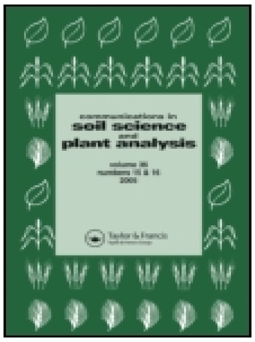

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Effect of Glyphosate and Zinc Application on Yield, Soil Fertility, Yield Components, and Nutritional Status of Soybean

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ABSTRACT

Glyphosate is a widely used nonselective herbicide for the control of agricultural weeds. It is being increasingly used in glyphosate resistant genetically modified plants. However, there are few studies on its effects on the nutritional status of soybean, particularly on the uptake of zinc (Zn). Two experiments were conducted under field conditions in a Typic Quartzipsamment and an Orthic Ferralsol to investigate the effect of glyphosate application × Zn interaction on soil fertility, yield components, seed yield (SY), shoot dry weight (SDW) yield, and nutritional status of soybean. The five Zn rates 0, 3, 6, 9, and 12 kg ha^{-1} were used in two soybean varieties [BRS 133 (conventional—NGM) and its essentially derived transgenic line BRS 245RR (GM), which was divided into: with (+Gly) and without (–Gly) glyphosate application. Only the P (phosphorus) and Zn available concentrations in the soil were impacted by Zn rates. However, the available P concentration only decreased in the soil planted with GM soybean. Mehlich 1 and diethylenetriaminepenta acetic acid–triethanolamine (DTPA–TEA), 7.3 extractants were effective to determine the available Zn. In the two crop sites, the number of pods per plant (NPP) and the SDW yield were affected by the interaction varieties × Zn. SY was influenced by the application of the herbicide, reducing a potential phytotoxic effect with the use of high rates. Regarding the nutrients, only the foliar calcium (Ca), boron (B), iron (Fe), and manganese (Mn) concentrations were negatively affected by glyphosate, and in the case of Zn, the difference occurred only between the varieties BRS 133 and BRS 245RR.

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Acid soils; effect of the herbicide; Glycine max; uptake of nutrients; Zn extractants

Introduction

The glyphosate [(N-phosphonomethyl-glycine)] use resistant genetically modified soybean [Glycine max (L.) Merril] by 85% of Brazilian farmers (Ikeda 2013) resulted in a considerable increase in the use of this herbicide alone or combined with other products, particularly in post-emergence control of weeds, with three to four applications during the crop cycle.

Glyphosate is the world's most widely used systemic nonselective herbicide, recommended for the control of both annual and perennial weeds (Ikeda 2013; Rodrigues and Almeida 1998). Its mechanism of action is based on the inactivation of 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (Coupland 1985), which prevents the synthesis of aromatic amino acids (AAs) (tryptophan, tyrosine, phenylalanine, and histidine) (Delannay et al. 1995; Graham and Webb 1991) and adverse effects on photosynthetic carbon (C) metabolism, and translocation of sucrose in the plants (Cakmak et al. 2009).

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Glyphosate resistant soybean was developed with the insertion of strain cp4 from *Agrobacterium* sp, which encodes a variety resistant to excitatory postsynaptic potential (EPSPS). Notwithstanding, according to some reports, the use of the herbicide may interfere with nutrient absorption (Cakmak et al. 2009; Zobiole et al. 2012). Zinc participates in the synthesis of aromatic AAs such as tryptophan, which is a precursor of indole-3-acetic acid (IAA), a plant hormone required for cell growth, maintenance of apical dominance, among other physiological processes (Davies 1995; Marschner 1995).

Zinc and boron (B) are the most commonly deficient micronutrients in the tropics (Fageria 2009). In the plant, Zn is generally uptake as a divalent cation zinc (Zn^{2+}) (Havlin et al. 1999), and acts as a constituent or activator of several enzymes, being directly involved in the metabolism of nitrogen (N), photosynthesis, respiration, synthesis of AAs and proteins, and hormone control. The lack of Zn causes a decrease of flowering and fruiting, reduced elongation of the cells, resulting in shorter internodes and poor root system development (Fageria 2009; Loué 1993; Marschner 1995). In the soil, different extractants were evaluated (Moreira, Moraes, and Fageria 2015; Oliveira et al. 2003), as well as the Zn concentrations in soybean leaves to determine the most appropriated soil extractants for available Zn in this crop production system.

The present study aimed to assess the effects of glyphosate application and Zn interaction, soil fertility, yield components, seed yield (SY), shoot dry weight (SDW), and nutritional status of two soybean varieties: one conventional (NGM) and its glyphosate resistant essentially derived transgenic line (GM) grown under two types of soil (Typic Quartzipsamment and Orthic Ferralsol) and climate conditions. In both soils was also evaluated the Zn available with Mehlich 1 and DTPA–TEA, pH 7.3 extractants.

Material and methods

Field experiments

Experiments were done at two Brazilian sites: Ponta Grossa, Paraná State (25º05′42”S and 50º10′43”W) on an Orthic Ferralsol (pH calcium chloride ($CaCl_2$) = 3.8, C = 10.9 g kg^{-1}, P = 1.2 g kg^{-1}, potassium (K^+) = 0.04 $cmol_c$ kg^{-1}, Ca^{2+} = 0.2 $cmol_c$ kg^{-1}, magnesium (Mg^{2+}) = 0.2 $cmol_c$ kg^{-1}, aluminum (Al^{3+}) = 14 $cmol_c$ kg^{-1}, potential acidity (H^++Al^{3+}) = 7.4 $cmol_c$ kg^{-1}, cation exchange capacity (CEC) = 7.8 $cmol_c$ kg^{-1}, base saturation (V) = 5.6%, B = 0.4 mg kg^{-1}, copper (Cu) = 1.7 mg kg^{-1}, iron (Fe) = 35.4 mg kg^{-1}, manganese (Mn) = 8.9, mg kg^{-1}, Zn = 0.8 mg kg^{-1}, clay = 350 g kg^{-1}, and sand = 573 g kg^{-1}), and Três Lagoas, Mato Grosso do Sul State (20°45′04”S and 51°40′42”W) on a Typic Quartzipsamment (pH $CaCl_2$ = 4.4, C = 10.9 g kg^{-1}, P = 3.0 g kg^{-1}, K^+ = 0.11 $cmol_c$ kg^{-1}, Ca^{2+} = 1.0 $cmol_c$ kg^{-1}, Mg^{2+} = 0.3 $cmol_c$ kg^{-1}, Al^{3+} = 0.3 $cmol_c$ kg^{-1}, H^++Al^{3+} = 3.1 $cmol_c$ kg^{-1}, CEC = 4.5 $cmol_c$ kg^{-1}, V = 31.0%, B = 0.2 mg kg^{-1}, cooper (Cu) = 0.5 mg kg^{-1}, Fe = 73.0 mg kg^{-1}, Mn = 18.4, mg kg^{-1}, Zn = 0.5 mg kg^{-1}, clay = 141 g kg^{-1}, and sand = 723 g kg^{-1}), in 4 m × 8 m plots in randomized block design in 3 × 5 factorial arrangement, with four replicates. The treatments consisted of five Zn rates (0, 3, 6, 9, and 12 kg ha^{-1}—source: zinc sulfate heptahydrate ($ZnSO_4$ × $7H_2O$)) and two parental varieties (BRS 133 and its essentially derived transgenic line BRS 245RR), and the latter was divided into two other varieties, resulting in three treatments: BRS 133, BRS 245RR with glyphosate (+Gly) and BRS 245RR without glyphosate (–Gly) application.

Fertilization and Soybean planting

In the total area, dolomite limestone (magnesium oxide (MgO) > 13%) was applied to the soil at 0–20 cm depth to raise base saturation to 60%. Except for N and Zn, the fertilizations were performed according to TPS (2013). The micronutrients (B, Cu, Fe, and Mn) and Zn rates corresponding to the treatments were mixed with gypsum calcium sulfate dihydrate

($CaSO_4 \times 2H_2O$) and incorporated with the use of a revolving hoe. The seeds were inoculated with Bradyrhizobium elkanii—SEMIA 587 and SEMIA 5019 (4.0×10^9 viable cells g^{-1}) and treated with a solution containing molybdenum (Mo), cobalt (Co), and nickel (Ni) (TPS 2013).

Crop management and Chlorophyll concentration

At the V4 growth stage (Fehr et al. 1971), half of the plants with treatment BRS 245RR received glyphosate application (Roundup Ready®) at the rate of 1.5 L/ha/application (540 g a.i. ha^{-1}), by spraying at constant pressure of 276 kPa, maintained by compressed CO_2. Following the glyphosate application, the plants were photographed for monitoring of visual symptoms of deficiency or phytotoxicity. At the R2 growth stage, the SPAD unit was measured from the third and fourth fully expanded trifoliate leaves from the apex of 20 plants per plot, and the values were later converted into chlorophyll concentration units (mg cm^{-2}) through equation $\hat{y} = 16.033 + (7.5774 \times SPAD)$ (Fritschi and Ray 2007).

Analysis of soil chemical properties

Soil samples were collected at 0–20 cm depth for each treatment and they were air-dried and passed through a 2.0 mm sieve for quantification of pH in $CaCl_2$ 0.01 mol L^{-1} using a 1:2.5 ratio, soil organic matter (SOM) (Walkley–Black—$C \times 1.724$), available P and exchangeable K^+ (extracted by Mehlich 1) (0.025 mol L^{-1} of H_2SO_4 + 0.05 mol $L^{-1}$of HCl). Exchangeable Ca^{2+} and Mg^{2+} extracted by potassium chloride (KCl) 1.0 mol L^{-1}, potential acidity (H^++Al^{3+}) estimated by SMP (Shoemaker, MacLean and Pratt) buffer, B extracted by hot water, Cu, Fe, and Mn available were extracted using the Mehlich 1 method and available Zn by Mehlich 1 (Mehlich 1978) and diethylene tetramine pentaacetic acid (DTPA)–triethanolamine (TEA)—pH 7.3, (Lindsay and Norvell 1978). CEC was calculated by summing the exchangeable $\sum Ca^{2+}$, Mg^{2+}, K^+, and H^++Al^{3+}, according to methodologies described by Embrapa (1997).

Collection of plant materials and foliar analysis

In the R2 growth stage (Fehr et al. 1971), random samplings of the third and fourth fully expanded trifoliate leaves from the apex were performed in each treatment for foliar diagnosis. In the R7 growth stage, five plants were collected per plot (∑stems, leaves, and pods) to quantify the SDW yield. These plant parts were then dried in forced ventilation oven at 65º±3 C until reaching constant weight. After drying, the samples were weighed for determination of SDW yield. Only the 3rd and 4th trifoliate leaves were ground and subjected to chemical analyzes (Malavolta, Vitti, and Oliveira 1997). Total N was extracted by sulfuric (H_2SO_4) digestion and determined by the micro-Kjeldahl method; P, K, Ca, Mg, S, Cu, Fe, Mn, and Zn were extracted by nitric (HNO_3)–perchloric ($HClO_4$) acid digestion (2:1, v:v), with P and sulfur (S) determined by the spectrophotometric method using the blue molybdenum procedure and turbidimetry, respectively. Foliar B was obtained by incineration at 500°C and determined using the colorimetric reagent Azomethine-H. The other nutrients were determined by atomic absorption spectrophotometry (Malavolta, Vitti, and Oliveira 1997).

Before harvest, 10 plants were randomly collected from each treatment to quantify the number of seeds per plant and per pod. Seed yield (SY) was determined after mechanized harvesting of the plots at the end of the cycle (R8 growth stage). SY data was converted into kg/ha and corrected to 13% moisture, and the 100-seed weight was later quantified.

Statistical analyzes

According to the proposed design, the normality test was conducted for the assessed variables, which were subsequently subjected to analysis of variance (ANOVA–F test), mean comparison by Scott–

Knott at the 5% probability. Regression and correlation analyzes ($P \leq 0.05$) were used to investigate the relationship between Zn rates and glyphosate application and the nutrients uptake, with, yield components, soil fertility, and physiological data and SY and SDW yield of the different parts of the plant and total. Correlations were established between Zn foliar concentration in each treatment, SDW yield and SY with the available Zn concentrations extracted with DTPA–TEA, 7.3, and Mehlich 1 extractants.

Results and discussion

Soil chemical properties and evaluation of extractants

The varieties NGM (BRS 133) and GM (BRS 245RR) without (–Gly) and with (+Gly) glyphosate application showed no varieties × Zn rates on the nutrients concentrations in the soil. Regarding Zn rates (Tables 1 and 2 and Figures 1 and 2), only P and Zn concentration were significantly affected. The P available in the treatment –Gly in the GM variety grown in a Typic Quartzipsamment soil and in the NGM and GM (–Gly) grown in Orthic Ferralsol were affected by Zn rates ($\hat{y} = 15.352 + 0.441x$, $R^2 = 0.94$, $\hat{y} = 1.666 + 0.357x$, $R^2 = 0.80$, and $\hat{y} = 2.008 + 0.269x$, $R^2 = 0.76$, $P \leq 0.05$). Such results demonstrate the effects of the negative Zn × P interaction described by Lopez-Gorostiaga and Malavolta (1974), Loneragan et al. (1982), Loué (1993), and Moreira and Malavolta (2001). However, the glyphosate use minimized this effect regardless of the type of soil. The absence of a significant effect of Zn rates on the other soil chemical properties was also reported by Ritchey et al. (1986) in corn (Zea mays), soybean and sorghum (Sorghum bicolor) crops grown in soil with low nutrients availability. The pH value and P, K^+, Ca^{2+}, Mg^{2+}, $S–SO_4^{2-}$, B, Cu, Fe, Mn, and Zn concentration in a Typic Quartzipsamment were within or close to the levels indicated as suitable for the crops, while in an Orthic Ferralsol, only P concentration were below the minimum suitable concentration for soybean cultivation under the climate and soil conditions of the tropics (TPS 2013).

Mehlich 1 (M1) and DTPA–TEA, 7.3 extractants had high correlation coefficient in the available Zn determination, regardless of the type of soil and glyphosate application (Figure 2). Similarly to Fageria and Santos (2011), M1 extractant showed, in average, a higher rate of recovery of Zn than DTPA–TEA (higher than 63.9%). This result also corroborates Abreu and van Raij (1996) and Moreira, Moraes, and Fageria (2015), who compared both extractants and found that the higher extraction capacity of M1 solution was due to the high acidity of the medium (0.0625 mol H^+ L^{-1}), that made occluded forms of Zn in soil that were unavailable for plant uptake more soluble and mobile, while the DTPA–TEA solution did not become more soluble because of its alkaline reaction (pH = 7.3).

The correlations between foliar Zn concentration and the available concentration in the soil with M1 and DTPA–TEA, 7.3 extractants were similar to those observed by Abreu and van Raij (1996), Oliveira et al. (2003) and Moreira, Moraes, and Fageria (2015), with a significant correlation ($P \leq 0.05$). This was also reported for Zn foliar concentration. However, DTPA–TEA solution showed higher correlation coefficients ($r = 0.78$, 0.81, and 0.78, $P \leq 0.05$), in the two types of soil and different varieties and managements adopted (Figures 1 and 2).

The two extractants obtained similar linear relations, with positive significance with the rates applied. The following equations were obtained for varieties BRS 133, BRS 245RR (–Gly), BRS 245RR (+Gly), and the average of the three types of management in the two sites. x = Zn rate in kg/ha and $\hat{y}$ = soil concentration in mg kg^{-1}:

a) Três Lagoas, Mato Grosso do Sul State (Typic Quartzipsamment)
 Mehlich 1 extractant
 BRS 133—$\hat{y} = 0.529 + 0.201^*x$, $R^2 = 0.40$, $P \leq 0.05$;
 BRS 245RR (–Gly)—$\hat{y} = 0.389 + 0.195^*x$, $R^2 = 0.48$, $P \leq 0.05$;

Table 1. Chemical properties of a Typic Quartzipsamment as influenced by zinc rates within the NGM–BRS 133 and GM–BRS 245RR [without (Gly) and with (+Gly) glyphosate application] soybean cultivars in Mato Grosso do Sul State, Brazil.

Soil properties	Zinc, kg ha^{-1} 0	3	6	9	12	Average	F-test
			BRS 133				
pH, $CaCl_2$	5.72	5.83	5.72	5.94	5.90	5.82	NS
C, g kg^{-1}	7.81	7.71	7.66	7.65	6.87	7.54	NS
P–M1, mg kg^{-1}	20.06	20.21	20.41	19.95	19.32	19.99	NS
K, cmol$_c$ kg^{-1}	0.12	0.11	0.11	0.10	0.08	0.10	NS
Ca, cmol$_c$ kg^{-1}	1.55	1.55	1.49	1.46	1.27	1.46	NS
Mg, cmol$_c$ kg^{-1}	0.78	0.77	0.75	0.74	0.68	0.74	NS
Al, cmol$_c$ kg^{-1}	0.00	0.00	0.00	0.00	0.00	0.00	NS
H+Al, cmol$_c$ kg^{-1}	1.94	1.86	1.93	1.76	1.69	1.83	NS
CEC, cmol$_c$ kg^{-1}	4.38	4.29	4.28	4.06	3.71	4.14	NS
V, %	55.96	57.07	55.04	57.06	54.79	55.98	NS
S–SO_4, mg kg^{-1}	5.84	5.59	5.49	5.27	5.46	5.53	NS
B, mg kg^{-1}	0.20	0.19	0.19	0.17	0.18	0.19	NS
Cu–M1, mg kg^{-1}	0.68	0.62	0.69	0.73	0.85	0.71	NS
Fe–M1, mg kg^{-1}	52.00	48.70	47.13	44.13	46.50	47.69	NS
Mn–M1, mg kg^{-1}	23.75	22.95	23.03	22.03	20.03	22.36	NS
			BRS 245RR (–Gly)				
pH, $CaCl_2$	5.82	5.85	5.89	5.67	5.64	5.59	NS
C, g kg^{-1}	7.54	7.02	6.95	7.27	7.33	7.34	NS
P–M1, mg kg^{-1}	19.99	20.07	18.25	16.57	15.12	13.76	*
K, cmol$_c$ kg^{-1}	0.10	0.10	0.08	0.10	0.10	0.09	NS
Ca, cmol$_c$ kg^{-1}	1.46	1.27	1.27	1.23	1.26	1.22	NS
Mg, cmol$_c$ kg^{-1}	0.74	0.66	0.66	0.65	0.64	0.64	NS
Al, cmol$_c$ kg^{-1}	0.00	0.00	0.00	0.00	0.00	0.00	NS
H+Al, cmol$_c$ kg^{-1}	1.83	1.72	1.69	1.88	1.94	1.98	NS
CEC, cmol$_c$ kg^{-1}	4.14	3.74	3.69	3.86	3.94	3.92	NS
V, %	55.98	54.34	54.54	51.42	51.06	49.85	NS
S–SO_4, mg kg^{-1}	5.53	5.35	5.50	5.78	5.68	5.58	NS
B, mg kg^{-1}	0.19	0.19	0.19	0.22	0.22	0.24	NS
Cu–M1, mg kg^{-1}	0.91	0.80	0.81	0.72	0.75	0.80	NS
Fe–M1, mg kg^{-1}	45.53	43.45	44.15	44.45	42.43	44.00	NS
Mn–M1, mg kg^{-1}	19.68	18.65	19.03	18.90	18.30	18.91	NS
			BRS 245RR (+Gly)				
pH, $CaCl_2$	5.45	5.64	5.64	5.53	5.72	5.59	NS
C, g kg^{-1}	7.77	7.56	7.35	7.44	7.03	7.43	NS
P–M1, mg kg^{-1}	18.90	21.28	22.08	22.03	14.96	19.85	NS
K, cmol$_c$ kg^{-1}	0.10	0.09	0.11	0.11	0.10	0.10	NS
Ca, cmol$_c$ kg^{-1}	1.19	1.27	1.21	1.18	1.23	1.22	NS
Mg, cmol$_c$ kg^{-1}	0.64	0.67	0.66	0.63	0.65	0.65	NS
Al, cmol$_c$ kg^{-1}	0.00	0.00	0.00	0.00	0.00	0.00	NS
H+Al, cmol$_c$ kg^{-1}	2.14	1.96	1.89	1.97	1.77	1.95	NS
CEC, cmol$_c$ kg^{-1}	4.07	3.99	3.87	3.89	3.74	3.91	NS
V, %	47.48	51.03	51.14	49.37	52.68	50.34	NS
S–SO_4, mg kg^{-1}	5.39	5.23	4.98	4.84	4.97	5.08	NS
B, mg kg^{-1}	0.28	0.27	0.27	0.26	0.22	0.26	NS
Cu–M1, mg kg^{-1}	1.05	0.99	0.99	0.93	0.65	0.92	NS
Fe–M1, mg kg^{-1}	44.33	43.00	43.70	45.65	45.15	44.37	NS
Mn–M1, mg kg^{-1}	19.23	18.20	16.65	16.63	15.83	17.31	NS

*, NS Significant at the 5% probability, respectively. CEC—cation exchange capacity, V%—base saturation [CEC/Σ(K, Mg, Ca)] × 100. M1—Mehlich 1 extractant.

BRS 245RR (+Gly)—$\hat{y} = 0.555 + 0.151^{*}x$, $R^2 = 0.66$, $P \leq 0.05$;
Total—$\hat{y} = 0.491 + 0.182^{*}x$, $R^2 = 0.41$, $P \leq 0.05$.
DTPA–TEA, pH 7.3 Extractant
BRS 133—$\hat{y} = 0.488 + 0.115^{*}x$, $R^2 = 0.44$, $P \leq 0.05$;
BRS 245RR (–Gly)—$\hat{y} = 0.416 + 0.126^{*}x$, $R^2 = 0.50$, $P \leq 0.05$;
BRS 245RR (+Gly)—$\hat{y} = 0.561 + 0.092^{*}x$, $R^2 = 0.57$, $P \leq 0.05$;
Total—$\hat{y} = 0.489 + 0.111^{*}x$, $R^2 = 0.48$, $P \leq 0.05$.

Table 2. Chemical properties of an Orthic Ultisol as influenced by zinc rates within the NGM–BRS 133 and GM–BRS 245RR [without (Gly) and with (+Gly) glyphosate application] soybean cultivars in Paraná State, Brazil.

Soil properties	Zinc, kg ha^{-1} 0	3	6	9	12	Average	F-test
			BRS 133				
pH, $CaCl_2$	5.12	5.25	5.13	5.33	5.17	5.20	NS
C, g kg^{-1}	20.54	23.35	23.85	23.91	23.02	22.93	NS
P–M1, mg kg^{-1}	1.91	3.80	2.80	4.12	5.79	3.68	*
K, cmol$_c$ kg^{-1}	0.20	0.22	0.22	0.22	0.21	0.21	NS
Ca, cmol$_c$ kg^{-1}	2.70	2.95	2.90	3.26	2.79	2.92	NS
Mg, cmol$_c$ kg^{-1}	1.51	1.68	1.55	1.68	1.65	1.61	NS
Al, cmol$_c$ kg^{-1}	0.08	0.06	0.06	0.01	0.02	0.05	NS
H+Al, cmol$_c$ kg^{-1}	5.34	5.01	5.19	4.69	5.06	5.06	NS
CEC, cmol$_c$ kg^{-1}	9.74	9.86	9.86	9.85	9.71	9.80	NS
V, %	45.63	49.16	47.39	52.15	47.79	48.42	NS
S–SO_4, mg kg^{-1}	12.84	10.47	13.38	19.68	14.18	14.11	NS
B, mg kg^{-1}	0.36	0.36	0.40	0.45	0.37	0.39	NS
Cu–M1, mg kg^{-1}	1.87	1.73	1.76	1.80	2.01	1.83	NS
Fe–M1, mg kg^{-1}	51.60	48.83	48.38	48.53	51.75	49.82	NS
Mn–M1, mg kg^{-1}	10.35	10.95	11.58	12.60	11.70	11.44	NS
			BRS 245RR (–Gly)				
pH, $CaCl_2$	5.62	5.13	5.42	4.98	5.31	5.29	NS
C, g kg^{-1}	22.96	23.14	22.91	21.27	24.06	22.87	NS
P–M1, mg kg^{-1}	1.77	1.99	5.11	4.11	6.07	3.81	*
K, cmol$_c$ kg^{-1}	0.20	0.22	0.22	0.17	0.20	0.20	NS
Ca, cmol$_c$ kg^{-1}	3.45	2.74	2.95	2.46	2.97	2.91	NS
Mg, cmol$_c$ kg^{-1}	2.00	1.44	1.78	1.34	1.72	1.66	NS
Al, cmol$_c$ kg^{-1}	0.00	0.10	0.06	0.08	0.08	0.06	NS
H+Al, cmol$_c$ kg^{-1}	4.03	5.32	4.68	5.57	5.00	4.92	NS
CEC, cmol$_c$ kg^{-1}	9.68	9.71	9.62	9.54	9.88	9.69	NS
V, %	58.36	45.50	51.45	41.55	49.63	49.30	NS
S–SO_4, mg kg^{-1}	12.38	13.61	13.43	14.09	12.23	13.15	NS
B, mg kg^{-1}	0.30	0.34	0.30	0.46	0.36	0.35	NS
Cu–M1, mg kg^{-1}	1.67	1.86	1.76	1.92	1.94	1.83	NS
Fe–M1, mg kg^{-1}	40.55	54.60	51.73	51.05	67.85	53.16	NS
Mn–M1, mg kg^{-1}	12.13	11.65	12.13	10.23	15.08	12.24	NS
			BRS 245RR (+Gly)				
pH, $CaCl_2$	5.24	5.28	5.22	5.11	5.29	5.23	NS
C, g kg^{-1}	22.45	23.21	23.88	22.44	22.00	22.80	NS
P–M1, mg kg^{-1}	2.28	1.76	2.69	1.66	2.19	2.12	NS
K, cmol$_c$ kg^{-1}	0.17	0.19	0.16	0.16	0.18	0.17	NS
Ca, cmol$_c$ kg^{-1}	2.76	2.99	2.71	2.65	3.00	2.82	NS
Mg, cmol$_c$ kg^{-1}	1.57	1.66	1.63	1.53	1.74	1.63	NS
Al, cmol$_c$ kg^{-1}	0.12	0.03	0.05	0.00	0.03	0.05	NS
H+Al, cmol$_c$ kg^{-1}	5.08	4.80	5.10	5.25	4.75	5.00	NS
CEC, cmol$_c$ kg^{-1}	9.57	9.64	9.60	9.58	9.67	9.61	NS
V, %	47.42	50.43	46.96	45.16	50.91	48.18	NS
S–SO_4, mg kg^{-1}	15.79	13.88	16.28	13.07	15.67	14.94	NS
B, mg kg^{-1}	0.45	0.35	0.40	0.38	0.34	0.38	NS
Cu–M1, mg kg^{-1}	2.24	2.04	1.90	1.83	1.84	1.97	NS
Fe–M1, mg kg^{-1}	63.45	58.88	51.58	50.45	48.20	54.51	NS
Mn–M1, mg kg^{-1}	11.85	12.15	11.53	10.58	11.25	11.47	NS

*, NS Significant at the 5% probability, respectively. CEC—cation exchange capacity, V%—base saturation [CEC/Σ(K, Mg, Ca)] × 100. M1—Mehlich 1 extractant.

b) Ponta Grossa, Paraná State (Orthic Ferralsol)
Mehlich 1 extractant
BRS 133—$\hat{y} = 0.515 + 0.263^{*}x$, $R^2 = 0.58$, $P \le 0.05$;
BRS 245RR (–Gly)—$\hat{y} = 0.919 + 0.185^{*}x$, $R^2 = 0.40$, $P \le 0.05$;
BRS 245RR (+Gly)—$\hat{y} = 1.019 + 0.123^{*}x$, $R^2 = 0.55$, $P \le 0.05$;
Total—$\hat{y} = 0.817 + 0.190^{*}x$, $R^2 = 0.44$, $P \le 0.05$.
DTPA–TEA, pH 7.3 extractant

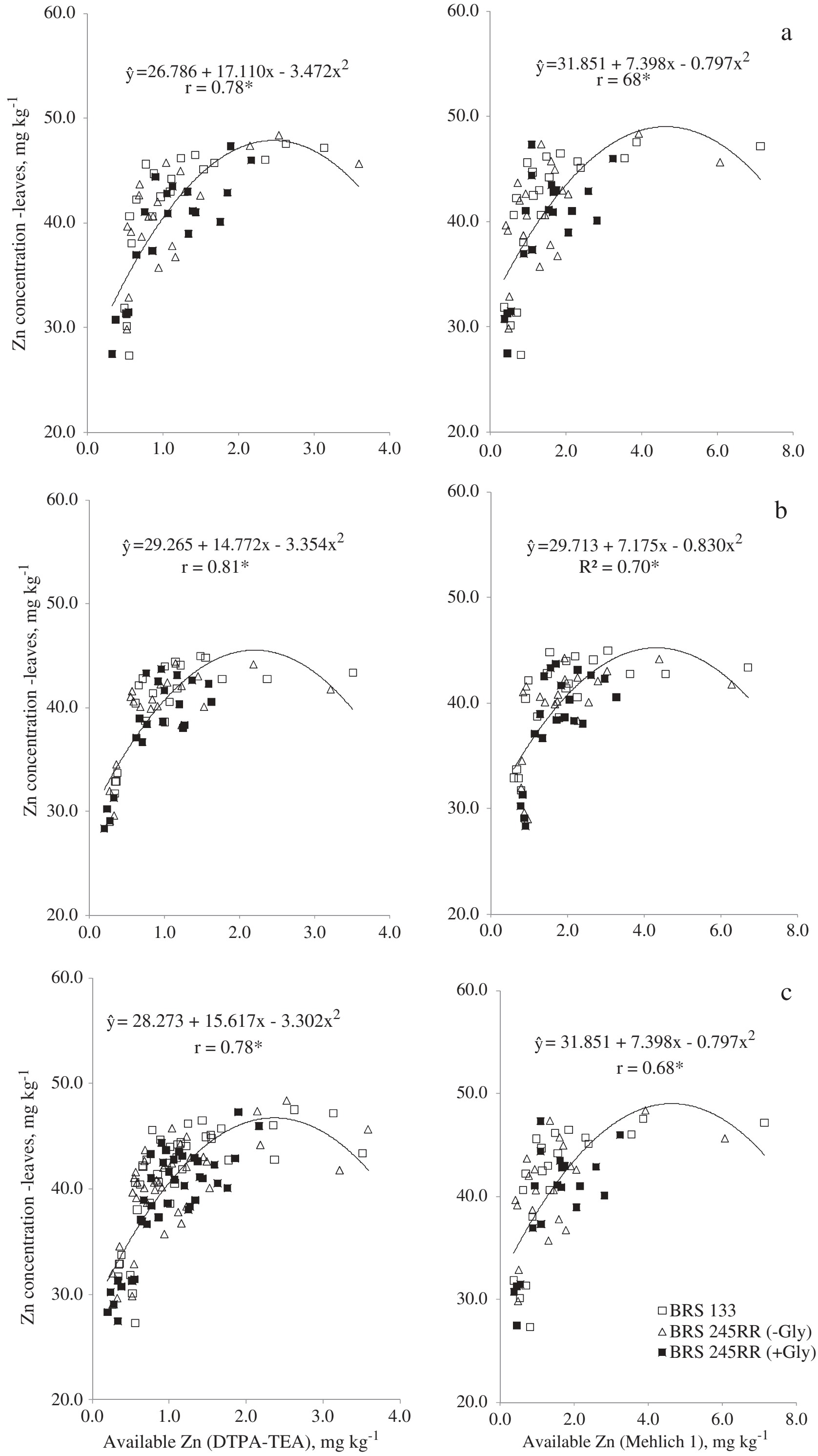

Figure 1. Relationship between the concentration of Zn in the leaf and in the soil obtained with Mehlich 1 and DTPA–TEA extractants in aTypic Quartzipsamment (a), an Orthic Ferralsol (b) and the sum of the two types of soils (c). *Significant at the 5% probability.

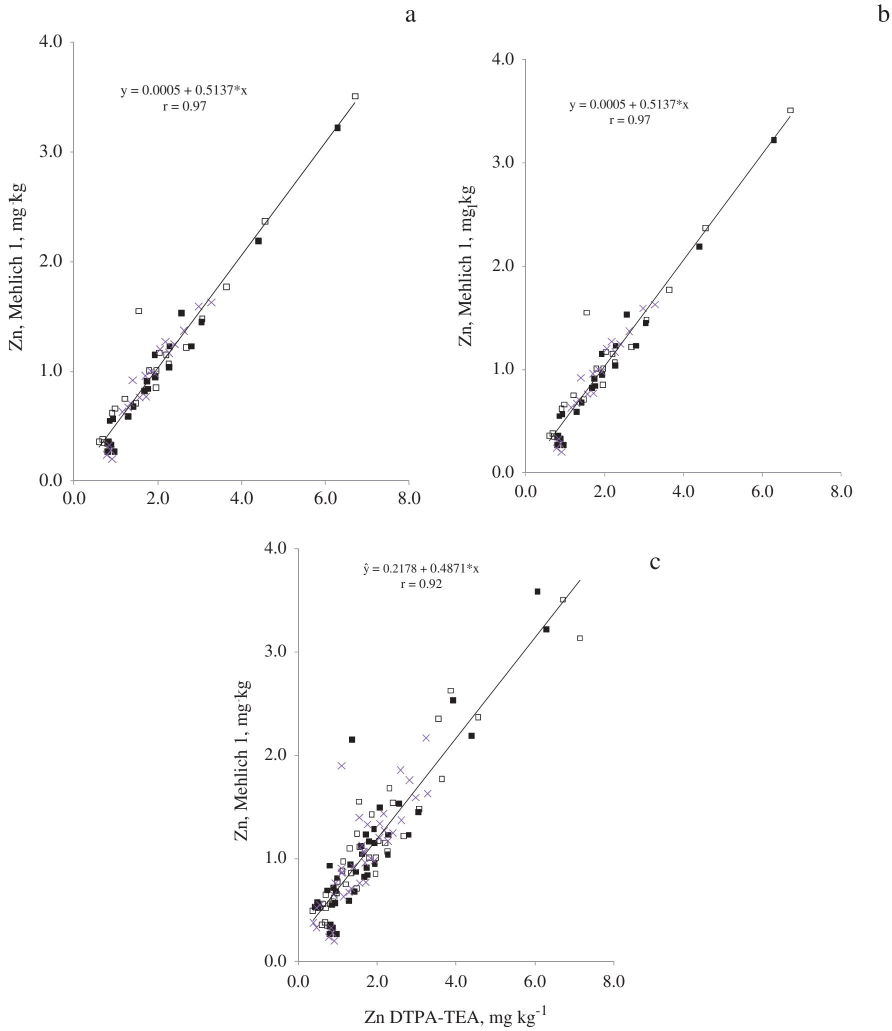

Figure 2. Relationship between Mehlich 1 and DTPA–TEA, 7.3 extractants for available Zn in Typic Quartzipsamment (a), Orthic Ultisol (b), and the sum of the two types of soils (c). *Significant at the 5% probability.

BRS 133—$\hat{y} = 0.361 + 0.125^{*}x$, $R^2 = 0.51$, $P \leq 0.05$;
BRS 245RR (−Gly)—$\hat{y} = 0.386 + 0.103^{*}x$, $R^2 = 0.40$, $P \leq 0.05$;
BRS 245RR (+Gly)—$\hat{y} = 0.403 + 0.082^{*}x$, $R^2 = 0.70$, $P \leq 0.05$;
Total—$\hat{y} = 0.383 + 0.104^{*}x$, $R^2 = 0.47$, $P \leq 0.05$.

The values obtained under the two climate and soil conditions indicate that Mehlich 1 (M1) and DTPA–TEA, 7.3 extractants provide similar information on the available Zn concentration in the soil. Regarding the selection of the extracting solutions, it should be considered, that although the coefficient values of M1 extractant are lower than those of DTPA–TEA, it can extract the available Zn and be used to determine P, K^+, Ca^{2+}, Mg^{2+}, Cu, Fe, and Mn concentration in the same extract (Moreira, Moraes, and Fageria 2015; Oliveira et al. 2003).

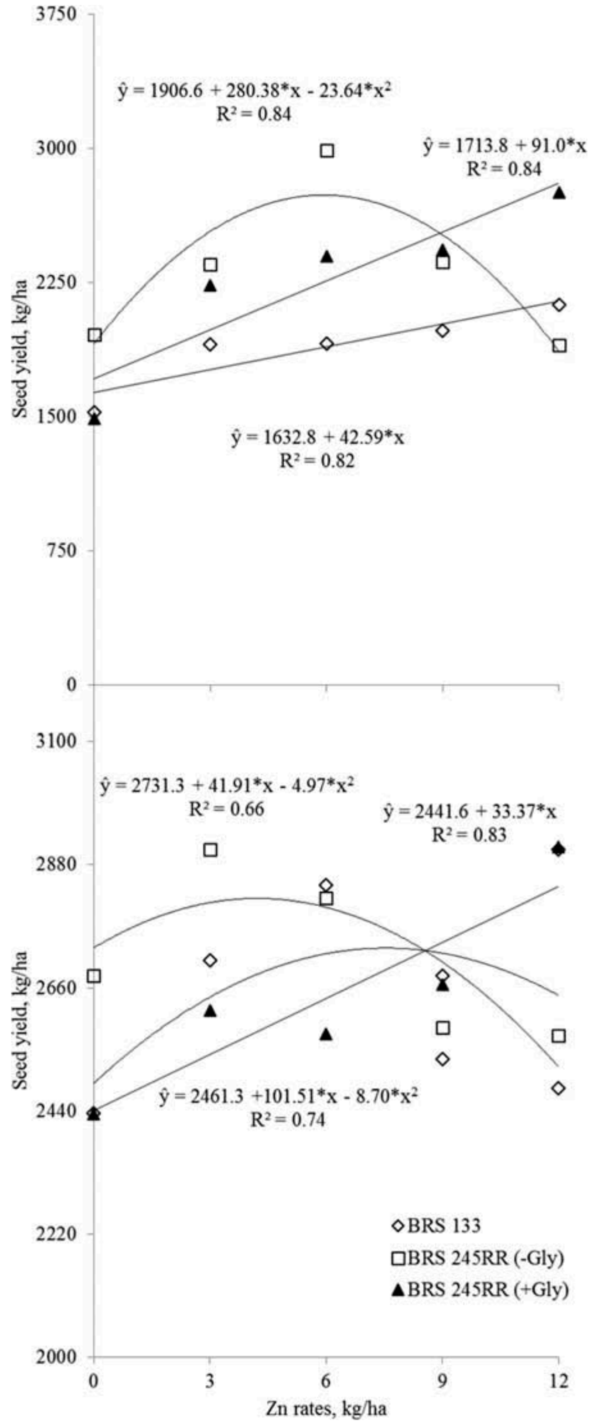

Figure 3. Effect of Zn rates on seed yield (SY) of the BRS 133 and BRS 245RR [with (+Gly) and without (–Gly) glyphosate application] soybean varieties cultivated in a Typic Quatzipsamment (a) and an Ortic Ultisol (b). *Significant at the 5% of probability.

Seed yield

The absence of glyphosate caused quadratic effect in SY, with the highest estimated yields obtained with the application of 12 kg ha^{-1} (BRS 133) and 5.9 kg ha^{-1} [BRS 245RR (–Gly)] of Zn in a Typic Quartzipsamment and 6.1 kg ha^{-1} (BRS 133) and 4.2 kg ha^{-1} [BRS 245RR (–Gly)] in an Orthic Ferralsol, respectively, while in treatment BRS 245RR (+Gly), there was a positive linear effect in the two types of soil, with the highest yield obtained with the 12 kg ha^{-1} of Zn application (Figure 3). This fact demonstrates the possible inhibitory effect in Zn uptake caused by glyphosate application described by Serra et al. (2011), reducing the possible phytotoxic effect of high Zn rates in the soil to meet the nutritional demands of plants, which was also reported by Zobiole et al. (2012) in glyphosate-resistant (GR2) second generation soybean crop.

The introduction of the gene that confers glyphosate resistance did not affect SY, and the GM variety had the highest SY compared to convention variety (BRS 133), with estimated values of 2,738.0 kg ha^{-1} (–Gly), 2,815.8 kg ha^{-1} (+Gly) and 2,143.9 kg ha^{-1} in NGM variety in a Typic Quartzipsamment and 2,819.6 kg ha^{-1} in (–Gly), 2,842.0 kg ha^{-1} (+Gly) and 2,757.4 kg ha^{-1} in NGM in an Orthic Ferralsol. The highest yield reported for BRS 245RR +Gly (Figure 3) can be probably due to the more efficient weed control. Rosolem et al. (2010) and Gonçalves et al. (2014) also found that the presence of the gene that confers resistance to glyphosate herbicide (RR) in the plant did not affect soybean development and yield.

Yield components

Plant height, chlorophyll content, number of pods per plant (NPP), number of seeds per pod (NSP), SDW, and 100-seed weight are shown in Table 3. The SDW yield and NPP were affected ($P \leq 0.05$) by Zn rates ant type of variety in the two crop sites, with significant interaction of varieties × rates, indicating variability

Table 3. Plant height, chlorophyll, seed per pod, pod per plant, shoot dry weight (SDW) yield and 100-seed weight of NGM–BRS 133 and GM–BRS 245RR [without (–Gly) and (+Gly) glyphosate application] soybean cultivars as influenced by Zn rates cultivated in Mato Grosso do Sul State (Typic Quartzipsamment—TQ) and Paraná States (Orthic Ultisol—OU), Brazil.

Cultivar	Zn rates	Plant height		Chlorophyll		Seed per pod		Pod per plant		SDW		100-seed weight	
		(cm)		(mg m^{-2})		(n)		(n)		(g/plant)		(g)	
	kg ha^{-1}	TQ	OU	TQ	OU	TQ	OU	TQ	OU	TQ	OU	TQ	OU
BRS 133	0	44.1	86.6	369.0	372.2	1.5	2.5	36.8	65.3	19.2	102.1	15.1	14.2
	3	40.9	85.7	363.6	381.0	1.8	2.5	84.3	75.1	51.0	125.1	15.1	14.8
	6	40.9	89.9	354.7	383.9	2.2	2.4	78.0	64.5	34.1	114.2	14.8	14.8
	9	47.0	86.3	381.7	377.7	2.0	2.4	93.7	61.2	37.7	92.0	15.0	14.3
	12	45.3	84.5	386.6	379.0	1.8	2.5	59.0	74.2	24.1	131.3	14.9	14.6
	Average	43.6b	86.6a	371.1a	378.8a	1.9a	2.5a	70.4c	68.1b	33.2b	112.9b	15.0a	14.5a
BRS 245RR	0	47.1	84.5	374.0	388.8	2.1	2.5	62.7	82.9	22.0	150.9	14.0	14.6
(–Gly)	3	52.0	87.1	380.5	379.4	2.1	2.5	88.7	50.4	37.2	109.7	12.8	14.7
	6	47.0	87.1	374.1	374.4	1.8	2.5	158.3	73.7	68.8	115.4	14.2	14.4
	9	48.4	89.0	376.4	386.0	2.1	2.5	127.7	103.6	54.9	157.1	14.0	14.8
	12	50.3	88.8	377.5	372.4	2.4	2.4	111.0	94.3	37.1	149.7	13.1	15.1
	Average	49.0a	87.3a	376.5a	380.2a	2.1a	2.5a	109.7a	81.0a	44.0a	136.6a	13.6a	14.7a
BRS 245RR	0	47.4	85.2	381.8	363.4	2.1	2.4	85.0	72.1	31.1	124.0	13.1	14.7
(+Gly)	3	47.5	88.2	395.4	378.1	2.1	2.5	115.3	65.0	49.3	105.8	13.6	15.1
	6	49.4	85.0	388.8	377.1	2.2	2.4	93.7	59.8	28.5	103.4	13.7	14.5
	9	46.8	86.6	373.9	382.2	2.2	2.4	67.0	76.3	23.4	119.1	13.6	14.7
	12	51.0	88.2	372.3	365.6	2.3	2.5	87.7	66.1	38.8	107.0	13.4	14.4
	Average	48.4a	86.6a	382.4a	373.3a	2.2a	2.4a	89.7b	67.9b	34.2b	111.9b	13.5a	14.7a
F-test													
Cultivar (a)		*	NS	NS	NS	NS	NS	*	*	*	*	NS	NS
Rates (b)		NS	NS	NS	NS	NS	NS	*	*	*	*	NS	NS
a × b		NS	NS	NS	NS	NS	NS	*	*	*	*	NS	NS
CV (%)		16.4	17.6	22.1	19.7	17.2	16.4	12.1	18.6	19.8	22.1	20.7	14.2

*, NS Significant at the 5% probability levels, respectively. CV—coefficient of variation. Cultivar—NGM BRS 133 and GM BRS 245RR without (–Gly) and with (+Gly) glyphosate application.

Notes. Means followed by the same letter in the same column are not significantly different at the 5% probability by the Scott–Knott test.

between soybean varieties and glyphosate application or non-application. It was also found that glyphosate use reduced, in average, the SDW yield, but did not affect SY, which was also observed for NPP (Figure 3). Regarding plant height, when soybean was grown in Typic Quartzipsamment, variety BRS 245RR was significantly larger than BRS 133, which did not occur in the crop grown in Orthic Ferralsol, despite its 84.6% larger height (Table 3). This difference in plant height between the two sites is possibly due to the sensitivity of both varieties to latitude variation (4.5º), which resulted in poorer development and lower size of the plants (Xavier et al. 2008).

Unlike the findings of Zobiole et al. (2012), chlorophyll content in soybean leaves was not affected by the introduction of the gene that confers glyphosate resistance, and glyphosate rates for each variety and site ranged from 371.1 to 380.2 mg m^{-2} (Table 3). The 100 seed weight was affected by the treatments (Table 3) and ranged from 12.8 to 15.1 g, which is lower than the value of 15.3 g obtained by Fageria et al. (2014) in a crop grown in an Oxisol of 'Cerrado' with variety BRS 7860. According to Baligar, Fageria, and He (2001), genetic and environmental factors are the main explanations for the differences observed in these variables.

Foliar Nutrient concentration

There was no significant interaction between varieties × Zn rates on the foliar N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn concentration in the plants, in the two crop sites (Table 4). Zn rates caused a quadratic effect on foliar Zn concentration and lack of effect resulting from the application (+Gly) or non-application (–Gly) of glyphosate, with the highest estimated concentrations of 44.7, 43.1, and 43.9 mg kg^{-1} obtained with rates 9.8, 8.7, and 9.2 kg ha^{-1} in a Typic Quartzipsamment, Orthic Ferralsol, and in the average of the two soils, respectively (Figure 4). There were differences between the varieties for foliar K, Ca, S, B, Fe, Mn, and Zn concentration in soybean grown in a Typic Quartzipsamment, and in Ca, S, B, Cu, Fe, and Mn concentration for soybean grown in an Orthic Ferralsol (Figure 4 and Table 4). Despite these adequate soil levels (Tables 1 and 2), the application of glyphosate caused an average reduction of 9.8%, 6.2%, 6.7%, and 14.5% in the Ca, B, Fe, and Mn concentration (Table 4). Similar findings regarding the negative glyphosate effects on the uptake of some nutrients were also reported by Cakmak et al. (2009), Duke et al. (2012), and Zobiole et al. (2012). For Cakmak et al. (2009), the high sensitivity of reproductive organs to glyphosate could partially explain the lower Ca and B uptake by the plants.

The negative effects of glyphosate application on the Fe and Mn uptake (Table 4) were also described by Eker et al. (2006), Cakmak et al. (2009), Serra et al. (2011), and Duke et al. (2012), who report that, despite the introduction of the gene that confers glyphosate resistance, the herbicide acts in the chikimic acid pathway, impairing the synthesis of phenolic compounds such as phenylalanine, and Mn participates in this synthesis. As for Fe, it inhibits the action of the ferric reductase enzyme on the roots, reducing the uptake of the nutrient, among other things (Cakmak et al. 2009). The application of glyphosate did not change foliar concentrations (Figure 4). Despite the findings described by Serra et al. (2011) and Zobiole et al. (2012) concerning the negative effects of the glyphosate use on nutrient uptake, other studies indicate that mineral nutrition in GM plants is not affected by the glyphosate application (Duke et al. 2012; Gonçalves et al. 2014; Rosolem et al. 2010)

Despite the harmful effects observed with or without the glyphosate application, except for Zn at the rate of 0 kg ha^{-1} (control) on soybean grown in a Typic Quartzipsamment that showed visual symptoms of nutrient deficiency (Figure 5). N, P, K, Ca, Mg, S, B, Cu, Fe, and Mn concentration were within the 45.0 to 50.0 g kg^{-1} of N, 2.5 to 5.0 g kg^{-1} of P, 17.0 to 25.0 g kg^{-1}of K, 3.5 to 20.0 g kg^{-1} of Ca, 2.5 to 10.0 g kg^{-1} of Mg, 2.0 to 4.0 g kg^{-1} of S, 20 to 55 mg kg^{-1} of B, 6 to 14 mg kg^{-1} of Cu, 50 to 350 mg kg^{-1} of Fe, 20 to 100 mg kg^{-1} of Mn and 20 to 50 mg kg^{-1} of Zn concentration considered suitable by TPS (2013) for soybean cultivation.

Table 4. Foliar concentration (N, P, K, Ca, Mg, S, B, Cu, Fe, and Mn) of soybean cultivars [conventional—BRS 133 and GM—BRS 245 without (–Gly) and with (+Gly) glyphosate application] as influenced by Zn rates cultivated in Mato Grosso do Sul State (Typic Quartzipsamment—TQ) and Paraná State (Orthic Ultisol—OU), Brazil.

Zn rates	N		P		K		Ca		Mg		S		B		Cu		Fe		Mn	
	(g kg^{-1})		(g kg^{-1})		(g kg^{-1})		(g kg^{-1})		(g kg^{-1})		(g kg^{-1})		(mg kg^{-1})		(mg kg^{-1})		(mg kg^{-1})		(mg kg^{-1})	
kg ha^{-1}	TQ	OU	TQ	OU	TQ	OU	TQ	OU	TQ	OU	TQ	OU	TQ	OU	TQ	OU	TQ	OU	TQ	OU
											BRS 133									
0	52.6	48.9	3.7	3.0	23.7	25.5	6.2	8.4	3.7	5.3	2.6	3.1	74.6	61.6	12.3	10.8	128.9	113.6	75.1	49.8
3	55.9	50.8	4.2	3.4	26.5	26.2	6.2	8.8	3.6	5.4	3.0	2.9	74.6	61.5	10.7	11.1	140.9	113.1	90.0	44.5
6	53.3	51.1	3.7	3.3	25.1	25.6	6.7	8.8	3.8	5.4	2.9	3.2	78.4	60.1	10.1	12.0	155.1	120.8	98.1	53.0
9	51.5	51.5	3.8	3.4	26.5	26.5	6.2	8.4	3.8	5.5	2.9	3.0	77.2	62.4	10.7	11.0	157.2	106.8	102.6	49.3
12	47.9	50.2	3.9	3.0	28.4	23.4	6.7	8.2	3.6	5.2	3.2	2.9	73.5	61.7	10.2	13.0	163.4	116.1	94.0	49.9
Average	52.2a	50.6a	3.9a	3.2a	26.0a	25.4a	6.4b	8.5a	3.7a	5.4a	2.9b	3.0a	75.7a	61.5a	10.8a	11.6a	149.1a	114.1b	92.0b	49.2a
											BRS 245RR (–Gly)									
0	49.4	49.3	3.3	3.3	24.1	24.3	7.2	8,0	5.3	3.8	3.2	3.1	67.4	58.9	9.8	12.2	133.7	135.0	125.9	44.9
3	51.5	48.3	3.5	3.1	23.8	24.7	7.4	7.8	5.6	3.8	3.6	2.9	65.1	57.8	9.2	12.1	155.9	125.7	120.7	46.1
6	51.5	51.5	3.4	3.2	24.7	25.6	7.3	7.9	5.5	3.8	3.6	2.8	60.6	60.9	9.9	11.8	131.6	126.6	108.1	45.7
9	47.7	52.0	3.2	3.5	21.7	25.7	7.1	7.8	5.2	3.8	3.4	2.9	65.2	59.2	8.9	11.4	138.5	123.6	131.2	44.8
12	50.4	50.8	3.4	3.5	22.5	25.4	6.8	8.8	5.4	3.7	3.4	2.7	63.6	57.3	9.0	11.3	137.4	123.2	122.4	43.9
Average	50.1a	50.4a	3.4a	3.3a	23.4a	25.1a	7.2a	8.1a	5.4a	3.8a	3.4a	2.9a	64.4b	58.8b	9.4a	11.8a	139.4b	126.8a	121.7a	45.1b
											BRS 245RR (+Gly)									
0	49.9	49.4	3.3	3.1	20.3	22.9	6.4	7.4	5.2	3.7	3.2	2.8	64.9	53.0	9.3	11.2	126.9	118.4	103.8	40.4
3	46.3	48.6	3.4	3.2	21.3	24.5	6.6	7.4	5.2	3.6	3.5	2.6	62.8	55.4	9.2	10.8	136.7	113.1	99.9	40.9
6	50.0	51.8	3.5	3.1	24.1	23.3	6.5	7.6	5.1	3.8	3.7	2.7	57.6	53.7	9.6	10.9	130.6	113.0	111.0	41.1
9	52.2	50.1	3.2	3.0	22.4	24.1	6.3	7.4	5.2	3.7	3.1	2.6	60.8	55.0	9.3	10.8	133.1	117.9	102.4	39,5
12	49.6	48.4	3.1	2.9	22.4	22.1	6.3	7.3	4.7	3.6	2.8	2.5	60.5	54.5	9.3	10.2	124.6	127.1	97.2	36.8
Average	49.6a	50.0a	3.3a	3.1a	22.1a	23.4b	6.4b	7.4b	5.1a	3.7a	3.3a	2.6b	61.3c	54.3c	9.3a	10.8a	130.4c	117.9b	102.9b	39.7c
F-test																				
Cult (a)	NS	NS	NS	NS	NS	*	*	*	NS	NS	*	*	*	*	NS	*	*	*	*	*
Rates (b)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
a × b	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	18.7	13.2	16.9	18.7	5.5	12.2	8.0	9.2	11.0	17.6	8.4	8.1	9.5	6.8	11.1	10.9	15.5	16.3	24.0	20.3

*, NS Significant at the 5% probability levels, respectively. CV—coefficient of variation. Cult (cultivar)—NGM BRS 133 and GM BRS 245RR without (–Gly) and with (+Gly) glyphosate application.
Notes. Means followed by the same letter in the same column are not significantly different at the 5% probability by the Scott–Knott test.

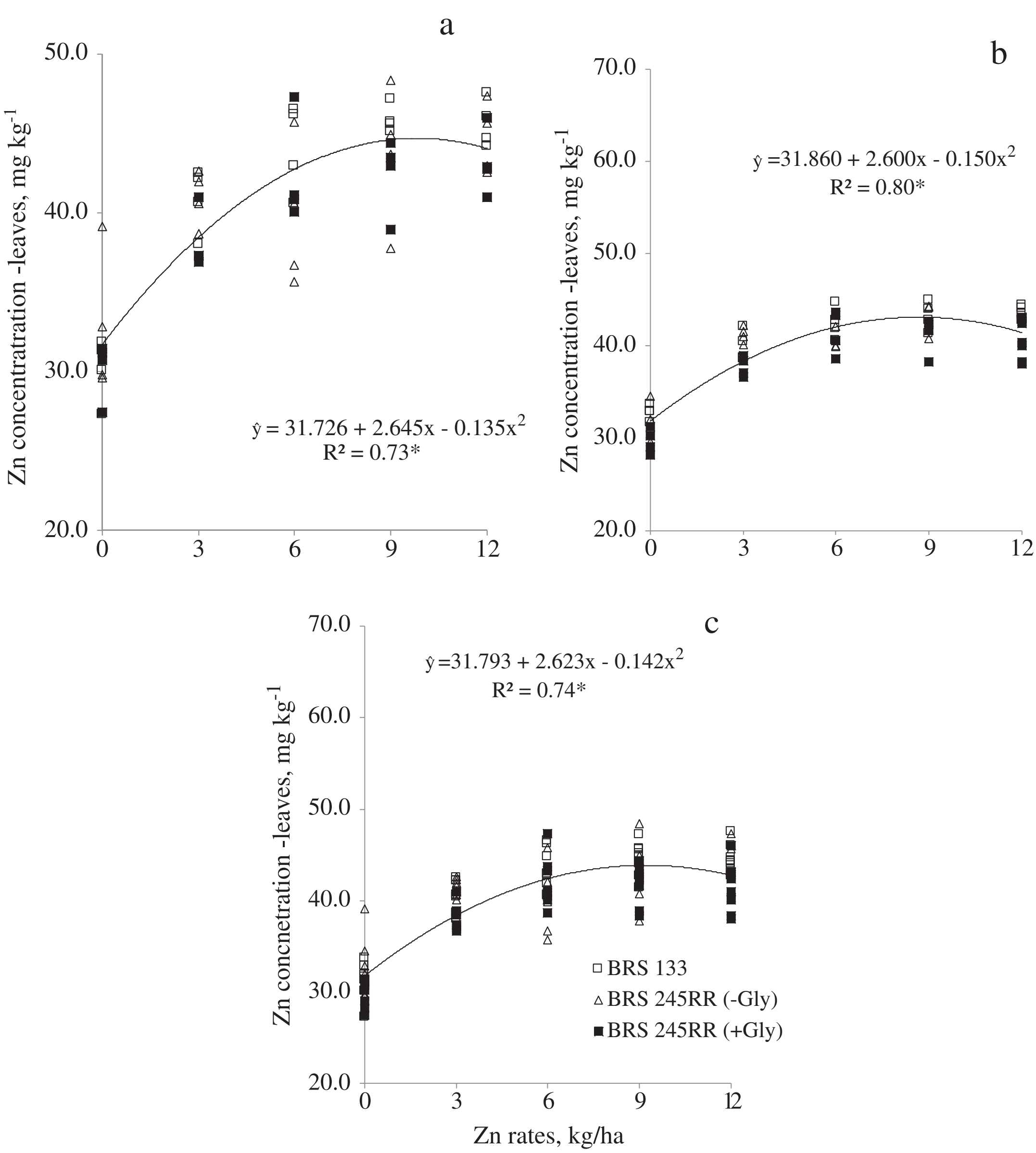

Figure 4. Effect of Zn rates on Zn concentration in BRS 133 and BRS 245RR [with (+Gly) and without (–Gly) glyphosate application] in soybean varieties cultivated in a Typic Quartzipsamment (a), an Orthic Ultisol (b), and the sum of the two soils (c). *Significant at the 5% probability.

Conclusions

Several authors have reported negative effects resulting from the glyphosate use, even in GM soybean. However, few studies investigate the effects of the herbicide on efficiency use of Zn by plants. The crops grown in a Typic Quartzipsamment and an Orthic Ferralsol showed that Mehlich 1 and DTPA–TEA, pH 7.3 extractants effectively determined the available Zn in soil. The highest estimated yields were obtained with of 12.7 kg ha^{-1} (BRS 133) and 5.9 kg ha^{-1} in BRS 245RR (–Gly) applications in a Typic Quartzipsamment (Mato Grosso do Sul State) and 6.1 kg ha^{-1} and 4.2 kg ha^{-1} in an Orthic Ferralsol (Parana State), respectively, while in the treatment BRS 245RR (+Gly), a linear

Figure 5. Symptoms of zinc (Zn) deficiency in soybean leaves grown in a Typic Quartzipsamment (Mato Grosso do Sul State) without Zn application.

effect was observed, and so the intersection point of maximum SY was not reached with the application of up to 12 kg ha^{-1} of Zn. Except for the rate 0 kg ha^{-1} of Zn (control) in the crop grown in a Typic Quartzipsamment, the foliar N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn concentration were within or slightly above the levels considered suitable for soybean cultivation. The introduction of the gene that confers glyphosate resistance only affected plant height, number of pods per plant (NPP) and SDW yield. Except for P and Zn available, the soil chemical properties were not influenced by the treatments. There was a significant interaction of varieties × Zn rates, with negative effect of the glyphosate application on the foliar Ca, B, Fe, and Mn concentration.

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