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Glyphosate Resistance Technology Has Minimal or No Effect on Maize Mineral Content and Yield

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Supporting Information

ABSTRACT: Controversy continues to exist regarding whether the transgene for glyphosate resistance (GR) and/or glyphosate applied to GR crops adversely affect plant mineral content. Field studies were conducted in 2013 and 2014 in Stoneville, MS and Urbana, IL to examine this issue in maize. At each location, the experiment was conducted in fields with no history of glyphosate application and fields with several years of glyphosate use preceding the study. Neither glyphosate nor the GR transgene affected yield or mineral content of leaves or seed, except for occasional (<5%) significant effects that were inconsistent across minerals, treatments, and environments. Glyphosate and AMPA (aminomethylphosphonic acid), a main degradation product of glyphosate, were found in leaves from treated plants, but little or no glyphosate and no AMPA was found in maize seeds. These results show that the GR transgene and glyphosate application, whether used for a single year or several years, have no deleterious effect on mineral nutrition or yield of GR maize.

KEYWORDS: *aminomethylphosphonic acid, glyphosate, glyphosate resistance, mineral content, transgenic crop, Zea mays*

INTRODUCTION

Worldwide, glyphosate (*N*-(phosphonomethyl)glycine) is used more than any other herbicide[1] due in large part to the widespread adoption of transgenic, glyphosate-resistant (GR) crops.[1−3] GR crops include alfalfa, canola, cotton, maize, soybean, and sugar beet with maize and soybean accounting for most GR hectares. All GR crops are made resistant to glyphosate by a transgene encoding a GR form of 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS), the enzyme target of glyphosate. In the majority of cases, the transgene encodes a resistant EPSPS from the soil microbe *Agrobacterium* sp. (*cp4 epsps*). One exception is that some maize cultivars contain the modified maize GR GA21 EPSPS.[4] GR crops are about 50-fold more resistant to glyphosate than nontransgenic crops.[5]

Some researchers have claimed glyphosate causes mineral deficiencies in GR crops that can lead to increased plant disease, impaired physiology, and reduced yield.[6−17] Others have not found negative effects of glyphosate on mineral content, plant disease, or yield of GR crops.[18−28] In a recent literature review,[29] the authors concluded glyphosate does not adversely affect mineral nutrition, disease, or yield of GR crops. Since then, additional studies have found no adverse effects of glyphosate on GR crops.[30−32] In one of these papers, the effects of glyphosate on mineral content of GR soybean was examined over three years in five US states and one Canadian province.[31] There were no effects of glyphosate treatment on Mn content at any site in any year. Potential effects of glyphosate on Mn content have been the focus of several papers claiming adverse effects on mineral nutrition of GR crops, for example, refs 8, 10, and 17. A few inconsistent effects on Fe, P, and Zn occurred in this study.[31] For example, the only significant effect on Zn content was higher levels in glyphosate treatments than in treatments with other herbicides in Ontario in one year of the study.

Previous studies were mostly on GR soybean. The GR trait is equally important in maize;[2,3] however, there are only two studies of glyphosate effects on mineral content of GR maize, and they were both limited in scope (one year and one research site).[33,34] More definitive studies of possible effects of glyphosate on mineral nutrition in GR maize are needed. In this paper, we investigate whether the GR transgene or glyphosate adversely affects plant mineral nutrition and yield of maize. The study was conducted over two growing seasons at two geographically distinct regions in fields both with and without a history of continuous glyphosate use. Our results provide further evidence for the null hypothesis that GR crop technology, whether used over one or several years, has no significant effect on mineral nutrition or yield of maize.

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Table 1. Maize Leaf Mineral Content 4 Weeks after Treatment in Isogenic Cultivars Grown on Glyphosate History and No-History Fields at Stoneville, MS in 2013 and 2014[a,b]

	glyphosate history field				no-history field			
leaf mineral content[c]	non-GR, no Gly	GR, no Gly	GR + Gly	LSD (0.05)	non-GR, no Gly	GR, no Gly	GR + Gly	LSD (0.05)
Al	180 a	159 a	156 a	60	386 a	323 a	369 a	114
Ba	4.8 a	3.8 a	4.5 a	1.5	7.9 a	9.5 a	7.0 a	4.2
Cd	0.05 a	0.10 a	0.04 a	0.11	0.05 a	0.06 a	0.05 a	0.02
Co	0.15 a	0.39 a	0.34 a	0.53	1.21 a	1.00 a	1.17 a	1.30
Cr	45.9 a	53.0 a	52.0 a	43.7	142 a	114 a	160 a	125
Cs	0.09 a	0.16 a	0.09 a	0.11	0.05 a	0.07 a	0.08 a	0.04
Cu	13.2 a	13.4 a	13.8 a	1.7	14.6 a	14.8 a	15.1 a	3.7
Fe	401 a	390 a	379 a	173	822 a	676 a	926 a	515
Ga	0.4 a	0.09 a	0.02 a	0.11	0.08 a	0.07 a	0.08 a	0.05
K	18.7 a	17.9 a	17.7 a	1.8	18.0 a	19.6 a	18.7 a	1.7
Mg	2020 a	1810 b	1820 b	130	1960 a	1950 a	1920 a	1230
Mn	80.8 a	67.6 ab	64.3 b	10.6	83.8 a	84.9 a	77.4 a	14.5
Ni	11.6 a	18.8 a	16.1 a	22.3	56.8 a	41.8 a	48.4 a	51.0
Pb	0.06 a	0.12 a	0.05 a	0.12	0.10 a	0.09 a	0.09 a	0.04
Rb	13.7 a	15.6 a	14.9 a	2.9	13.1 a	16.6 a	15.4 a	4.7
Se	13.8 a	11.6 a	12.4 a	3.9	9.5 a	11.8 a	9.8 a	3.7
Sr	18.5 a	16.5 a	15.8 a	3.5	17.5 a	16.0 a	15.2 a	2.7
U	0.03 a	0.07 a	0.02 a	0.09	0.03 a	0.03 a	0.03 a	0.01
V	0.26 a	0.40 a	0.32 a	0.32	1.03 a	0.85 a	1.03 a	0.76
Zn	54.7 a	55.9 a	50.8 a	7.6	52.5 a	52.6 a	50.1 a	4.7

[a]Abbreviations: GR, glyphosate-resistant; non-GR, nonglyphosate-resistant; Gly, glyphosate; LSD, least significant difference. [b]Means within a row for each field followed by same letter are not significantly different at $\alpha = 0.05$. [c]All units are μg/g, except K, which is mg/g.

MATERIALS AND METHODS

Field Experiments. Field trials were conducted in the Southern and Midwestern U.S. maize production regions over two years. Maize grown for grain was investigated in the Southern region at Stoneville, MS. Maize grown for sweet corn was investigated in the Midwestern region at Urbana, IL. At each location, trials were conducted in two field types: (1) fields with a history of glyphosate use (hereafter called "glyphosate history" fields), and (2) fields with no recent history of glyphosate use (hereafter called "no-history" fields).

Stoneville. Field experiments were conducted in 2013 and 2014 at the USDA-ARS Crop Production Systems Research farm, Stoneville, Mississippi, U.S.A. The glyphosate history field had grown either GR soybean or GR cotton the previous 15 years. The no-history field had cogongrass [*Imperata cylindrica* (L.) Beauv.] grown without herbicides the previous 12 years. In 2012, cogongrass was killed with repeated tillage, and then non-GR soybean and non-GR maize were planted in alternate rows to prepare the land. Maize and soybean were grown until maturity and flail mowed. Fields were prepared by disking and bedding in the fall of 2012 and 2013. The soil in both fields was a Dundee silt loam (fine-silty, mixed, active, thermic Typic Endoqualf) with pH 6.7, 1.1% organic carbon, and a cation exchange capacity of 15 mequiv 100 g^{-1}. At planting, soil samples from the top 15 cm depth were collected by taking four random cores (7.5 cm diameter) from all plots. Soil samples were analyzed for mineral content as before.[32]

The treatments were a non-GR cultivar without glyphosate, a GR cultivar without glyphosate, and a GR cultivar with glyphosate (application details below). The experimental design was a randomized complete block with four replications. Each plot consisted of four rows spaced 102 cm apart and 15.2 m long. Maize isogenic cultivars, DKC65-17 RR2 (GR, containing the *cp4 epsps* transgene) and DKC65-18 (non-GR) were planted at 70 000 plants/ha on April 8, 2013 and April 2, 2014 and grown using standard production practices under irrigation. Herbicides (pendimethalin and *S*-metolachlor), plus hand weeding were used to keep the experimental area weed-free during the experiment.

A commercial formulation of potassium salt of glyphosate (Roundup WeatherMAX, Monsanto Agricultural Co., St. Louis, MO) was used in the treatments. Consistent with label recommendations, the first glyphosate application (0.87 kg ae/ha) was applied over the top and the second application (0.87 kg ae/ha) was applied as post directed to the base of the maize plants. Glyphosate was applied with a tractor-mounted sprayer with TeeJet 8004 standard flat fan nozzles (TeeJet Spraying Systems Co., Wheaton, IL) delivering 187 L/ha spray volume at 179 kPa.

At R2 (blister stage, the silks are beginning to dry and darken to a brownish color) maize growth stage (~4 weeks after second glyphosate application and 11 weeks after planting), 40 flag (uppermost) leaves were sampled randomly from the middle two rows of each plot. At physiological maturity, 20 ears were sampled randomly from the middle two rows, and seed were collected from all the ears for chemical analysis. Grain from all four rows of each plot was harvested using a combine, and grain yield was adjusted to 15% moisture. Leaf and seed samples were stored in sealed plastic bags at 4 °C and room temperature, respectively.

Urbana. Similar field experiments were conducted in 2013 and 2014 at two fields at the University of Illinois Crop Sciences Research and Education Center near Urbana, IL, U.S.A. A GR soybean crop had been grown regularly on the glyphosate history field the previous 15-years, while the no-history field had been used as a perennial grass pasture for decades. Soil samples were analyzed by the Agricultural Analytical Services Laboratory, Pennsylvania State University. P, K, Mg, and Ca are Mehlich 3 extractable, and all other metals are total sorbed metals by the EPA 3050 method. The Urbana location differed from Stoneville in terms of isogenic maize cultivars (glyphosate-sensitive sweet corn "Passion" and GR sweet corn "Passion II"), glyphosate rate (a single application of 1.68 kg ae/ha glyphosate applied at the V4−5 growth stage), and timing of yield measurements (R3 growth stage). Additional details are published in the plant pathology results from this same study which addressed the influence of glyphosate and the GR transgene on Goss's wilt incidence and yield in maize.[35]

Mineral Analyses. *Sample Preparation.* Leaves were dried at 60 °C for 24 h to constant weight prior to digestion. Seeds were digested without drying. Samples of each treatment were triplicated. Samples were digested and prepared for analysis as before.[12,32]

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Table 2. Maize Leaf Mineral Content 4 Weeks after Treatment in Isogenic Cultivars Grown on Glyphosate History and No-History Fields at Stoneville, MS in 2013 or 2014[a,b]

		glyphosate history field				no-history field			
year	leaf mineral content[c]	non-GR, no Gly	GR, no Gly	GR + Gly	LSD (0.05)	non-GR, no Gly	GR, no Gly	GR + Gly	LSD (0.05)
2013	As	0.18 a	0.13 a	0.12 a	0.08	0.27 a	0.28 a	0.28 a	0.07
	Na	45.7 a	42.4 a	38.0 a	13.5	93.7 a	130.0 a	99.6 a	89.0
2014	Be	0.03 a	0.14 a	0.01 a	0.25	0.01 a	0.03 a	0.01 a	0.03
	Ca	2710 a	2600 a	2540 a	310	2290 a	2090 a	2110 a	690
	Li	0.23 a	0.36 a	0.17 a	0.36	0.22 a	0.22 a	0.22 a	0.07
	Tl	28.4 a	132.8 a	4.3 a	237	7.4 a	20.5 a	9.9 a	27.7

[a]Abbreviations: GR, glyphosate-resistant; non-GR, nonglyphosate-resistant; Gly, glyphosate; LSD, least significant difference. [b]Means within a row for each field followed by same letter are not significantly different at $\alpha = 0.05$. [c]All units are μg/g, except Tl, which is ng/g.

Table 3. Maize Seed Mineral Content after Treatment in Isogenic Cultivars Grown on Glyphosate History and No-History Fields at Stoneville, MS in 2013 and 2014[a,b]

	glyphosate history field				no-history field			
seed mineral content[c]	non-Gr, no gly	GR, no Gly	GR + Gly	LSD (0.05)	non-GR, no Gly	GR, no Gly	GR + Gly	LSD (0.05)
Al	8.4 a	6.6 a	14.1 a	7.2	8.4 a	10.0 a	15.6 a	16.5
Ba	0.28 a	0.27 a	0.29 a	0.08	0.41 a	0.40 a	0.34 a	0.16
Ca	27.5 a	24.3 a	52.3 a	46.5	26.6 a	36.9 a	32.4 a	3.49
Cd	0.08 b	0.09 ab	0.10 a	0.01	0.09 ab	0.10 a	0.08 b	0.01
Co	0.36 a	0.18 a	0.29 a	0.44	0.14 a	0.20 a	0.22 a	0.18
Cu	7.5 a	12.7 a	12.9 a	16.6	3.5 a	11.7 a	4.7 a	13.0
Fe	54.0 a	23.7 a	32.6 a	40.4	25.6 a	27.2 a	25.3 a	11.9
Mg	1110 a	1040 a	1100 a	80	1110 a	1130 a	1110 a	70
Mn	6.1 a	5.8 a	6.5 a	0.7	6.3 a	6.6 a	6.4 a	0.7
Ni	4.7 a	8.0 a	10.2 a	6.8	8.5 a	12.2 a	14.9 a	12.8
Sr	0.17 a	0.20 a	0.25 a	0.12	0.19 a	0.20 a	0.18 a	0.06
Zn	33.2 a	32.4 a	32.1 a	10.7	33.1 a	40.5 a	36.5 a	23.1

[a]Abbreviations: GR, glyphosate-resistant; non-GR, nonglyphosate-resistant; Gly, glyphosate; LSD, least significant difference. [b]Means within a row for each field followed by same letter are not significantly different at $\alpha = 0.05$. [c]All units are ng/g.

ICP-MS Analysis. The tissue concentrations of 26 minerals (Al, As, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Tl, U, V, Zn) were determined by sector field inductively coupled plasma mass spectrometry (SF-ICPMS) using a Thermo Fisher Element-XR as before.[32] Instrumental and data acquisition parameters are provided in Table S1.

Leaf sample data are provided on a dry-weight basis and seed data are on a wet-weight (fresh) basis. Reference material (NIST SRM 1547, Peach Leaves) recoveries generally ranged from 80 to 120%. Standard deviations between samples of the same treatment were generally less than 10%.

Analysis of Glyphosate and AMPA. Samples were prepared as before.[36] GC-MS analyses were performed as before[36] with modifications.[32]

Statistical Analyses. Because different types of maize were grown at Stoneville and Urbana, crop responses were analyzed separately by location. Within each location, glyphosate history and no-history fields were each represented once due to the difficulty of identifying fields without a recent history of glyphosate use. Therefore, data from glyphosate history and no-history fields were analyzed separately.

Response variables were individually analyzed by ANOVA using the Proc Mixed procedure of SAS, version 9.4 (SAS Institute, Cary, North Carolina). Years and replicates were considered random effects. The three treatments (i.e., non-GR cultivar without glyphosate, GR cultivar without glyphosate, and GR cultivar with glyphosate) were considered fixed effects. In a few instances, some minerals could not be quantified in one of the two years. In such cases, year was removed as a random effect in the mixed model. For all data, when the treatment effect was considered significant ($P < 0.05$), treatment means were compared using the Bonferroni-corrected multiple comparisons procedure. Yield data were subjected to analysis of variance using SAS PROC GLM and treatment means were separated at the 5% level of significance using Fisher's least significant difference test.

■ RESULTS

At planting, there were no differences in soil samples for pH, cation exchange capacity, organic matter, or content of 20 minerals among plots assigned to the three treatments at the Mississippi location (Table S2). There were no differences in soil composition between glyphosate history and no-history fields (Table S2). Soil mineral analyses at the Illinois location were similar (Table S3), one exception being lower Mn in soil of the no-history fields that were to be treated with glyphosate.

Mineral Content of Leaves and Seed. *Stoneville.* There were few treatment differences in leaf mineral content 4 weeks after glyphosate application, and none were consistent across both glyphosate history and no-history fields. Neither the GR transgene nor glyphosate had an effect on leaf content of 24 of 26 minerals (Tables 1 and 2). In the glyphosate history field, leaf Mg and Mn content in the GR with glyphosate treatment were significantly (10 and 20%, respectively) less than the non-GR, no-glyphosate treatment (Table 1). However, leaf Mg and Mn content were unaffected by treatments in the no-history field.

Similarly, few treatment differences in seed mineral content were observed and none were consistent across both glyphosate history and no-history fields. The transgene and glyphosate application had no effect on seed content of 21 of

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Table 4. Maize Seed Mineral Content after Treatment in Isogenic Cultivars Grown on Glyphosate History and No-History Fields at Stoneville, MS in 2013 or 2014[a,b]

		glyphosate history field				no-history field			
year	seed mineral content[c]	non-GR, no Gly	GR, no Gly	GR + Gly	LSD (0.05)	non-GR, no Gly	GR, no Gly	GR + Gly	LSD (0.05)
2013	Cr	0.13 a	0.26 a	0.15 a	0.36	0.14 a	0.13 a	0.19 a	0.21
	Pb	0.48 a	0.36 a	0.24 a	0.32	0.31 a	0.62 a	0.73 a	0.87
	Sr	8.9 a	10.2 a	7.9 a	3.0	7.3 a	7.6 a	7.6 a	2.8
	U	0.06 a	0.06 a	0.06 a	0.01	0.07 a	0.06 a	0.05 a	0.02
	V	0.07 a	0.07 a	0.08 a	0.01	0.07 a	0.07 a	0.07 a	0.01
2014	Ag	0.09 a	0.10 a	0.15 a	0.08	0.16 a	0.12 a	0.11 a	0.13
	Be	0.43 b	0.49 a	0.50 a	0.05	0.43 b	0.51 a	0.41 b	0.06
	Cs	0.23 a	0.26 a	0.27 a	0.04	0.23 a	0.27 a	0.22 a	0.04
	Ga	0.52 a	0.54 a	0.97 a	0.41	0.69 a	0.80 a	0.64 a	0.33
	K	5.62 a	4.76 a	5.36 a	0.72	5.13 a	5.84 a	5.23 a	0.71
	Li	0.57 b	0.65 ab	0.68 a	0.07	0.58 a	0.70 a	0.55 a	0.10
	Rb	5.9 a	5.7 a	5.6 a	1.2	6.2 ab	5.9 b	6.9 a	0.7
	Tl	0.12 b	0.14 a	0.14 a	0.01	0.12 b	0.15 a	0.12 b	0.02

[a]Abbreviations: GR, glyphosate-resistant; non-GR, nonglyphosate-resistant; Gly, glyphosate; LSD, least significant difference. [b]Means within a row for each field followed by same letter are not significantly different at α = 0.05. [c]All units are ng/g, except K, which is mg/g.

Table 5. Sweet Corn Leaf Mineral Content 5 Weeks after Treatment in Isogenic Cultivars Grown on Glyphosate History and No-History Fields at Urbana, IL in 2013 and 2014[a,b]

	glyphosate history field				no-history field			
leaf mineral content	non-GR, no Gly	GR, no Gly	GR + Gly	LSD (0.05)	non-GR, no Gly	GR, no Gly	GR + Gly	LSD (0.05)
Ag (ng/g)	11 a	14 a	27 a	27	4 a	7 a	4 a	4
Al (μg/g)	22.9 a	22.0 a	27.1 a	10.2	26.7 a	27.2 a	21.5 a	6.49
Ba (μg/g)	5.16 a	5.20 a	4.51 a	1.76	4.60 a	4.30 a	3.85 a	1.66
Ca (μg/g)	2810 a	2830 a	2780 a	630	3350 a	3240 a	3150 a	470
Cd (ng/g)	60 a	66 a	52 a	22	53 a	63 a	49 a	18
Co (ng/g)	18 a	19 a	19 a	3	23 a	20 a	19 a	8
Cr (ng/g)	499 a	493 a	638 a	278	510 a	584 a	512 a	80
Cs (ng/g)	36 a	32 a	39 a	16	<1 a	<1 a	<1 a	0.1
Cu (μg/g)	5.59 a	6.21 a	6.42 a	1.57	5.33 a	6.11 a	5.23 a	1.22
Fe (μg/g)	59.5 a	58.4 a	59.9 a	11.8	65.6 a	68.0 a	62.2 a	12.1
Li (μg/g)	5.57 a	3.05 a	7.21 a	5.59	6.23 a	6.77 a	4.76 a	3.79
Mg (μg/g)	1400 a	1330 a	1320 a	326	981 a	852 ab	812 b	133
Mn (μg/g)	53.1 a	54.5 a	45.5 a	23.5	25.9 a	24.3 a	24.9 a	6.28
Ni (ng/g)	738 a	848 a	861 a	318	773 a	734 a	651 a	302
Pb (ng/g)	107 a	110 a	87 a	46	99 a	93 a	91 a	36
Rb (μg/g)	5.43 a	4.84 a	5.17 a	1.44	1.15 a	1.03 a	0.95 a	0.29
Sr (μg/g)	6.78 a	6.60 a	6.39 a	1.40	7.82 a	7.76 a	7.46 a	1.52
Tl (ng/g)	<0.1 a	<0.1 a	0.1 a	0.1	<0.1 a	0.1 a	0.1 a	0.1
V (ng/g)	66 a	75 a	68 a	55	61 a	95 a	60 a	43
Zn (μg/g)	17.1 a	15.6 a	14.8 a	4.3	29.5 a	34.7 a	23.6 a	13.6

[a]Abbreviations: GR, glyphosate-resistant; non-GR, nonglyphosate resistant; Gly, glyphosate; LSD, least significant difference. [b]Means within a row for each field followed by same letter are not significantly different at α = 0.05.

26 minerals (Tables 3 and 4). Significant treatment effects were observed for Cd seed content; however, the pattern of treatment responses was inconsistent across fields with increases above non-GR technology plant levels with glyphosate treatment of GR plants in glyphosate history fields and decreases below nonsprayed GR plants with glyphosate treatment of GR plants in no-history fields (Table 3). Similarly, there were small treatment differences in Be, Li, Rb, and Tl seed content, but these effects were inconsistent across fields (Table 4).

Across leaf and seed samples, when treatment differences were observed, glyphosate or the GR transgene appeared to equally increase or decrease mineral content relative to the non-GR, no-glyphosate treatment. In the instances where treatment differences were observed, the range of differences was 10 to 20%.

Urbana. With one exception, there were no effects of glyphosate or the transgene on leaf mineral content 5 weeks after glyphosate was applied (Table 5). Glyphosate application to GR sweet corn in the no-history field reduced leaf Mg content 17% compared to the non-GR no-glyphosate treatment. This effect was not observed in the glyphosate history field.

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Table 6. Sweet Corn Seed Mineral Content after Treatment in Isogenic Cultivars Grown on Glyphosate History and No-History Fields at Urbana, IL in 2013 and 2014[a,b]

	glyphosate history field				no-history field			
seed mineral content	non-GR, no Gly	GR, no Gly	GR + Gly	LSD (0.05)	non-GR, no Gly	GR, no Gly	GR + Gly	LSD (0.05)
Ag (pg/g)	50 a	48 a	51 a	8	60 a	47 a	56 a	19
Al (ng/g)	7.2 a	26.7 a	8.4 a	32.4	16.0 a	21.7 a	18.5 a	13.7
Ba (pg/g)	539 a	539 a	584 a	75	774 a	570 a	743 a	236
Cd (pg/g)	385 a	394 a	399 a	63	428 a	361 a	360 a	75
Co (pg/g)	504 a	507 a	522 a	86	690 a	737 a	780 a	532
Cr (pg/g)	665 a	1018 a	598 a	909	710 a	536 a	1471 a	1270
Cs (pg/g)	192 a	193 a	200 a	32	190 a	155 a	156 a	36
Fe (ng/g)	26.5 a	27.4 a	29.1 a	5.8	43.3 a	59.0 a	57.4 a	19.1
Li (pg/g)	7 a	7 a	6 a	4	5 a	4 a	7 a	8
Mg (ng/g)	1620 a	1550 a	1640 a	190	1740 a	1660 a	1720 a	150
Mn (ng/g)	8.68 a	8.48 a	8.97 a	1.25	9.06 a	8.04 a	8.43 a	1.23
Na (ng/g)	6.78 a	8.86 a	6.85 a	4.67	13.88 a	6.90 a	9.71 a	11.48
Ni (ng/g)	3.82 a	2.19 a	3.50 a	3.41	3.81 a	4.01 a	10.14 a	8.12
Pb (pg/g)	330 a	351 a	369 a	73	390 a	319 a	324 a	67
Rb (ng/g)	7.98 a	7.22 a	8.34 a	2.06	1.48 a	1.57 a	1.54 a	0.41
Sr (pg/g)	208 a	210 a	245 a	48	321 a	276 a	332 a	98
Tl (pg/g)	283 a	283 a	290 a	48	303 a	249 a	248 a	56

[a]Abbreviations: GR, glyphosate-resistant; Non-GR, nonglyphosate resistant; Gly, glyphosate; LSD, least significant difference. [b]Means within a row for each field followed by same letter are not significantly different at $\alpha = 0.05$.

Table 7. Sweet Corn Seed Mineral Content after Treatment in Isogenic Cultivars Grown on Glyphosate History and No-History Fields at Urbana, IL in 2013[a,b]

	glyphosate history field				no-history field			
seed mineral content	non-GR, no Gly	GR, no Gly	GR + Gly	LSD (0.05)	non-GR, no Gly	GR, no Gly	GR + Gly	LSD (0.05)
Be (pg.g)	650 a	651 a	670 a	124	691 a	575 a	564 a	135
Ca (ng/g)	28.4 a	28.7 a	27.2 a	8.5	32.7 a	27.7 a	28.5 a	13.2
Cu (ng/g)	2.69 a	2.32 a	2.80 a	0.98	3.43 a	5.39 a	4.21 a	5.73
Ga (pg/g)	765 a	765 a	788 a	139	823 a	678 a	660 a	210
K (ng/g)	13600 a	13700 a	15200 a	1300	15500 a	15100 a	14500 a	3000
Se (ng/g)	7.21 a	7.26 a	8.02 a	5.33	7.70 a	3.81 a	3.66 a	4.13
U (pg/g)	725 a	728 a	745 a	134	778 a	643 a	630 a	150
V (pg/g)	799 a	798 a	819 a	146	859 a	709 a	695 a	166
Zn (ng/g)	17.1 a	15.4 a	15.5 a	2.2	20.0 a	21.2 a	19.9 a	4.7

[a]Abbreviations: GR, glyphosate-resistant; non-GR, nonglyphosate resistant; Gly, glyphosate; LSD, least significant difference. [b]Means within a row for each field followed by same letter are not significantly different at $\alpha = 0.05$.

Neither glyphosate nor the GR transgene affected content of the 26 minerals measured in sweet corn seed (Tables 6 and 7).

Glyphosate and AMPA Content of Leaves and Seed. *Stoneville.* Both glyphosate and AMPA were found in leaves of plants that were treated with glyphosate (Figure 1). Neither glyphosate nor AMPA were found in leaves or seed of plants not treated with glyphosate. The levels of AMPA were much lower than those of glyphosate. Both glyphosate and AMPA levels were similar in both years with the same treatments, but measured amounts were higher in glyphosate history field than the no-history field both years, although statistical design did not allow a rigorous comparison. Neither glyphosate nor AMPA was detected in maize seed in either year.

Urbana. Glyphosate levels were approximately 1 order of magnitude lower in the leaves of glyphosate-treated plants in Urbana than in Stoneville (Figure 1). No AMPA was detected in these samples. As with the Stoneville experiment, in 2013 neither glyphosate nor AMPA was detected in the seed of treated plants. In 2014, there were 24.9 ± 4.4 and 40.9 ± 2.4 ng g^{-1} glyphosate in the seed from the no glyphosate history and glyphosate history fields, respectively.

Yield. Stoneville yields were unaffected by the transgene or glyphosate (Figure 2). Sweet corn yield results of the experiment in Urbana, IL (data provided in Williams et al.[35]) were similar in that glyphosate use in GR maize did not reduce yield but was associated with a significant yield increase (8.3%) that was not related to weed control.[35]

DISCUSSION

As with soybean,[18,32] few significant effects of GR technology on mineral content in maize leaves or harvested seed were observed. The small number of significant differences appeared random because they represented less <5% of comparisons and were inconsistent across crop tissues and environments. In a similar study with GR soybean,[32] significant differences in GR technology treatments were also few (<5%) and inconsistent.

Claims of adverse effects of glyphosate on GR crop mineral content have focused on Mn.[8,10,17] The only effect we found on Mn was at the Stoneville glyphosate history fields in which there was a 20% reduction of leaf Mn with glyphosate treatment, compared to the non-GR, no glyphosate plants (Table 1). This effect was not seen in the seed of the same

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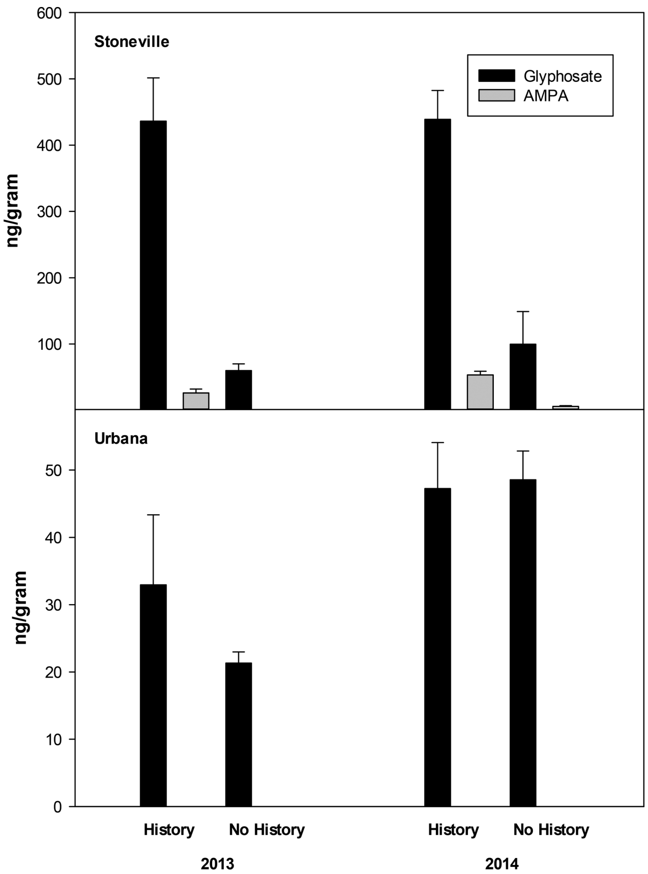

Figure 1. Glyphosate and AMPA content of glyphosate-treated GR maize leaf tissues from glyphosate history and no-history fields in 2013 and 2014 in Stoneville (upper) and Urbana (lower). Error bars are 1 SE of the mean.

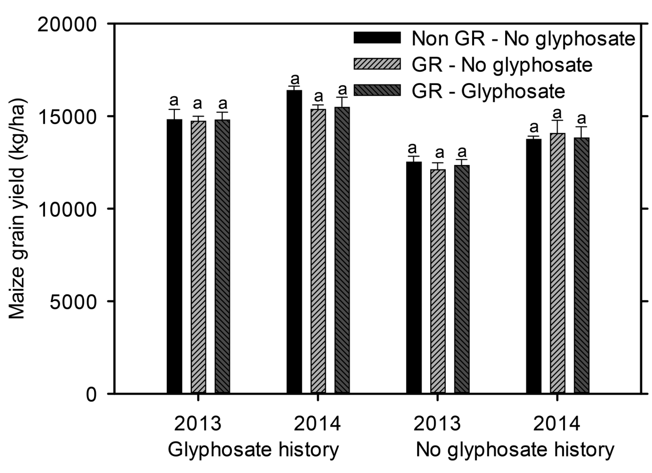

Figure 2. Yield of GR and non-GR maize grown following three treatments (non-GR cultivar without glyphosate, GR cultivar without glyphosate, and GR cultivar with glyphosate) on glyphosate history and no-history fields in 2013 and 2014 at Stoneville, MS. Means within each year followed by same letter are not significantly different at the 5% level as determined by Fisher's least significant difference (LSD) test. Error bars are 1 SE of the mean.

plants. At Urbana, even though there was less Mn in the no-history soil of the glyphosate treatment plots (Table S3), both leaf and seed tissues of glyphosate-treated plants from no-history fields had the same Mn levels as the other treatments (Tables 5 and 6).

In general, our findings are in agreement with the conclusions of Duke et al.[29] that an effect of GR technology on crop mineral nutrition is unlikely for a number of reasons, including lack of phytotoxicity and the much smaller number of herbicide molecules than mineral cations with which glyphosate could directly interact. The results are also in agreement with two different 1-year studies done in Brazil that found no effect of glyphosate on leaf mineral content of GR maize.[33,34]

Foliar glyphosate and AMPA combined were more than 10-fold lower than that found in soybean in a similar experiment performed during the same years at the same Stoneville location.[32] Although the statistical design does not allow a statistical comparison of glyphosate history and no-history fields, we found much higher levels of glyphosate and AMPA in GR plants sprayed with glyphosate grown on the glyphosate history field than the no-history field at Stoneville (Figure 1). We reported a similar finding with soybean in a companion study done the same years on the same fields.[32] Except for the no-history field in 2013, the glyphosate and AMPA results with the cultivar grown at Stoneville (DKC65-17 RR2) contrast with those of an earlier study in which no AMPA was found in glyphosate-treated leaves of the DKC69-72 RR2 cultivar.[37] However, the application rate was much lower in the earlier study. The higher glyphosate and AMPA levels from sprayed plants grown in soil with a glyphosate history were not due to uptake from soil, because nonsprayed plants from glyphosate history fields had neither glyphosate nor AMPA in them. To be sure, we assayed nonsprayed samples twice. The higher glyphosate and AMPA levels in tissues of both soybean and maize grown in the glyphosate history field in two consecutive years at Stoneville may have been due to an undetected difference in growing conditions.

Glyphosate and AMPA levels in leaf tissue were much smaller in plants from Urbana than from Stoneville (Figure 1). The total amount of glyphosate applied to the plants was about the same at the two locations. The difference, in part, may be due to the time between glyphosate application and leaf sampling, which was 4 weeks at Stoneville and 5 weeks at Urbana. Other factors, such as different cultivars or growing conditions, also may have accounted for some of the difference in glyphosate content in plant tissues across locations. No AMPA was detected in any sample that had a glyphosate concentration of about ≤50 ng g^{-1}, whether from Stoneville or Urbana.

Unlike soybean, there was little or no glyphosate and no AMPA in the maize seed. The only two studies of which we are aware that have tested seed from glyphosate-treated GR maize for glyphosate found either no detectable glyphosate[38] or a very low amount (0.12 ng g^{-1}) only from plants sprayed twice with glyphosate (520 and 980 g a.e. ha^{-1} at 14 and 21 days after emergence, respectively).[34] Furthermore, the annual USDA analysis of pesticide residues in maize in the USA has reported neither glyphosate, nor AMPA, in maize seed,[39] of which about 90% are GR maize.[3] The lack of glyphosate in seed of glyphosate-treated GR maize is surprising because of the very phloem-mobile nature of glyphosate.[40] A partial explanation for nondetection or very low concentrations of glyphosate in maize seed of glyphosate-treated GR maize could be more dilution than in soybean because maize produces

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about three times more grain (by weight) per unit area than soybean.

The lack of effect of GR technology on yield is not surprising, as there was no effect of this technology with soybeans in a similar experiment conducted during the same years at the same Stoneville site.[32] Furthermore, maize yields in the U.S. increased at the same rate before and after GR maize was introduced,[29] indicating that GR technology has not influenced this yield trend. Hormesis is enhancement of growth or stimulation of physiological processes (e.g., photosynthesis) by a subtoxic dose of a toxin such as a herbicide. The previously reported increased yield (8.3% increase in harvested kernel mass) in glyphosate-treated GR maize in this study in Urbana[35] may be a hormetic response to glyphosate, as hormetic responses to glyphosate are common.[41] Indeed, Silva et al.[42] recently reported that glyphosate at 1.44 kg ha^{-1} increased yield of GR soybean, independently of its effects on weed control. Because glyphosate is about 50-fold less effective on GR crops than on susceptible isogenic cultivars,[5] one might expect that the hormetic dose for GR crops to be much higher than a hormetic dose for a glyphosate-sensitive crop (about 20 g ha^{-1} for maize).[43]

In summary, for the vast majority of mineral-treatment comparisons (26 minerals, 2 locations, 2 years, 2 fields per location), there was no effect of the GR transgene or glyphosate. When differences were detected, they were not consistent across fields, locations, or plant tissues (leaves and grain). The only effect found in both Stoneville and Urbana was a small reduction in leaf Mg in GR maize leaves treated with glyphosate compared to non-GR maize. However, even this effect was inconsistent in that it was observed only in the glyphosate history but not the no-history field in Stoneville and vice versa in Urbana. Furthermore, the Mg response was independent of glyphosate treatment in Stoneville and dependent on glyphosate treatment in Urbana. Even if these responses are real and require unknown conditions to occur, they are unlikely to be biologically significant because yields were the same across treatments. We do not rule out that glyphosate could adversely affect mineral nutrition of GR crops under mineral deficiency conditions. However, in soils with adequate mineral composition for normal plant growth and development the environment (year and field) has larger effects on plant mineral contents than any aspect of glyphosate resistance technology.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b01655.

> Table S1. ICP-MS instrumental settings. Table S2. Chemical characteristics of soils from glyphosate history and no-history fields at Stoneville, MS in 2013 and 2014. Table S3. Chemical characteristics of soils from glyphosate history and no-history fields at Urbana, IL in 2013 (PDF)

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Notes

The authors declare no competing financial interest.

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