

EFFECT OF GLYPHOSATE ON NUTRIENT CONTENTS IN GLYPHOSATE-RESISTANT SOYBEANS

Luiz Henrique Saes Zobiolo^{1, 5}; Rubem Silvério de Oliveira Junior²; Jamil Constantin²;
Don M. Huber³, César de Castro⁴; Fábio Alvares de Oliveira⁴, Adilson de Oliveira Junior⁴

1. PhD student of Postgraduate Program in Agronomy, Center for Advanced Studies in Weed Research (NAPD), State University of Maringá (UEM), Colombo Av., 5790, 87020-900, Maringá, Paraná, Brazil
2. Associated Professor, Center for Advanced Studies in Weed Research (NAPD), Agronomy Department, State University of Maringá (UEM), Colombo Av., 5790, 87020-900, Maringá, Paraná, Brazil
3. Botany & Plant Pathology, 915 W. State Street, Purdue University, West Lafayette, IN, USA
4. Embrapa Soybean Researcher, Postcode 231, 86001-970, Londrina, Paraná, Brazil
5. National Council for Scientific and Technology Development (CNPq) scholarship
lhzobiolo@uol.com.br

Abstract Although GR soybean technology is spread all over the countries involved in soybean production, there is no particular fertilizing recommendation under glyphosate use and not much has been reported in the influence of glyphosate on GR soybean status. The objective of this work was to evaluate the mineral status of GR soybeans and their non-GR parental lines under glyphosate use. Considering different soil types and cultivar maturity groups, a significative decrease in macro and micronutrients leaf contents and in photosynthetic parameters (chlorophyll, photosynthetic rate, transpiration and stomatal conductance) was observed under glyphosate use (single or sequential application), both when compared to their respective non GR parental lines and GR soybeans not submitted to glyphosate use. Shoot and root dry biomass productions were affected by glyphosate presence for all cultivars evaluated in both soils, probably because of a sum of effects: decrease of photosynthetic parameters, nutrient uptake and content into the plants.

Keywords GR soybean; glyphosate; nutritional status; photosynthesis

Introduction

The cultivation of glyphosate-resistant (GR) soybeans has continuously increased in recent years, however many farmers report that the initial development of some GR soybean varieties is visually injured by glyphosate (Zablotowicz and Reddy, 2007). 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.

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 that is, responsible for the biosynthesis of aromatic amino acids, plant defense mechanisms, and phenolic compounds (Singh et al. 1991; Hernandez et al. 1999). However, 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).

Nutrient sufficiency of plants is directly related to production potential; therefore, foliar analysis can be an important instrument to evaluate nutrient status of plants (Oliveira et al. 2007). There are very few reports of the effects of glyphosate on mineral nutrition of GR soybeans. The objective of this research was to evaluate the mineral status of GR soybeans and their near-isogenic non-GR parental lines under glyphosate use.

Materials and Methods

Growth conditions and soil

A greenhouse experiment was conducted at the State University of Maringá, Paraná State, Brazil, between October 14th, 2007 and February 15th, 2008 (location: 23° 25' S, 51° 57' W), with different soybean (*Glycine max* L.) maturity groups growing in 5.0 dm³ polyethylene pots filled with either of two different soils. Soil was 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 CaCl₂: 5.40; Al: 0.0; Ca: 8.22; Mg: 3.03; K: 0.47 cmolc.dm⁻³; P: 10.90; S: 5.47; Fe: 88.02; Zn: 11.98; Cu: 32.38; 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 CaCl₂:

5.10; Al: 0.0; Ca: 1.85; Mg: 1.24; K: 0.26 cmolc.dm⁻³; P: 18.10; S: 27.06; Fe: 264.30; Zn: 1.73; Cu: 3.08; Mn: 32.82 mg.dm⁻³ and C_{org}: 7.82 g.dm⁻³.

Glyphosate application

Plants were sprayed 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 CO₂. The environmental conditions during the applications 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 cloudiness. 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

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 LCpro+ (Analytical Development Co. Ltd, Hoddesdon, UK), in the diagnostic leaf of all three plants in each pot. After these assessments, the last fully expanded trifolium (diagnostic) leaf was collected from all three plants in each pot to determine their macro and micronutrient content using an AES Perkin Elmer ICP (inductively coupled plasma) spectrometry.

SPAD readings were taken on the terminal leaflet of the diagnostic leaf by placing the SPAD sensor randomly only on leaf mesophyll tissue to avoid veins. Two readings were chosen 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 cm² of leaf tissue. All harvested materials (aerial part and root) 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.

Data analysis

Treatments were distributed in a randomized block experimental design using a factorial scheme 4x3x2 with four replicates. The first factor was represented by four herbicide treatments, using the commercially formulated isopropylamine salts of glyphosate (360 g a.e. L⁻¹) as recommended. Individual treatments to GR soybean consisted of 1) single application of glyphosate (900 g a.e. ha⁻¹) at the four-leaf stage, 2) a sequential application (450 + 450 g a.e. ha⁻¹) at the four-leaf and five-leaf stages, 3) a non-glyphosate control, 4) non-GR parental line. The no-GR parental line was considered the treatment control for each cultivar, and did not receive any glyphosate. The second factor was the cultivar maturity groups where cv. BRS 242 GR types represented the early maturity group, cv. BRS 245 GR types represented the medium maturity group and cv. BRS 247 GR represented the late maturity group along with their respective non-GR parental lines cv. Embrapa 58, cv. BRS 133 and cv. BRS 134, respectively. The last design factor was soil type. All data were subjected to analysis of variance and then tested by Scott Knott groupment test to 5% probability by SISVAR variance analysis software.

Results and Discussion

Nutritional status

Currently the same macro and micronutrient values are used as a reference to interpret leaf analysis of both GR and conventional soybeans (Oliveira et al. 2007); however these reference data were generated with conventional materials. Although most of the nutrients fell within the sufficiency level recommended by Oliveira et al. (2007), the intensity of responses to glyphosate varied with the cultivar maturity group in question. All of the macronutrients except nitrogen in the early cultivar maturity group (cv. BRS 242 GR) were reduced by glyphosate compared with the non-glyphosate (untreated) GR soybean and non-GR parental line. Macronutrients in the non-GR parental cultivar always had higher values than its GR soybean derivatives, with or without glyphosate (Table 1). Only the N, Zn and Mn contents were affected by glyphosate in the medium cultivar maturity group (cv. BRS 245 GR) (Tables 1 and 2). The K and Mn were the elements reduced in the late cultivar maturity group (cv. BRS 247 GR), when treated with glyphosate (Tables 1 and 2).

All micronutrients were reduced by glyphosate in the early GR cultivar maturity group (Table 2) compared with the non-treated GR soybean its non-GR parental line. Similarly, all micronutrients in the medium cultivar maturity group also reduced, with exception of Fe, by glyphosate presence compared to the non-glyphosate treated GR soybean and its non-GR parental line (Table 2). The late cultivar maturity

group was not affected as severely by glyphosate; however Zn and Mn were still reduced by glyphosate (Table 2). 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 (Glass 1984; Coutinho and Mazo 2005).

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 cultivar maturity group, where all macronutrients, except N, and all micronutrients were affected by glyphosate (Tables 1 and 2). This data suggests that early cultivar maturity group cultivars maybe predisposed to more severe injury after herbicide use. The strong severe injury of early maturity group cultivars may be due to the shorter period for detoxification of glyphosate or to toxicity of one of its metabolites such as aminomethylphosphonic acid (AMPA) (Duke et al. 2003; Reddy et al. 2004) which could increase the chelating effect (Jaworski 1972; Glass 1984; Bromilow et al. 1993; Coutinho and Mazo 2005; Eker et al. 2006).

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 Hapludox and Cu in the Rhodic Ferralsol soil. Furthermore, the non-GR parental soybean line generally had higher levels of the macro and micronutrients compared to its near-isogenic GR derivative, whether glyphosate was applied or not (Tables 3 and 4). The immobilizing of glyphosate was more intense for P, K and S on the clay soil - Typic Hapludox (Tables 3).

Photosynthetic parameters

GR soybean cultivars treated with glyphosate had less chlorophyll than its non-treated control, except for the early cultivar maturity group which had significantly reduced chlorophyll only with sequential application of glyphosate (Table 5). In this same cultivar maturity group, the same behavior was also obtained for stomatal conductance (gs); however, stomatal conductance was lower in the presence of glyphosate than in its absence in GR soybeans and their near-isogenic non-GR parental lines in the medium and late maturity group cultivars (Table 5).

The photosynthetic rate (A) was lower in glyphosate treated than in non-treated cultivars in the early and medium cultivar maturity groups, not with the late cultivar maturity group (Table 5). Transpiration (E) was decreased by glyphosate in all cultivars evaluated (Table 5). The photosynthetic parameters (A, E, gs) were severely affected by glyphosate in the different maturity groups of GR soybeans growing in different soil types evaluated (Table 6) although there were no differences between the non-treated GR soybeans and their respective near-isogenic non-GR parental line. 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. 1981; Reddy et al. 2004) or immobilization of Mg and Mn required for chlorophyll production and function (Beale 1978). The main metabolite of glyphosate in plants AMPA may also cause injury to GR-soybeans treated with glyphosate and contribute to the chlorosis (Pline et al. 1999; Duke et al. 2003; Reddy et al. 2004).

Biomass production

Shoot and root biomass of all cultivars evaluated were reduced by glyphosate (Table 7) except for root biomass of the early cultivar maturity group where the non-GR parental line had less biomass than its GR derivative without glyphosate. There was no difference between single or sequential application on biomass accumulation of plants. Similar behavior was found for soils 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).

Thus, decreased A, E and gs (Table 5 and 6), could explain the lower nutrient content (Tables 1, 2, 3 and 4) and biomass production in GR soybeans treated with glyphosate (Tables 7 and 8). The multiple effects of glyphosate on nutrient availability, uptake, translocation, and physiological efficiency, indicate that different values from non-GR soybeans are needed for recommending fertilizer applications to GR soybeans. The higher nutrient levels in non-GR parental soybeans also indicate a greater efficiency in nutrient uptake and physiological function than their GR derivatives.

Conclusions

The nutritional status of GR soybeans is strongly affected by glyphosate. Non-GR parental lines and GR soybean varieties of different maturity groups without glyphosate generally have higher levels of tissue

macro and micronutrients and also greater physiological activity (photosynthesis and respiration) and chlorophyll content.

Acknowledgement

We thank the National Council for Scientific and Technology Development (CNPq), for the scholarship and financial support.

References

Arnon DI, 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol* 24:1-15

Beale SI, 1978. δ -Aminolevulinic acid in plants: its biosynthesis, regulation and role in plastid development. *Annu Rev Plant Physiol* 29:95-120

Bromilow RH, Chamberlain K, Tench AJ, Williams RH, 1993. Phloem translocation of strong acids: Glyphosate, substituted phosphonic, and sulfonic acids in *Ricinus communis* L. *Pestic Sci* 37:39-47

Campbell WF, Evans JO, Reed SC, 1976. Effect of glyphosate on chloroplast ultrastructure of quackgrass mesophyll cells. *Weed Sci* 24:22-25

Coutinho CFB and Mazo LH, 2005. Complexos metálicos com o herbicida glyphosate: Revisão. *Química Nova* 28:1038-1045

Duke SO, Rimando AM, Pace PF, Reddy KN, Smeda RJ, 2003. Isoflavone, glyphosate, and aminomethylphosphonic acid levels in seeds of glyphosate-treated, glyphosate-resistant soybean. *J Agric Food Chem* 51:340-344

Eker S, Ozturk L, Yazici A, Erenoglu B, Romheld V, Cakmak I, 2006. Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus* L.) plants. *J Agric Food Chem* 54:10019-10025

Glass RL, 1984. Metal complex formation by glyphosate. *J Agric Food Chem* 32:1249-1253

Hernandez A, Garcia-Plazaola JI, Bacerril JM, 1999. Glyphosate effects on phenolic metabolism of nodulated soybean (*Glycine max* L. Merrill). *J Agric Food Chem* 47:2920-2925

Jaworski EG, 1972. Mode of action of N-phosphonomethyl-glycine: inhibition of aromatic amino acid biosynthesis. *J. Agri. Food Chem* 20:1195-1198

Kitchen LM, Witt WW, Rieck CE, 1981. Inhibition of chlorophyll accumulation by glyphosate. *Weed Sci* 29:513-516

Oliveira FA, Sfredo GJ, Castro C, Klepker D, 2007. Fertilidade do solo e nutrição da soja. Londrina: Embrapa Soja, Circular Técnica 50, 8

Pline WA, Wu J, Hatzios KK, 1999. Effects of temperature and chemical additives on the response of transgenic herbicide-resistant soybeans to glufosinate and glyphosate applications. *Pestic Biochem Physiol* 65:119-131

Reddy KN, Rimando AM, Duke SO, 2004. Aminomethylphosphonic acid, a metabolite of glyphosate, causes injury in glyphosate-treated, glyphosate-resistant soybean. *J Agric Food Chem* 52:5139-5143

Singh BK, Siehl DL, Connelly JA, 1991. Shikimate pathway: why does it mean so much to so many? *Oxf Surv Plant Mol Cell Biol* 7:143-185

Zablotowicz RM and Reddy KN, 2007. Nitrogenase activity, nitrogen content, and yield responses to glyphosate in glyphosate-resistant soybean. *Crop Protec* 26:370-376

Table 1 Macronutrient content of GT soybean cultivars and their respective near-isogenic non-GT parental lines at R1 growth stage

Cultivar maturity group	Herbicide treatment	N	P	K	Ca	Mg	S	Total macronutrients
		g kg ⁻¹						
Early – non-GT	Without glyphosate	32.62 a*	3.16 a	27.04 a	13.61 a	5.19 a	2.56 a	84.18 a
Early GT	Without glyphosate	33.70 a	2.14 b	23.11 b	10.65 b	3.70 b	1.83 b	75.14 b
Early GT	Sequential (450 / 450 g a.e. ha ⁻¹)	30.74 a	2.06 b	17.80 c	9.59 c	3.28 c	1.63 c	65.11 c
Early GT	Single (900 g a.e. ha ⁻¹)	29.55 a	1.76 c	20.47 c	8.28 d	2.89 d	1.48 d	64.45 c
Medium – non-GT	Without glyphosate	34.87 a	2.18 a	23.74 a	12.02 a	3.94 a	2.00 a	78.76 a
Medium GT	Without glyphosate	33.77 a	2.08 a	24.40 a	11.22 a	3.58 b	1.88 a	76.94 a
Medium GT	Sequential (450 / 450 g a.e. ha ⁻¹)	28.45 b	2.04 a	21.96 a	11.04 a	3.38 b	1.96 a	68.84 b
Medium GT	Single (900 g a.e. ha ⁻¹)	28.02 b	1.93 a	22.66 a	11.31 a	3.53 b	2.05 a	69.51 b
Late – non-GT	Without glyphosate	32.56 a	1.74 a	21.85 a	11.00 a	3.06 a	1.67 a	71.68 a
Late GT	Without glyphosate	31.35 a	1.96 a	22.78 a	9.61 b	3.03 a	1.75 a	70.49 a
Late GT	Sequential (450 / 450 g a.e. ha ⁻¹)	30.42 a	2.01 a	18.34 b	9.58 b	2.97 a	1.78 a	65.11 b
Late GT	Single (900 g a.e. ha ⁻¹)	29.45 a	1.85 a	19.25 b	8.69 b	2.74 a	1.70 a	63.69 b
CV (%)		15.90	10.36	12.65	8.77	9.22	7.85	7.92

*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 content of GT soybean cultivars and their respective near-isogenic non-GT parental lines at R1 growth stage

Cultivar maturity group	Herbicide treatment	Zn	Mn	Fe	Cu	B	Total micronutrients
		mg kg ⁻¹					
Early – non-GT	Without glyphosate	72.67 a*	270.27 a	219.28 a	24.21 a	49.79 a	636.33 a
Early GT	Without glyphosate	44.18 b	232.73 b	168.00 b	22.11 a	34.18 b	501.24 b
Early GT	Sequential (450 / 450 g a.e. ha ⁻¹)	42.43 b	181.67 c	127.60 c	9.55 b	29.38 c	390.72 c
Early GT	Single (900 g a.e. ha ⁻¹)	37.64 c	163.67 c	127.15 c	13.55 b	28.53 c	370.61 c
Medium – non-GT	Without glyphosate	53.17 a	204.72 a	74.95 a	12.40 a	43.46 a	388.84 a
Medium GT	Without glyphosate	49.02 a	198.75 a	75.87 a	5.90 b	39.10 b	368.74 a
Medium GT	Sequential (450 / 450 g a.e. ha ⁻¹)	44.61 b	179.71 b	71.03 a	6.03 b	35.76 b	337.20 b
Medium GT	Single (900 g a.e. ha ⁻¹)	44.06 b	168.41 b	80.62 a	5.38 b	36.88 b	335.41 b
Late – non-GT	Without glyphosate	56.13 a	236.70 a	117.28 a	18.22 a	33.79 a	462.21 a
Late GT	Without glyphosate	49.92 b	214.21 a	95.49 a	21.39 a	35.27 a	416.21 a
Late GT	Sequential (450 / 450 g a.e. ha ⁻¹)	47.03 b	187.61 b	98.36 a	14.74 a	34.23 a	382.06 b
Late GT	Single (900 g a.e. ha ⁻¹)	47.92 b	189.56 b	103.36 a	9.60 a	31.34 a	381.86 b
CV (%)		11.01	15.64	27.12	77.48	10.08	11.08

Table 3 Macronutrient content of GT soybean cultivars and their respective near-isogenic non-GT parental lines at R1 growth stage

Soil type	Herbicide treatment / Cultivar maturity group	N	P	K	Ca	Mg	S	Total macronutrient
		-----g kg ⁻¹ -----						
Typic Hapludox	Without glyphosate / non-GT	32.90 a*	2.39 a	23.18 a	13.19 a	4.35 a	1.80 a	77.82 a
	Without glyphosate / GT	32.21 a	2.10 b	22.05 a	11.19 b	3.69 b	1.62 b	72.81 b
	Sequential (450 / 450 g a.e. ha ⁻¹) / GT	31.35 a	1.92 c	17.35 c	10.81 b	3.15 c	1.49 c	66.09 c
	Single (900 g a.e. ha ⁻¹) / GT	30.61 a	1.76 c	19.67 b	10.01 c	3.01 c	1.46 c	66.54 c
Rhodic Ferralsol	Without glyphosate / non-GT	33.67 a	2.33 a	25.24 a	11.23 a	3.77 a	2.35 a	78.60 a
	Without glyphosate / GT	33.67 a	2.01 a	24.81 a	9.79 b	3.24 b	2.02 b	75.57 a
	Sequential (450 / 450 g a.e. ha ⁻¹) / GT	28.39 b	2.16 b	21.37 b	9.32 b	3.26 b	2.09 b	66.62 b
	Single (900 g a.e. ha ⁻¹) / GT	27.41 b	1.93 b	21.91 b	8.84 b	3.09 b	2.03 b	66.23 b
CV (%)		15.90	10.36	12.65	8.77	9.22	7.85	7.92

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

Table 4 Micronutrient content of GT soybean cultivars and their respective near-isogenic non-GT parental lines at R1 growth stage

Soil type	Herbicide treatment / Cultivar maturity group	Zn	Mn	Fe	Cu	B	Total amount of micronutrient
		-----mg kg ⁻¹ -----					
Typic Hapludox	Without glyphosate / non-GT	72.20 a*	276.95 a	93.56 a	18.16 a	40.08 a	501.13 a
	Without glyphosate / GT	52.16 b	246.60 b	99.97 a	13.99 a	32.08 b	444.91 b
	Sequential (450 / 450 g a.e. ha ⁻¹) / GT	48.47 b	218.00 c	83.49 b	9.62 b	27.25 c	386.95 c
	Single (900 g a.e. ha ⁻¹) / GT	47.66 b	211.60 c	85.14 b	6.38 b	26.93 c	377.80 c
Rhodic Ferralsol	Without glyphosate / non-GT	48.11 a	197.50 a	180.77 a	18.39 a	44.61 a	490.46 a
	Without glyphosate / GT	43.21 b	183.87 a	126.21 b	18.94 a	40.29 b	412.60 b
	Sequential (450 / 450 g a.e. ha ⁻¹) / GT	40.91 b	148.00 b	114.50 b	10.59 a	38.99 b	353.03 c
	Single (900 g a.e. ha ⁻¹) / GT	38.76 b	136.16 b	122.29 b	12.63 a	37.57 b	347.46 c
CV (%)		11.01	15.64	27.12	77.48	10.08	11.08

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

Table 5 Photosynthetic parameters of GT soybean cultivars and their respective non-GT parental lines at R1 growth stage

Cultivar maturity group	Herbicide treatment	Chlorophyll content	Stomatal conductance (gs)	Photosynthetic Rate (A)	Transpiration Rate (E)
		-----mg cm ² -----	---H ₂ O mol m ⁻² s ⁻¹ --	micro mol CO ₂ m ⁻² s ⁻¹	mmol H ₂ O m ⁻² s ⁻¹
Early – non-GT	Without glyphosate	0.017 a*	0.53 a	20.97 a	13.06 a
Early GT	Without glyphosate	0.019 a	0.49 a	16.49 a	11.86 a
Early GT	Sequential (450 / 450 g a.e. ha ⁻¹)	0.010 b	0.28 b	12.02 b	8.29 b
Early GT	Single (900 g a.e. ha ⁻¹)	0.015 a	0.41 a	14.42 b	9.96 b
Medium – non-GT	Without glyphosate	0.017 a	0.38 a	15.37 a	9.57 a
Medium GT	Without glyphosate	0.014 b	0.43 a	15.79 a	10.36 a
Medium GT	Sequential (450 / 450 g a.e. ha ⁻¹)	0.008 c	0.25 b	12.10 b	7.21 b
Medium GT	Single (900 g a.e. ha ⁻¹)	0.011 c	0.27 b	11.81 b	7.70 b
Late – non-GT	Without glyphosate	0.018 a	0.40 a	12.52 a	9.99 a
Late GT	Without glyphosate	0.015 b	0.37 a	14.52 a	8.93 a
Late GT	Sequential (450 / 450 g a.e. ha ⁻¹)	0.010 c	0.26 b	13.06 a	8.00 b
Late GT	Single (900 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

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

Table 6 Photosynthetic parameters of GT soybean cultivars and their respective non-GT parental lines at R1 growth stage

Soil type	Herbicide treatment / Cultivar maturity group	Chlorophyll content	Stomatal conductance (gs)	Photosynthetic Rate (A)	Transpiration Rate (E)
		-----mg cm ² -----	---H ₂ O mol m ⁻² s ⁻¹ --	micro mol CO ₂ m ⁻² s ⁻¹	mmol H ₂ O m ⁻² s ⁻¹
Typic Hapludox	Without glyphosate / non-GT	0.015 a*	0.43 a	14.42 a	10.46 a
	Without glyphosate / GT	0.017 a	0.38 a	15.11 a	9.94 a
	Sequential (450 / 450 g a.e. ha ⁻¹) / GT	0.010 b	0.24 b	12.08 b	7.25 b
	Single (900 g a.e. ha ⁻¹) / GT	0.013 b	0.30 b	12.88 b	8.33 b
Rhodic Ferralsol	Without glyphosate / non-GT	0.018 a	0.44 a	18.18 a	11.28 a
	Without glyphosate / GT	0.017 a	0.48 a	16.09 a	10.83 a
	Sequential (450 / 450 g a.e. ha ⁻¹) / GT	0.009 b	0.29 b	12.72 b	8.41 b
	Single (900 g a.e. ha ⁻¹) / GT	0.010 b	0.34 b	13.00 b	8.72 b
CV (%)		23.68	36.09	24.26	23.29

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

Table 7 Shoot and root dry biomass, of GT soybean cultivars and their respective near-isogenic non-GT parental lines at R1 growth stage

Cultivar maturity group	Herbicide treatment	Shoot	Root
		-----g plant ⁻¹ -----	
Early – non-GT	Without glyphosate	13.54 a*	4.48 b
Early GT	Without glyphosate	12.62 a	7.24 a
Early GT	Sequential (450 / 450 g a.e. ha ⁻¹)	7.92 b	4.35 b
Early GT	Single (900 g a.e. ha ⁻¹)	9.62 b	5.08 b
Medium – non-GT	Without glyphosate	9.33 a	6.94 a
Medium GT	Without glyphosate	11.20 a	6.66 a
Medium GT	Sequential (450 / 450 g a.e. ha ⁻¹)	7.15 b	3.72 b
Medium GT	Single (900 g a.e. ha ⁻¹)	8.17 b	4.54 b
Late – non-GT	Without glyphosate	12.17 a	6.63 a
Late GT	Without glyphosate	11.76 a	5.47 a
Late GT	Sequential (450 / 450 g a.e. ha ⁻¹)	8.24 b	4.36 b
Late GT	Single (900 g a.e. ha ⁻¹)	9.04 b	4.33 b
CV (%)		20.49	24.91

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

Table 8 Shoot and root dry biomass, of GT soybean cultivars and their respective near-isogenic non-GT parental lines at R1 growth stage

Soil type	Herbicide treatment / Cultivar maturity group	Shoot	Root
		-----g plant ⁻¹ -----	
Typic Hapludox	Without glyphosate / non-GT	11.19 a*	5.19 a
	Without glyphosate / GT	11.42 a	5.80 a
	Sequential (450 / 450 g a.e. ha ⁻¹) / GT	7.53 b	3.89 b
	Single (900 g a.e. ha ⁻¹) / GT	8.93 b	4.31 b
Rhodic Ferralsol	Without glyphosate / non-GT	12.17 a	6.84 a
	Without glyphosate / GT	12.29 a	7.11 a
	Sequential (450 / 450 g a.e. ha ⁻¹) / GT	8.00 b	4.40 b
	Single (900 g a.e. ha ⁻¹) / GT	8.95 b	4.99 b
CV (%)		20.49	24.91

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