Plant Soil
DOI 10.1007/s11104-009-0081-3

REGULAR ARTICLE

Glyphosate reduces shoot concentrations of mineral nutrients in glyphosate-resistant soybeans

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Received: 30 March 2009 / Accepted: 17 June 2009

Abstract Although glyphosate-resistant (GR) technology is used in most countries producing soybeans (*Glycine max* L.), there are no particular fertilize recommendations for use of this technology, and not much has been reported on the influence of glyphosate on GR soybean nutrient status. An evaluation of different cultivar maturity groups on different soil types, revealed a significant decrease in macro and micronutrients in leaf tissues, and in photosynthetic parameters (chlorophyll, photosynthetic rate, transpiration and stomatal conductance) with glyphosate use (single or sequential application). Irrespective of glyphosate applications, concentrations of shoot macro- and micronutrients were found lower in the near-isogenic GR-cultivars compared to their respective non-GR parental lines Shoot and root dry biomass were reduced by glyphosate with all GR cultivars evaluated in both soils. The lower biomass in GR soybeans compared to their isogenic normal lines probably represents additive effects from the decreased photosynthetic parameters as well as lower availability of nutrients in tissues of the glyphosate treated plants.

Keywords Glyphosate resistant soybean (*Glycine max* L.) · Glyphosate · Nutrient status · Photosynthesis

Abbreviations

A	net photosynthesis
DAS	days after sowing
E	transpiration rate
Gs	stomatal conductance
GR	glyphosate-resistant soybean
Non-GR	conventional soybean near-isogenic parental line
ICP-OES	(inductively coupled plasma-optical emission spectrometer)

Introduction

The cultivation of glyphosate-resistant (GR) soybeans in Brazil has continuously increased in recent years, however many farmers report that the initial development of some GR soybean varieties is visually injured

Responsible Editor: Ismail Cakmak.

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by glyphosate (Santos et al. 2007; Zablotowicz and Reddy 2007).

Glyphosate is a wide spectrum, foliar-applied herbicide that is translocated throughout the plant to actively growing tissues where it inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate pathway. This pathway is responsible for the biosynthesis of aromatic amino acids, plant defense mechanisms, and phenolic compounds (Sprankle et al. 1975; Boocock and Coggins 1983; Singh et al. 1991; Hernandez et al. 1999).

The typical visual symptom noticed in the field after glyphosate application to GR soybeans is known as "yellow flashing" or yellowing of the upper leaves. Some varieties of GR soybeans have little visible yellowing while others may be extensively injured by glyphosate. To relieve yellow flashing, many farmers and agronomists recommend the application of manganese (Mn) before or after applying glyphosate in Brazil. There are studies reporting that glyphosate increases the population of oxidant microorganisms and decreases Mn-reducing microorganisms in the soil making this essential micronutrient unavailable to the plant (Johal and Huber 2009). Very low rates of glyphosate also reduce the root uptake and translocation of Mn and other essential micronutrients in plants (Eker et al. 2006; Ozturk et al. 2008).

Previous studies have demonstrated that GR soybeans respond positively to foliar application of Mn even when the conventional soybean parent variety does not require additional Mn (Gordon 2007a). Organophosphorus complexes such as amino-phosphonic acids present in glyphosate are a new group of highly effective compounds capable of binding metal ions in aqueous media (Kabachnik et al. 1974). Since glyphosate is a phosphonic acid (Franz et al. 1997) and strong "chelator" of metallic cations (Kabachnik et al. 1974; Coutinho and Mazo, 2005), this property might be another cause of Mn decrease in tissue in glyphosate-applied GR soybeans. The first mode of action reported for glyphosate was as a metal chelator, and the molecule was initially patented for that purpose (Jaworski 1972; Bromilow et al. 1993). A current concern related to the use of glyphosate on GR crops, including soybeans, is related to a higher incidence of many diseases that are influenced by the reduced nutritional status of the plant, and the effect of glyphosate on many beneficial soil microorganisms (Kremer et al. 2005; Johal and Huber 2009).

The nutritional status of plants is usually determined through foliar diagnosis, analysis of newly-matured leaves (Mills and Jones 1996; Malavolta et al. 1997). Nutrient sufficiency of plants is directly related to production potential; therefore, foliar analysis can be an important instrument to evaluate nutrient status of plants (Marschner 1995; Mills and Jones 1996; Oliveira et al. 2007). However, there are very few reports about the effects of glyphosate on mineral nutrition of GR soybeans. The objective of this research was to evaluate the mineral status of GR soybeans with glyphosate use compared to their near-isogenic non-GR parental lines.

Materials and methods

Growth conditions

A greenhouse experiment was conducted at the State University of Maringá, PR, between October 14th, 2007 and February 15th, 2008 (location: 23° 25′ S, 51° 57′ W), with soybean (*Glycine max* L.) plants growing in 5.0 dm^{-3} polyethylene pots filled with either of two different soils.

Treatments were combined in a factorial scheme 4×3×2 with four replicates. The first factor was represented by four herbicide treatments, using the commercially formulated isopropylamine salt of glyphosate (480 g a.e. L^{-1}) as recommended. Individual treatments to GR soybean consisted of 1) the non-GR parental line, 2) a non-glyphosate control, 3) a sequential application (600+600 g a.e. ha^{-1}) at the four-leaf and five-leaf stages, 4) a single application of glyphosate (1200 g a.e. ha^{-1}) at the four-leaf stage (Gazziero et al. 2008). The none-GR parental line was considered as the treatment control for each cultivar, and did not receive glyphosate.

The second factor was the cultivar maturity group. Three near-isogenic pairs of soybean cultivars consisting of the glyphosate-resistant and normal parent of each were selected from early, medium, and late maturity groups commonly grown in Brazil. Embrapa 58 and BRS 242 GR are early maturity cultivar, BRS 133 and BRS 245 GR are medium maturity cultivar, and BRS 134 and BRS 247 GR are late maturity cultivar.

The last design factor was soil type. The two soil types evaluated were collected from the A horizon (0 – 20 cm) and sieved to pass through a 5 mesh screen. The Typic Hapludox soil contained 75% clay, 16% sand, pH $CaCl_2$: 5.40; Al: 0.0 cmolc.dm^{-3}; Ca: 8.22 cmolc.dm^{-3}; Mg; 3.03 cmolc.dm^{-3}; K: 0.47 cmolc.dm^{-3}; P: 10.90 mg.dm^{-3}; S: 5.47 mg.dm^{-3}; Fe: 88.02 mg.dm^{-3}; Zn: 11.98 mg.dm^{-3}; Cu: 32.38 mg.dm^{-3}; Mn: 95.04 mg. dm^{-3} and C_{org}: 7.82 g.dm^{-3} while the Rhodic Ferralsol soil was of much lower fertility containing 21% clay, 71% sand, pH $CaCl_2$: 5.10; Al: 0.0 cmolc.dm^{-3}; Ca: 1.85 cmolc.dm^{-3}; Mg: 1.24 cmolc.dm^{-3}; K: 0.26 cmolc.dm^{-3}; P: 18.10 mg.dm^{-3}; S: 27.06 mg.dm^{-3}; Fe: 264.30 mg.dm^{-3}; Zn: 1.73 mg.dm^{-3}; Cu: 3.08 mg. dm^{-3}; Mn: 32.82 mg.dm^{-3} and C_{org}: 7.82 g.dm^{-3}. The characteristics of each soil, organic matter (Corg) content and pH in $CaCl_2$ were determined according to procedures established by Embrapa (1997).

Independent of chemical analyses, the Typic Hapludox was fertilized with 100 mg K_2O and 250 mg P_2O_5 per kg of soil and the Rhodic Ferralsol with 80 mg K_2O, 80 mg P_2O_5 and 1 mg $ZnSO_4$ per kg of soil. Main effects and two factor interactions accounted for 96 experimental units, which were distributed in a randomized block experimental design.

Cultivation practices and glyphosate application

Prior to sowing, soybean seeds were treated with 200 mL 100 kg^{-1} seed of a mixture of 200 g L^{-1} carboxim 200 g L^{-1} thiram (concentrated suspension of systemic and contact fungicide), 13.5 g L^{-1} cobalt and 135.0 g L^{-1} molybdenum, before inoculating with 300 mL 100 kg $^{-1}$ of seeds of a culture of *Bradyrhizobium elkanii*, strains SEMIA 587 and SEMIA 5019 (5×10^9 bacteria per gram). Six seeds were sown per pot at 3 cm depth and thinned to three plants per pot at the one-leaf stage.

Plants at the V4 growth stage were sprayed at 7:00 AM with glyphosate at 190 L ha^{-1} outside the greenhouse using a backpack sprayer with SF110.02 nozzles under a constant pressure of 2 kgf cm^{-2} of CO_2. Environmental conditions during glyphosate application were air temperature between 25 and 29 ° C, humidity between 80 and 89%, wet soil, wind speed between 5 and 10 km h^{-1} and open sky without clouds. The sprayed solution did not cause run-off from leaves. After each herbicide application, the pots were returned to the greenhouse and irrigated the following day to ensure leaf absorption of the herbicide. Thereafter, the pots were irrigated daily in order to keep the soil moist, and kept free of weeds by hand weeding.

Analysis of photosynthesis and mineral nutrients

Just prior to collecting leaves at the R1 stage, the photosynthetic parameters of net photosynthesis (A), transpiration rate (E) and stomatal conductance (gs) were evaluated using an infrared gas analyzer (IRGA) or ADC model LC*pro*+(Analytical Development Co. Ltd, Hoddesdon, UK). The evaluations were made between 7:00 and 11:00 am, using the diagnostic leaf of all three plants in each pot. After photosynthesis parameter analyses were completed, the last fully expanded trifolium (diagnostic leaf) was collected from all three plants in each pot to determine their macro and micronutrient concentration. The R1 stage was slightly different for each cultivar with cultivar BRS 242 GR being 46 days after sowing (DAS); cultivar BRS 245 GR, 54 DAS and cultivar BRS 247 GR, 65 DAS, providing by Embrapa Soja. After complete dry digestion, N was determined by the Kjeldahl method (Baker and Thompson 1992) and the concentration of P, K, Ca, Mg, S, Zn, Mn, Cu and B were measured by ICP-OES (model Optima 3300 DV, Perkin Elmer, USA).

The chlorophyll content was measured (Minolta SPAD-502 meter) on the terminal leaflet of the diagnostic leaf (Singh et al. 2002; Richardson et al. 2002; Pinkard et al. 2006). Two readings were taken per plant in each pot and measurements were averaged to provide a single SPAD unit reading. Chlorophyll content was calculated using the equation of Arnon (1949) and expressed as milligrams of chlorophyll per cm^2 of leaf tissue.

After photosynthetic evaluation, aerial parts of soybean plants were clipped close to the soil and roots were gently washed under running water to remove the soil. All harvested materials were then packed in paper bags and dried in a circulating air oven at 65 – 70°C until a constant weight was achieved. Biomass was determined by weighing plant parts.

Statistic

Data errors of the experimental design were passed through the test of Shapiro and Wilk (1965), in order to evaluate their normality. All data were subjected to analysis of variance and then tested by Scott Knott groupment test to 5% probability by SISVAR variance analysis software (Ferreira 1999).

Results

Nutritional status

Leaf tissue analyses results obtained in this study were compared with values generally considered adequate for physiological sufficiency (Mills and Jones 1996; Oliveira et al. 2007). Although most of the nutrients fell within the broad range considered sufficient by Oliveira et al. (2007), the intensity of decrease caused by glyphosate varied with the cultivar. The data clearly show that there are two factors influencing nutrient efficiency of GR crops. The first factor is the reduced nutrient efficiency imposed by the presence of the glyphosate-resistant gene(s) independent of whether glyphosate is applied or not, and the second factor is the additive impact of glyphosate applied to GR plants. Presence of the GR gene(s) reduced the level of both macro and micronutrients, with the effect being most pronounced in the early maturity group cultivar (Tables 1 and 2). Calcium, Mg, Zn, Mn and Cu were the most commonly reduced mineral nutrients. Most of the nutrients that were reduced by the GR gene were reduced further when glyphosate was applied.

All macronutrients except nitrogen in the early maturity group cultivar (BRS 242 GR) were reduced by glyphosate compared to the non-glyphosate (untreated) GR soybean and its near-isogenic non-GR parental line. Macronutrients in the non-GR parental cultivars always had higher nutrient values than their GR soybean derivatives, with or without glyphosate (Table 1). Only the N, Zn and Mn concentrations were affected by glyphosate in the medium maturity group cultivar (BRS 245 GR) (Tables 1 and 2). Potassium and Mn were reduced in the late maturity group cultivar (BRS 247 GR), when treated with glyphosate (Tables 1 and 2).

All micronutrients were reduced by glyphosate in the early GR maturity group cultivar (Table 2) compared with the non-treated GR soybean and its non-GR parental line. Similarly, all micronutrients except for Fe in the medium maturity group GR cultivar also were reduced, with the exception of Fe, by glyphosate compared to the non-glyphosate treated GR soybean or its non-GR parental line (Table 2). Not as many nutrients in the late maturity group cultivar were affected by glyphosate; however Zn and Mn were still reduced by glyphosate (Table 2).

Glyphosate significantly reduced the macro- and micronutrients in leaf tissues of soybeans grown on both soil types (Tables 3 and 4), with the exception of N in the Typic Hapdulox and Cu in the Rhodic Ferralsol soil. Furthermore, the non-GR parental soybean lines generally had higher levels of the macro and micronutrients compared to their near-isogenic GR derivatives, whether glyphosate was applied or not (Tables 3 and 4).

Photosynthetic parameters

Plants from all maturity groups exposed to a single or sequential application of glyphosate frequently had chlorophyll concentrations lower than plants that were not exposed to this herbicide (Table 5). GR soybean cultivars treated with glyphosate had less chlorophyll than their non-treated control; however the early maturity group GR cultivar had significantly lower chlorophyll and lower stomatal conductance (gs) only with a sequential application of glyphosate (Table 5). In this same cultivar maturity group, the same behavior was also obtained for stomatal conductance (gs); however, in the medium and late maturity group cultivars stomatal conductance was lower in the presence of glyphosate and also in the near-isogenic non-GR parental lines (Table 5)

The photosynthetic rate (A) was lower in glyphosate treated than in non-treated cultivars in the early and medium maturity groups cultivar, but not in the late maturity group cultivar (Table 5). Transpiration (E) was decreased by glyphosate in all cultivars (Table 5).

The photosynthetic parameters (A, E, gs) were severely affected by glyphosate in the different maturity group of GR soybeans growing in different soil types; however there were no differences between the non-treated GR soybeans and their respective near-isogenic non-GR parental lines (Table 6).

Table 1 Macronutrient concentrations at the last fully expanded trifolium (diagnostic leaf) in GR soybean cultivars and their respective near-isogenic non-GR parental lines

Cultivar type	Herbicide treatment	N	P	K	Ca	Mg	S
		g kg^{-1}					
Early - non-GR parent	Without glyphosate	32.62 a*	3.16 a	27.04 a	13.61 a	5.19 a	2.56 a
Early GR	Without glyphosate	33.70 a	2.14 b	23.11 b	10.65 b	3.70 b	1.83 b
Early GR	Sequential (600 + 600 g a.e. ha^{-1})	30.74 a	2.06 b	17.80 c	9.59 c	3.28 c	1.63 c
Early GR	Single (1200 g a.e. ha^{-1})	29.55 a	1.76 c	20.47 c	8.28 d	2.89 d	1.48 d
Medium - non-GR parent	Without glyphosate	34.87 a	2.18 a	23.74 a	12.02 a	3.94 a	2.00 a
Medium GR	Without glyphosate	33.77 a	2.08 a	24.40 a	11.22 a	3.58 b	1.88 a
Medium GR	Sequential (600 + 600 g a.e. ha^{-1})	28.45 b	2.04 a	21.96 a	11.04 a	3.38 b	1.96 a
Medium GR	Single (1200 g a.e. ha^{-1})	28.02 b	1.93 a	22.66 a	11.31 a	3.53 b	2.05 a
Late - non-GR parent	Without glyphosate	32.56 a	1.74 a	21.85 a	11.00 a	3.06 a	1.67 a
Late GR	Without glyphosate	31.35 a	1.96 a	22.78 a	9.61 b	3.03 a	1.75 a
Late GR	Sequential (600 + 600 g a.e. ha^{-1})	30.42 a	2.01 a	18.34 b	9.58 b	2.97 a	1.78 a
Late GR	Single (1200 g a.e. ha^{-1})	29.45 a	1.85 a	19.25 b	8.69 b	2.74 a	1.70 a
CV (%)		15.90	10.36	12.65	8.77	9.22	7.85

At R1 growth stage (46 DAS for early maturity group, 54 DAS for medium maturity group and 65 DAS for late maturity group)

*Data represent the average of two soil types and four independent replicates. For each column, within each cultivar maturity group, statistically significant differences are indicated by different characters according to the Scott-Knott test at $P<0.05$

Table 2 Micronutrient concentrations at the last fully expanded trifolium (diagnostic leaf) in GR soybean cultivars and their respective near-isogenic non-GR parental lines

Cultivar type	Herbicide treatment	Zn	Mn	Fe	Cu	B
		mg kg^{-1}				
Early - non-GR parent	Without glyphosate	72.67 a*	270.27 a	219.28 a	24.21 a	49.79 a
Early GR	Without glyphosate	44.18 b	232.73 b	168.00 b	22.11 a	34.18 b
Early GR	Sequential (600 + 600 g a.e. ha^{-1})	42.43 b	181.67 c	127.60 c	9.55 b	29.38 c
Early GR	Single (1200 g a.e. ha^{-1})	37.64 c	163.67 c	127.15 c	13.55 b	28.53 c
Medium - non-GR parent	Without glyphosate	53.17 a	204.72 a	74.95 a	12.40 a	43.46 a
Medium GR	Without glyphosate	49.02 a	198.75 a	75.87 a	5.90 b	39.10 b
Medium GR	Sequential (600 + 600 g a.e. ha^{-1})	44.61 b	179.71 b	71.03 a	6.03 b	35.76 b
Medium GR	Single (1200 g a.e. ha^{-1})	44.06 b	168.41 b	80.62 a	5.38 b	36.88 b
Late - non-GR parent	Without glyphosate	56.13 a	236.70 a	117.28 a	18.22 a	33.79 a
Late GR	Without glyphosate	49.92 b	214.21 a	95.49 a	21.39 a	35.27 a
Late GR	Sequential (600 + 600 g a.e. ha^{-1})	47.03 b	187.61 b	98.36 a	14.74 a	34.23 a
Late GR	Single (1200 g a.e. ha^{-1})	47.92 b	189.56 b	103.36 a	9.60 a	31.34 a
CV (%)		11.01	15.64	27.12	77.48	10.08

At R1 growth stage (46 DAS for early maturity group, 54 DAS for medium maturity group and 65 DAS for late maturity group)

*Data represent the average of two soil types and four independent replicates. For each column, within each cultivar maturity group, statistically significant differences are indicated by different characters according to the Scott-Knott test at $P<0.05$

Biomass production

Shoot and root biomass of all GR cultivars were reduced by glyphosate (Table 7), although the non-GR parental line of the early maturity group cultivar had less root biomass than its GR derivative without glyphosate. There was no difference between a single or sequential application of glyphosate on biomass accumulation of GR plants. Similar behavior was found with the different soils used in this study, where glyphosate reduced shoot and root biomass of all treated GR soybeans compared with non-treated GR plants or their non-GR parental lines (Table 8).

Table 3 Macronutrient concentrations at the last fully expanded trifolium (diagnostic leaf) in GR soybean cultivars and their respective near-isogenic non-GR parental lines grown on two soil types

Soil type	Herbicide treatment / Cultivar type	N	P	K	Ca	Mg	S
		g kg^{-1}					
Typic Hapludox	Without glyphosate / non-GR parent	32.90 a*	2.39 a	23.18 a	13.19 a	4.35 a	1.80 a
	Without glyphosate / GR	32.21 a	2.10 b	22.05 a	11.19 b	3.69 b	1.62 b
	Sequential (600 + 600 g a.e. ha^{-1}) / GR	31.35 a	1.92 c	17.35 c	10.81 b	3.15 c	1.49 c
	Single (1200 g a.e. ha^{-1}) / GR	30.61 a	1.76 c	19.67 b	10.01 c	3.01 c	1.46 c
Rhodic Ferralsol	Without glyphosate / non-GR parent	33.67 a	2.33 a	25.24 a	11.23 a	3.77 a	2.35 a
	Without glyphosate / GR	33.67 a	2.01 a	24.81 a	9.79 b	3.24 b	2.02 b
	Sequential (600 + 600 g a.e. ha^{-1}) / GR	28.39 b	2.16 b	21.37 b	9.32 b	3.26 b	2.09 b
	Single (1200 g a.e. ha^{-1}) / GR	27.41 b	1.93 b	21.91 b	8.84 b	3.09 b	2.03 b
CV (%)		15.90	10.36	12.65	8.77	9.22	7.85

At R1 growth stage (46 DAS for early maturity group, 54 DAS for medium maturity group and 65 DAS for late maturity group)

*Data represent the average over three maturity group cultivars and four independent replicates. For each column, within each soil type, statistically significant differences at $P<0.05$ are indicated by different characters according to the Scott-Knott test

Table 4 Micronutrient concentrations at the last fully expanded trifolium (diagnostic leaf) in GR soybean cultivars and their respective near-isogenic non-GR parental lines grown on two soil types

Soil type	Herbicide treatment / Cultivar type	Zn	Mn	Fe	Cu	B
		mg kg^{-1}				
Typic Hapludox	Without glyphosate / non-GR parent	72.20 a*	276.95 a	93.56 a	18.16 a	40.08 a
	Without glyphosate / GR	52.16 b	246.60 b	99.97 a	13.99 a	32.08 b
	Sequential (600 + 600 g a.e. ha^{-1}) / GR	48.47 b	218.00 c	83.49 b	9.62 b	27.25 c
	Single (1200 g a.e. ha^{-1}) / GR	47.66 b	211.60 c	85.14 b	6.38 b	26.93 c
Rhodic Ferralsol	Without glyphosate / non-GR parent	48.11 a	197.50 a	180.77 a	18.39 a	44.61 a
	Without glyphosate / GR	43.21 b	183.87 a	126.21 b	18.94 a	40.29 b
	Sequential (600 + 600 g a.e. ha^{-1}) / GR	40.91 b	148.00 b	114.50 b	10.59 a	38.99 b
	Single (1200 g a.e. ha^{-1}) / GR	38.76 b	136.16 b	122.29 b	12.63 a	37.57 b
CV (%)		11.01	15.64	27.12	77.48	10.08

At R1 growth stage (46 DAS for early maturity group, 54 DAS for medium maturity group and 65 DAS for late maturity group)

*Data represent the average over three maturity group cultivars and four independent replicates. For each column, within each soil type, statistically significant differences at $P<0.05$ are indicated by different characters according to the Scott-Knott test

Discussion

Currently the same macro and micronutrient levels in soybean tissues are used by agronomists to determine nutrient sufficiency of both GR and conventional soybeans (Mills and Jones 1996; Oliveira et al. 2007) even though these reference values were generated with conventional cultivars. The results presented in Tables 1 and 2 show that non-GR soybean parental lines generally had higher nutrient concentrations than their respective near-isogenic GR derivatives, independent of glyphosate application. Gordon (2007a, 2007b) also reported that the presence of the GR gene (s) in soybeans reduce the plant's nutrient efficiency compared with near-isogenic non-GR (conventional) soybeans.

Various studies and field observations have reported that glyphosate affects micronutrient nutrition of plants and has been correlated with its ability to form insoluble glyphosate-metal complexes (Madsen et al. 1978; Glass 1984; Coutinho and Mazo 2005; Gordon 2007a; Gordon 2007b). According to Eker et al. (2006), after absorption of glyphosate into the plant, the uptake and transport of cationic micronutrients may be inhibited by the formation of poorly soluble glyphosate-metal complexes within plant tissues. This also could explain the lower micronutrient concentration of GR soybeans after glyphosate application when compared to GR soybeans without glyphosate or their near-isogenic non-GR parental lines (Table 2).

Field observations in Brazil and the North Central United States have reported that frequent applications of glyphosate induce Fe, Zn, and Mn deficiencies in GR- soybean (Franzen et al. 2003; Gordon 2007a; Johal and Huber 2009). In the current study, glyphosate decreased not only the total amount of micronutrients but also the total amount of macronutrients in GR-soybean tissues (Tables 1, 2, 3 and 4); however, some differences were observed between the different maturity group cultivars. These differences could reflect different cultivar efficiencies or a more generalized maturity effect reflected by the more severe effect on the shorter maturity group cultivar which would have a shorter time to compensate or recover from the glyphosate effect. Future research, with more isolines within each maturity group as they become available, can quantify the cultivar versus maturity group-glyphosate effects more thoroughly. It is clear that glyphosate applied at the recommended herbicidal rate can exert negative side-effects on plant growth and micronutrient status, even in transgenic, GR soybeans (Bott et al. 2008).

Since there were no differences generally between the effect of a single or a sequential glyphosate application on the macronutrient concentration of medium and late maturity group cultivars, the initially applied glyphosate apparently remained active in the soybean tissue for more than a week. In contrast, the single glyphosate application to the early maturity group cultivar significantly reduced P, K, Ca, Mg and

Table 5 Photosynthetic parameters of GR soybean cultivars and their respective non-GR parental lines

Cultivar type	Herbicide treatment	Chlorophyll content ——mg cm^2——	Stomatal conductance (gs) ——H_2O mol $m^{-2}s^{-1}$——	Photosynthetic Rate (A) micro mol CO_2 $m^{-2}s^{-1}$	Transpiration Rate (E) mmol H_2O $m^{-2}s^{-1}$
Early - non-GR parent	Without glyphosate	0.017 a*	0.53 a	20.97 a	13.06 a
Early GR	Without glyphosate	0.019 a	0.49 a	16.49 a	11.86 a
Early GR	Sequential (600 + 600 g a.e. ha^{-1})	0.010 b	0.28 b	12.02 b	8.29 b
Early GR	Single (1200 g a.e. ha^{-1})	0.015 a	0.41 a	14.42 b	9.96 b
Medium - non-GR parent	Without glyphosate	0.017 a	0.38 a	15.37 a	9.57 a
Medium GR	Without glyphosate	0.014 b	0.43 a	15.79 a	10.36 a
Medium GR	Sequential (600 + 600 g a.e. ha^{-1})	0.008 c	0.25 b	12.10 b	7.21 b
Medium GR	Single (1200 g a.e. ha^{-1})	0.011 c	0.27 b	11.81 b	7.70 b
Late - non-GR parent	Without glyphosate	0.018 a	0.40 a	12.52 a	9.99 a
Late GR	Without glyphosate	0.015 b	0.37 a	14.52 a	8.93 a
Late GR	Sequential (600 + 600 g a.e. ha^{-1})	0.010 c	0.26 b	13.06 a	8.00 b
Late GR	Single (1200 g a.e. ha^{-1})	0.010 c	0.28 b	12.58 a	7.92 b
CV (%)		23.68	36.09	24.26	23.29

At R1 growth stage (46 DAS for early maturity group, 54 DAS for medium maturity group and 65 DAS for late maturity group);

*Data represent the average of two soil types and four independent replicates. For each column, within each cultivar maturity group, statistically significant differences are indicated by different characters according to the Scott-Knott test at $P<0.05$

Table 6 Photosynthetic parameters of GR soybean cultivars and their respective non-GR parental lines grown on two different soils

Soil type	Herbicide treatment / Cultivar type	Chlorophyll content	Stomatal conductance (gs)	Photosynthetic Rate (A)	Transpiration Rate (E)
		———mg cm^2———	——H_2O mol $m^{-2}s^{-1}$——	micro mol CO_2 $m^{-2}s^{-1}$	mmol H_2O $m^{-2}s^{-1}$
Typic Hapludox	Without glyphosate / non-GR parent	0.015 a*	0.43 a	14.42 a	10.46 a
	Without glyphosate / GR	0.017 a	0.38 a	15.11 a	9.94 a
	Sequential (600 + 600 g a.e. ha^{-1}) / GR	0.010 b	0.24 b	12.08 b	7.25 b
	Single (1200 g a.e. ha^{-1}) / GR	0.013 b	0.30 b	12.88 b	8.33 b
Rhodic Ferralsol	Without glyphosate / non-GR parent	0.018 a	0.44 a	18.18 a	11.28 a
	Without glyphosate / GR	0.017 a	0.48 a	16.09 a	10.83 a
	Sequential (600 + 600 g a.e. ha^{-1}) / GR	0.009 b	0.29 b	12.72 b	8.41 b
	Single (1200 g a.e. ha^{-1}) / GR	0.010 b	0.34 b	13.00 b	8.72 b
CV (%)		23.68	36.09	24.26	23.29

At R1 growth stage (46 DAS for early maturity group, 54 DAS for medium maturity group and 65 DAS for late maturity group)

*Data represent the average over three maturity group cultivars and four independent replicates. For each column, within each soil type, statistically significant differences at $P<0.05$ are indicated by different characters according to the Scott-Knott test

S more than the sequential application (Table 1). This cultivar may be especially nutrient inefficient or there was less time to recover from the likely chelating effects of the higher rate of glyphosate applied early on these plants (Jaworski 1972; Kabachnik et al. 1974; Madsen et al. 1978; Glass 1984; Bromilow et al. 1993; Coutinho and Mazo 2005; Eker et al. 2006).

Application of a single full rate of glyphosate early generally reduced Zn more than a comparable total rate applied in sequential applications in the early maturity group cultivar (Table 2). In general, the timing of glyphosate application had a more specific effect on micronutrients than on macronutrients.

Glyphosate decreased the total amount of macro and micronutrients absorbed by all GR soybeans evaluated (Tables 1 and 2). This reduction was more pronounced in the early maturity group cultivars in which all macronutrients, except N, and all micronutrients were affected by glyphosate (Tables 1 and 2). This data suggests that early maturity group cultivars may be predisposed to more severe injury after herbicide use. The more severe injury of the early maturity group cultivar may be due to the shorter period for detoxification of glyphosate or one of its metabolites such as aminomethylphosphonic acid (AMPA) (Duke et al. 2003; Reddy et al. 2004) which could extend the chelating effect (Jaworski 1972; Kabachnik et al. 1974; Madsen et al. 1978; Glass 1984; Bromilow et al. 1993; Coutinho and Mazo 2005; Eker et al. 2006).

The extent of injury in glyphosate-treated GR soybean is correlated with levels of AMPA formed within the plant (Zablotowicz and Reddy 2007). This primary phytotoxic metabolite is also toxic to GR soybean as evidenced by the reduction in chlorophyll and shoots fresh weight (Reddy et al. 2004); however, glyphosate-immobilized Mg could also be a mechanism, since chlorophyll is dependent on Mg for its formation (Beale 1978; Taiz and Zeiger 1998).

The fact that non-GR parental lines always had a higher nutrient concentration than their near-isogenic GR derivatives treated with glyphosate, independent of the soil type in which they were grown (Tables 3 and 4), indicates that glyphosate reduces the uptake, translocation, or availability of nutrients. Thus, conventional cultivars (non-GR) may have lower critical nutrient levels for physiological sufficiency compared to GR soybeans as reported by Gordon (2007a, 2007b) whether treated with glyphosate or

Table 7 Shoot and root dry biomass, of GR soybean cultivars and their respective near-isogenic non-GR parental lines

Cultivar type	Herbicide treatment	Shoot	Root
		g plant^{-1}	
Early - non-GR parent	Without glyphosate	13.54 a*	4.48 b
Early GR	Without glyphosate	12.62 a	7.24 a
Early GR	Sequential (600 + 600 g a.e. ha^{-1})	7.92 b	4.35 b
Early GR	Single (1200 g a.e. ha^{-1})	9.62 b	5.08 b
Medium - non-GR parent	Without glyphosate	9.33 a	6.94 a
Medium GR	Without glyphosate	11.20 a	6.66 a
Medium GR	Sequential (600 + 600 g a.e. ha^{-1})	7.15 b	3.72 b
Medium GR	Single (1200 g a.e. ha^{-1})	8.17 b	4.54 b
Late - non-GR parent	Without glyphosate	12.17 a	6.63 a
Late GR	Without glyphosate	11.76 a	5.47 a
Late GR	Sequential (600 + 600 g a.e. ha^{-1})	8.24 b	4.36 b
Late GR	Single (1200 g a.e. ha^{-1})	9.04 b	4.33 b
CV (%)		20.49	24.91

At R1 growth stage (46 DAS for early maturity group, 54 DAS for medium maturity group and 65 DAS for late maturity group)

*Data represent the average of two soil types and four independent replicates. For each column, within each cultivar maturity group, statistically significant differences are indicated by different characters according to the Scott-Knott test at $P<0.05$

not. Application of glyphosate exacerbated this interaction since the critical level of a particular nutrient in the plant may be variable depending on the ability to absorb and/or use the nutrient (Fageria 1976; Fageria 1987; Muniz et al. 1985; Fonseca et al. 1988, Scherer 1998). The lower biomass production in glyphosate treated GR cultivars indicates that a higher level of nutrient may actually be required for GR cultivars to achieve physiologically sufficiency.

Nutrient immobilization by glyphosate was more intense for P, K and S on the clay soil — (Typic Hapludox — Table 3). The low availability of P, probably from higher adsorption on clay soils (Novais and Kamprath 1979; Seybold et al. 1999), could have accentuated the lower P in glyphosate treated leaves. The lower S in glyphosate-treated plants on the clay soil may be because its concentration there was lower than in the Rhodic Ferralsol.

The effect of glyphosate on photosynthetic parameters probably reflects lower chlorophyll glyphosate treated plants (Tables 5 and 6) as a result of direct damage of glyphosate to chlorophyll (Kitchen et al. 1981a; Kitchen et al. 1981b; Lee 1981; Reddy et al. 2004) or immobilization of Mg and Mn required for chlorophyll production and function (Beale 1978; Taiz and Zeiger 1998). The main metabolite of glyphosate in plants AMPA may also cause injury to GR-soybeans treated with glyphosate and contribute to observed chlorosis (Pline et al. 1999; Reddy et al. 2001; Duke et al. 2003; Reddy et al. 2004).

Eker et al. (2006) attributed the chlorosis on younger leaves and shoot tips caused by glyphosate to the physiological inactivation of Fe and Mn in these glyphosate-accumulating tissues. In the work reported here, microelement levels of GR soybeans were greatly reduced in the presence of glyphosate compared to treatments without glyphosate (Tables 2 and 4).

The reduced photosynthetic rate (A), stomatal conductance (gs), and transpiration rate (E) in the presence of glyphosate (Tables 5 and 6), could be due to direct damage of chloroplasts (Campbell et al. 1976; Pihakaski and Pihakaski 1980; Nilsson 1985) or immobilization of essential micronutrients by glyphosate. The chloroplast is sensitive to Zn (Homann 1967) and Mn (Thomson and Weier, 1962) deficiency, both of which are reduced by glyphosate (Nilsson 1985). Therefore, the reduction of these essential microelements by glyphosate in GR soybeans (Tables 2 and 4), also could be one of the reasons for the low A, gs and E in GR soybeans treated with glyphosate compared to the GR soybeans without glyphosate or the non-GR parental lines (Tables 5 and 6).

Table 8 Shoot and root dry biomass, of GR soybean cultivars and their respective near-isogenic non-GR parental lines

Soil type	Herbicide treatment / Cultivar type	Shoot	Root
		g plant^{-1}	
Typic Hapludox	Without glyphosate / non-GR parent	11.19 a**	5.19 a
	Without glyphosate / GR	11.42 a	5.80 a
	Sequential (600 + 600 g a.e. ha^{-1}) / GR	7.53 b	3.89 b
	Single (1200 g a.e. ha^{-1}) / GR	8.93 b	4.31 b
Rhodic Ferralsol	Without glyphosate / non-GR parent	12.17 a	6.84 a
	Without glyphosate / GR	12.29 a	7.11 a
	Sequential (600 + 600 g a.e. ha^{-1}) / GR	8.00 b	4.40 b
	Single (1200 g a.e. ha^{-1}) / GR	8.95 b	4.99 b
CV (%)		20.49	24.91

At R1 growth stage (46 DAS for early maturity group, 54 DAS for medium maturity group and 65 DAS for late maturity group)

*Data represent the average over three maturity group cultivars and four independent replicates. For each column, within each soil type, statistically significant differences at $P<0.05$ are indicated by different characters according to the Scott-Knott test

Since glyphosate forms insoluble glyphosate-metal complexes (Madsen et al. 1978; Glass 1984; Coutinho and Mazo 2005), the decrease in microelements could be affecting the main function of chloroplast, i.e. photosynthesis, as evidenced by the severe reduction in photosynthetic parameters (Tables 5 and 6).

The reduction of photosynthetic parameters in GR soybeans by glyphosate at the R1 stage (Tables 5 and 6), long after herbicide application, suggests that both glyphosate and its metabolites have long residual impact on the plant's physiology late into the crop cycle. In either case, glyphosate molecules can remain in plants until complete physiological maturity (Duke et al. 2003; Arregui et al. 2004).

Shibles and Weber (1965) concluded that the total biomass of soybean depends on energy supplied by the photosynthetic process in synthesizing carbon compounds. With the lower availability of energy in glyphosate-treated GR plants, this carbon cannot be formed as efficiently (Taiz and Zeiger 1998). Thus, decreased A, E and gs (Table 5 and 6), could explain the lower nutrient concentration (Tables 1, 2, 3 and 4) and biomass production in GR soybeans treated with glyphosate (Tables 7 and 8).

Decreased shoot and root dry weight by glyphosate also probably occurred because of additive effects from decreased photosynthetic parameters (Tables 5 and 6) and lower nutrient concentration (Tables 1, 2, 3 and 4). Other authors also have pointed out that glyphosate could cause a lower availability of nutrients in plants (Franzen et al. 2003; Eker et al. 2006; Huber 2006; Bott et al. 2008). Reduced nodule formation in GR plants (Jaworski 1972; Moorman et al. 1992; Hernandez et al. 1999; Reddy et al. 2001; King et al. 2001; Reddy et al. 2004; Zablotowicz and Reddy 2004; Bellaloui et al. 2006; De Maria et al. 2006; Zablotowicz and Reddy 2007) may be by direct toxicity of glyphosate in root exudates to *Bradyrhizobium* spp. or by toxicity of aminomethylphosphonic acid (AMPA) formed during glyphosate degradation (Reddy et al. 2004).

The multiple effects of glyphosate on the concentration of nutrients in shoots indicate that GR soybeans require different fertilizer recommendations than non-GR soybeans. The higher nutrient levels in non-GR parental soybeans also indicate that they have a greater efficiency for nutrient uptake and physiological function than their GR derivatives. Lower Mg, Zn, Mn, Fe, and Cu in GR crops could have implications for animal and human health since these nutrients are frequently deficient among people and crops provide the primary dietary source for these essential minerals (Ames 1998). Future research should evaluate the potential impact of glyphosate on seed nutrients since it could affect dietary recommendations as well as seedling health (McCay-Buis et al. 1995)

Conclusions

The nutritional status of GR soybeans is strongly affected by glyphosate. Non-GR parental lines and GR soybean cultivars of different maturity groups without glyphosate generally have higher concentrations of tissue macro and micronutrients than glyphosate-treated near-isogenic GR cultivars. Non glyphosate-treated plants also have greater physiological activity (photosynthesis and respiration) and functional chlorophyll. Fertilize recommendations for GR crops should consider the reduced nutrient efficiency imposed by the presence of the GR gene as well as the further impact of glyphosate on nutrient efficiency.

Acknowledgements We thank the National Council for Scientific and Technology Development (CNPq), for the graduate scholarship for the senior author and financial support for this research.

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