used as the level of significance.

RESULTS AND DISCUSSION

Field Experiment. Analysis of variance showed that year and Gly treatment had a significant ($p < 0.0001$) effect on Fe concentrations in leaves and seed (**Table 1**). Because there was significant ($p = 0.003$) interaction between year × treatment for leaf Fe (**Table 1**), data for each year were analyzed separately.

There were no differences in soybean yield among the four treatments, regardless of time of Gly application in both years (data not shown). The soybean yield ranged from 3780 to 3920 kg/ha in 2005 and from 4490 to 4760 kg/ha in 2006. These results are similar to that reported on GS soybean by other researchers, such as Ellis and Griffin (*7*), who observed that there was no significant yield reduction in soybean at simulated Gly drift of 0.8−12.5% of the usage rate of 1.12 kg ai/ha.

Fe Concentration in Leaves and Seed. Results of mean values (**Table 2**) showed that application of Gly at 3 WAP significantly ($p < 0.0001$) decreased the Fe concentration in leaves of Gly-treated plants compared to the control. The percentage decrease in the Fe concentration in leaves in Gly-treated plants was high at both early (4 WAP) and late stage (10 WAP), and the Fe concentration in leaves did not recover during the course of the experiment. Gly application at a later stage (8 WAP) resulted in a lower Fe concentration in seed than those of Gly application at an earlier stage (3 and 6 WAP) (**Figure 1**). The decrease in the Fe concentration in leaves and seed by Gly application may suggest a decrease in Fe uptake, Fe translocation from root to shoot, and Fe translocation from leaves to seed. A previous study on GS sunflower (*H. annuus* L., cv. TR-3080) showed that Gly application altered uptake and translocation of micronutrients, such as Fe, Mn, and Zn (*3*). In addition, it was reported that the effect of Gly on Fe reduction was due to inhibition of soil Fe uptake and Fe translocation within the plants. This inhibitions resulted from the formation of Fe−Gly complexes (*3*, *16*), Gly drift to nontarget plants (*18*), and Gly residue in soil or root exudates of treated weed (*18*, *19*). Although the current study did not determine the uptake of Fe by roots and its translocation within the plants, the concentration levels of Fe in leaves and seed, measured in the current study, may reflect the reduction of Fe uptake and translocation by Gly application. Neumann et al. (*20*), working on target and nontarget plants [soybean (*G. max* L., cv. BRSMG68; Nidera A8000 RR) and sunflower (*H. annuus* L., cv. TR 6149 SA)] showed that Gly in the rhizosphere can inhibit acquisition of micronutrients, such as Mn, Zn, Fe, and B, which are involved in plant-disease-resistance mechanisms.

Table 1. Analysis of Variance with *F* and *p* Values of Gly Application (Treatment), Year, and Their Interactions for Leaf Fe and Seed Fe Concentrations [mg of Fe/kg of Dry Weight (dwt)] in Soybean Grown in Field 2005 and 2006 at Stoneville, MS[a]

	leaf Fe (mg of Fe/kg of dwt)		seed Fe (mg of Fe/kg of dwt)	
source of variance	*F*	*p*	*F*	*p*
year	1.47	0.229	0.07	0.799
treatment	48.52	<0.0001	4.08	0.025
year × treatment	5.15	0.003	0.23	0.875
replications (year)	0.30	0.97	1.81	0.196

[a] The $p \leq 0.05$ level was considered significant.

In a hydroponic experiment, it was shown that 1.25−6% of the recommended dose of Gly resulted in a significant decline in acquisition, root uptake and root−shoot translocation of radiolabeled Fe, Zn, and Mn in GS sunflower (*3*, *12*). Recently, Bott et al. (*13*), studying the detrimental side effects of Gly on plant growth and micronutrients (Mn and Zn) of a GR soybean variety (*G. max*, cv. Valiosa), showed that Gly application significantly inhibited root biomass, root elongation, and lateral root formation of the GR line, associated with a 50% reduction of Mn shoot concentrations. They concluded that Gly application at the recommended rate can negatively affect plant growth and micronutrient status, even in GR soybean. They further reported that development of strategies to avoid the negative effects of Gly requires characterization of responsible factors and mechanisms and their degree of expression under field conditions.

In the current experiment, the leaf Fe concentration decreased at both early and late stages and this was observed in both GS soybean with the drift rate and GR soybean with the commercial rate. Our results indicate that the Fe concentration in leaves could be more sensitive to Gly and the period for Fe recovery may be longer than it was expected in comparison to other research on other nutrients, such as nitrogen. For example, Zablotowicz and Reddy (*9*) studied the effect of four Gly treatments (0.84, 1.68, 2.52 + 2.52, and 0.84 + 0.84 kg ae/ha) on nitrogen accumulation in GR soybean. In comparison to nontreated (hand-weeded) soybean, all Gly treatments reduced foliar nitrogen content (26−42%) in 1 year of 3 years (*9*). Total seed nitrogen (kg/ha) as calculated as the product between the seed yield and nitrogen concentration was reduced by 32 and 17% in 2 years of 3 years when two applications of 2.52 kg ae/ha Gly were used compared

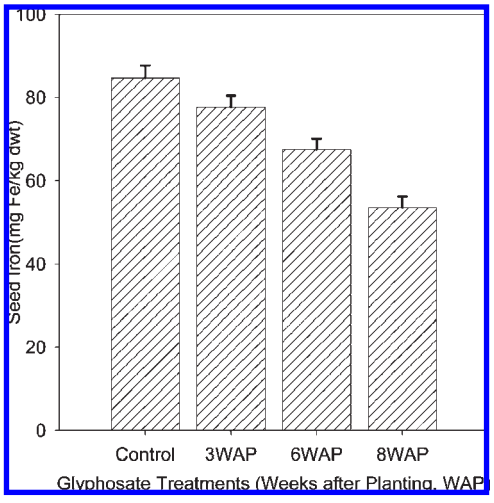

Figure 1. Effects of Gly treatment (WAP) on soybean seed iron concentrations (mg of Fe/kg of dwt). The control did not receive any Gly treatment. Bars represent a mean value of eight replicates ± standard error (SE).

Table 2. Effect of Gly Application on Fe Concentrations (mg of Fe/kg of dwt) in Leaves[a]

		year 2005						year 2006					
		time of sampling (WAP)						time of sampling (WAP)					
treatment	time of application	4	5	7	8	9	10	4	5	7	8	9	10
control		110 a	141 a	153 a	151 a	149 a	159 a	141 a	159 a	162 a	160 a	163 a	150 a
Gly	3	74 b	80 b	115 b	103 b	117 b	89 b	64 b	72 b	70 b	83 b	99 b	104 b
Gly	6			80 c	94 b	94 c	86 b			69 b	65 c	96 b	93 c
Gly	8					77 c	67 c					63 c	57 d

[a] Gly at 0.105 kg/ha was applied at 3, 6, and 8 WAP, and samples were taken 1 and 2 WAT after each Gly application. The experiment was conducted in Stoneville in 2005 and 2006. Means within a column followed by the same letter (a, b, c, or d) are not significantly different at the $p \leq 0.05$ level as determined by Fisher's LSD test.

Table 3. Analysis of Variance with *F* and *p* Values of Gly Application (Treatment), Experiment, and Their Interactions for the Leaf Fe Concentration and FRA**a**

	leaf Fe (mg of Fe/kg of dwt)		FRA (nmol of Fe^{2+} $gfwt^{-1}$ h^{-1})	
source of variance	*F*	*p*	*F*	*p*
variety	0.46	0.631	24.80	<0.0001
treatment	40.27	<0.0001	38.23	<0.0001
experiment	2.09	0.154	0.14	0.715
variety × treatment	2.06	0.075	1.61	0.165
variety × experiment	0.63	0.538	0.99	0.379
variety × treatment × experiment	2.24	0.0548	1.28	0.283
replications (experiment)	0.25	0.783	1.17	0.318

[a] Soybean was grown under greenhouse conditions. The $p \leq 0.05$ level was considered significant.

Table 4. Effect of the Gly Application on the Fully Expanded Leaf Fe Concentration (mg of Fe/kg of dwt) in Soybean[a]

variety	control (2 DAT)	treatment (2 DAT)	control (5 DAT)	treatment (5 DAT)
Hutcheson	89 b	31 c	104 a	31 c
DP 5110STS	75 b	40 c	87 a	40 c
AG 4604RR	83 b	45 c	97 a	37 d

[a] Gly was applied at a single application of 12.5% of use rate of 0.84 kg ae/ha at 18 DAP (V2, first trifoliate). Samples were taken 2 and 5 DAT. The experiment was conducted under greenhouse conditions. Means within a row for each variety separately followed by the same letter (a, b, c, or d) are not significantly different at the $p \leq 0.05$ level as determined by Fisher's LSD test.

to hand-weeded soybean. They concluded that commercial rates of Gly had minimal effects on nitrogen assimilation (leaf nitrogen and seed nitrogen) in GR soybean, but Gly application above the commercial rate significantly reduced nitrogen, especially under water stress (*9*). Also, it is important to indicate that the physiological responses of soybean cultivars to Gly may vary, depending upon geographical location, environmental conditions, soil types, and sensitivity of native populations of *B. japonicum* (*9*). In our experiment, both early and late application of the drift rate resulted in a significant Fe decrease in seed, especially when the application was at a late stage. This may be due to the fact that Gly application at a late stage (8−10 WAP) may have coincided with R2−R3 (full bloom to pod initiation). These stages could be important for Fe translocation and Fe seed accumulation.

Greenhouse Experiment. *Fe Concentrations in Leaves*. Analysis of variance showed that cultivar and Gly treatment were the main source of variability in leaf Fe and FRA. Because there were no significant interactions between experiments (two separate experiments were conducted in the greenhouse) and the other variables (Gly treatment and cultivar) (**Table 3**), the data were pooled across the two experiments. The mean values showed that Gly drift significantly decreased the Fe concentration in leaves at both sampling times (**Table 4**). The decrease in the Fe concentration in GS Hutcheson was greater (65%) than those of GS DP5110 (47%) compared to their control. Fe concentrations in yellow (with clear visual chlorosis) young leaves showed a significant decrease in both GS soybean variety, but the decrease in Hutcheson was more severe (**Table 5**). Also, the effect of Gly on the Fe concentration was more severe in young growing leaves than those of fully expanded leaves (**Tables 4** and **5**). Gly application of the commercial rate of 0.84 kg ai/ha decreased the Fe concentration in leaves of GR soybean AG 4604RR as well. The root Fe concentration was less affected by Gly compared to leaves (data not shown).

Table 5. Effect of the Gly Application on the Leaf Fe Concentration (mg of Fe/kg of dwt) in Young Growing Leaves of Soybean[a]

variety	control (2 DAT)	treatment (2 DAT)	control (5 DAT)	treatment (5 DAT)
Hutcheson	92 b	24 c	98 a	26 c
DP 5110STS	101 a	38 c	92 b	38 c
AG 4604RR	84 b	45 a	94 a	37 d

[a] Gly was applied at a single application of 12.5% of use rate of 0.84 kg ae/ha at 18 DAP (V2, first trifoliate). Samples were taken 2 and 5 DAT. The experiment was conducted under greenhouse conditions. Values are means of eight replicates. Means within a row for each variety followed by the same letter (a, b, c, or d) are not significantly different at the $p \leq 0.05$ level as determined by Fisher's LSD test.

Table 6. Effect of the Gly Application on FRA (nmol of Fe^{2+} $gfwt^{-1}$ h^{-1}) in Roots of Soybean[a]

variety	control (2 DAT)	treatment (2 DAT)	control (5 DAT)	treatment (5 DAT)
Hutcheson	202 a	100 c	179 b	87 d
DP 5110STS	236 b	138 d	253 a	160 c
AG 4604RR	220 a	139 c	225 a	155 b

[a] Gly was applied at a single application of 12.5% of use rate of 0.84 kg ai/ha at 18 DAP (V2, first trifoliate). Samples were taken 2 and 5 DAT. The experiment was conducted under greenhouse conditions. Values are means of eight replicates. Means within a row for each variety followed by the same letter (a, b, c, or d) are not significantly different at the $p \leq 0.05$ level as determined by Fisher's LSD test.

Gly application resulted in a decrease in the Fe concentration in leaves at two sampling times (2 and 5 DAT) after Gly treatment, supporting the results from the field experiment. Gly application resulted in significant decrease in the leaf Fe concentration in GS cultivar Hutcheson compared to GS cultivar DP 5110. Hutcheson showed more severe visual chlorosis symptoms and leaf damage than DP 5110. The degree of sensitivity of GR soybean may depend upon the Fe requirement for each cultivar. This suggestion may be supported by the lower Fe accumulation in Hutcheson (**Table 4**). Root Fe was less affected by Gly application than leaves in both GS and GR soybean (data not shown). The lower Fe concentration in leaves could be a result of Fe uptake and translocation, as discussed above. Young growing leaves appear to be the most sensitive for Fe nutrition compared to expanding leaves (**Table 5**).

FRA. Gly application resulted in a significant decrease in FRA in GS soybean cultivars compared to the control (**Table 6**), with the highest decrease being recorded in cultivar Hutcheson. FRA in AG 4604RR also showed a significant decrease without recovering to full activity during the period of the study, although there was less of a decrease compared to the GS soybean (**Table 6**). Whether or not FRA recovers after a longer period and at lower or higher Gly rates, further research needs to be conducted. A positive correlation was found between the FRA and Fe concentration in the fully expanded leaves in GS and GR cultivars, with the strongest being in GS cultivars ($p < 0.0001$, $r = 0.821$ for DP 51105; $p < 0.0001$, $r = 0.806$ for Hutcheson) (**Figure 2**). The strongest positive correlation was shown between the FRA and Fe concentration in the youngest growing (top) leaves as well (**Figure 3**), where visual Gly symptoms were observed. There are two clusters representing the control and treatments in each variety, and indicating that FRA increases with the increase of the Fe concentration in leaf tissues. The FRA−Fe pattern increases from low, representing Gly treatment, to high, representing the control (nontreated) plants. No correlation was found within each treatment, and this was expected because of the short time effect and the clustering pattern of each treatment individually. In our study, the correlation between

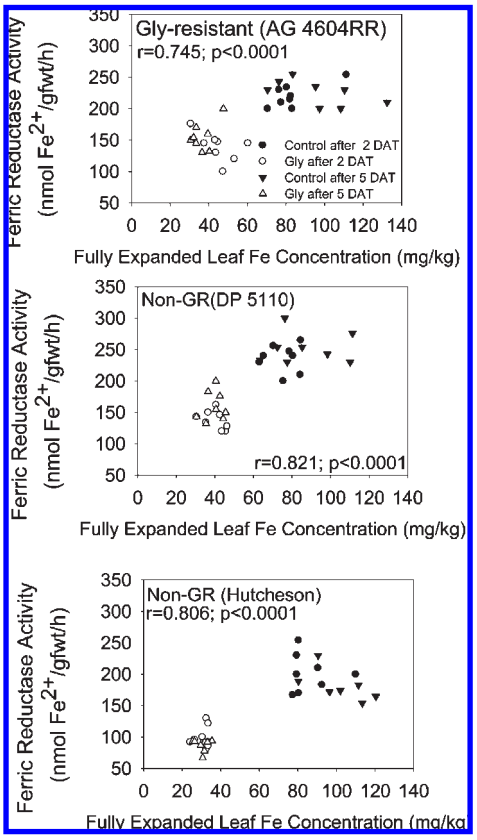

Figure 2. Correlation between the fully expanded leaf Fe concentration (mg/kg) and FRA (nmol of Fe^{2+} $gfwt^{-1}$ h^{-1}) in the GR soybean cultivar (AG 4604RR) and GS cultivars (DP5110STS and Hutcheson) in the control (C) and Gly treatment (Gly) after 2 and 5 DAT. The level of significance used was 5%.

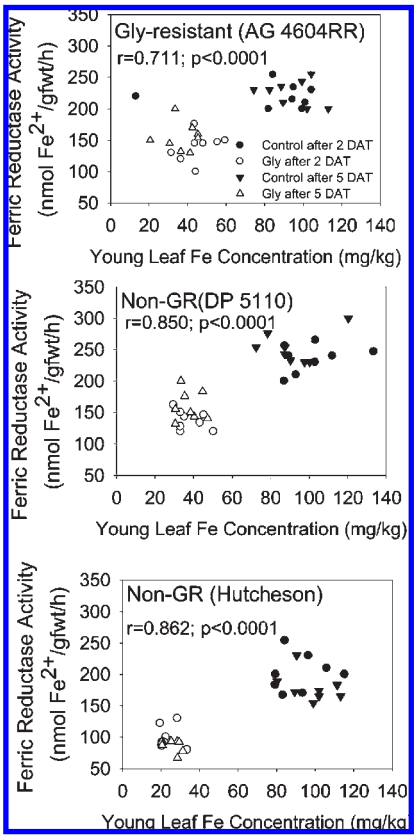

Figure 3. Correlation between the young leaf Fe concentration (mg/kg of dwt) and FRA (nmol of Fe^{2+} $gfwt^{-1}$ h^{-1}) in the GR soybean cultivar (AG 4604RR) and GS cultivars (DP5110STS and Hutcheson) in the control (C) and Gly treatment (Gly) after 2 and 5 DAT. The level of significance used was 5%.

FRA and Fe and between control and treatments is more relevant to the goal of our study.

The inhibition of FRA by Gly in other species was recently reported (*3*). It was found that Gly reduced FRA in GS sunflower (*H. annus* L., cv. TR-3080) (*3*) and altered uptake and translocation of micronutrients, such as Fe, Mn, and Zn (*13*). It was shown that Gly at 1, 3 and 6% of the recommended rate reduced FRA in GS sunflower roots under Fe deficiency and 1.89 mM Gly resulted in about 50% inhibition of FRA within 6 h and complete inhibition within 24 h after the treatment (*3*). Similar effects of Gly on other enzymes were also demonstrated. For example, it was found that the Gly drift rate (12.5% of the commercial use) reduced nitrate reductase and nitrogenase activities in GS soybean (*14*) and a rate of 1.12 and 3.36 kg ae/ha inhibited NRA in GR soybean (*15*). The decrease of FRA by Gly was attributed to impairment of soil Fe uptake resulting from the Fe−Gly complexes formed in soil, reduction of foliar Fe absorption by leaves, and translocation within the plant (*16*, *3*) or because of permanent or transient damage to ferric reductase enzyme by either blocking *de novo* synthesis of the enzyme or inhibition of essential amino acids or precursors for FRA enzyme synthesis. The inability of FRA to recover in GS soybean cultivars during the period of experiment could be due to either the short duration of the experiment (5 days) and/or the indirect negative effect of Gly on the amino acids involved in *de novo* synthesis of the FRA enzyme. In addition, it was shown that injury in GR soybean is caused by aminomethylphosphonic acid (AMPA) formed from Gly degradation (*30*, *31*). Because AMPA is the most frequently detected metabolite of Gly in plants and tends to accumulate at higher concentrations in GS soybean than in GR soybean (*31*), the possibility that AMPA may be involved indirectly in the uptake and translocation process of nutrients, such as Fe, cannot be excluded and needs further investigation.

In a recent study, Zobiole et al. (*32*) found that Gly application, either sequential (0.6 + 0.6 kg ae/ha) or single (1.2 kg ae/ha), decreased macro- and micronutrients and shoot and root biomass in GR soybean as compared to their near-isogenic nontreated non-GR soybean or nontreated GR soybean. They also found that Gly application decreased stomatal conductance, chlorophyll concentration, and photosynthesis rate (*32*). These findings are supported by Bott et al. (*13*), who showed that application of 450 g/L *N*-[phosphonomethyl]glycine isopropylamine salt as the active ingredient to a GR soybean variety (*G. max*, cv. Valiosa) significantly inhibited root biomass, root elongation, and lateral root formation of a GR line, associated with a 50% reduction of Mn shoot concentrations. The reduction in biomass was explained as a result of the reduction in photosynthesis parameters and nutrient efficiencies resulting from Gly effects (*32*). This reduction in biomass was in disagreement with the findings of Nandula et al. (*33*). Nandula et al. studied the effect of the dose response of GR and GS soybean [*G. max* (L.) Merr.] and applied Gly at a rate of 0.87, 1.73, 3.47, 6.93, 13.86, 27.72, 55.44, and 110.88 kg ae/ha to Asgrow 4603RR GR soybean and at a rate of 0.007, 0.015, 0.03, 0.06, 0.11, 0.22, 0.44, and 0.87 kg/ha to HBKC 5025 non-GR soybean. They found that these levels did not affect the growth. On the other hand, it was demonstrated that, although nitrate assimilation was negatively affected by the Gly drift rate (12.5% of use rate of 0.84 kg ae/ha) to GS soybean and full rate (0.84 kg ae/ha) to GR soybean, no yield differences between Gly and control plots were observed. It appears that Gly can severely affect the

physiology of GR and GS soybean without effecting the yield (*14*, *15*). The nonyield or growth differences in some GR or GS soybean may indicate that soybean can recover from the Gly effect. The above controversial literature may indicate that the recovery mechanism in soybean may depend upon cultivar and genotype differences in relation to mechanisms of Gly detoxification, detoxification period of either Gly or its metabolite, AMPA (*30*, *9*), the chelating (Gly−nutrient complexes) effect response (*12*, *32*), and growth conditions. The mechanism of Gly effects on soybean may be more complicated than previously thought, and further research is needed to investigate FRA recovery time under low and high Gly application in GS and GR soybean.

The present study demonstrated that Gly at drift rates decreased Fe concentrations in leaves and seed and inhibited FRA in GS and GR soybean. It appears that Gly results in physiological and biochemical disturbances in soybean, and controlling these factors to better understand the mechanisms of Gly effects is important to minimize Gly side effects, especially under field conditions (*13*). The current results advance our understanding of nontarget effects of the Gly moiety, especially in relation to maintaining adequate Fe nutrition to the plant and improving the nutritional quality of soybean seed.

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