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Effect of glyphosate and water stress on plant morphology and nutrient accumulation in soybean

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Abstract

Some RR® soybean cultivars show poor agronomic performance after glyphosate application under water stress. Thus, the objective of this trial was to evaluate the morpho-physiological responses and macro and micronutrient accumulations in soybean plants under normal conditions and water deficit in a savannah-like environment (Cerrado). The experimental design was a factorial under completely randomized block. We evaluated three cultivars Roundup Ready (RR®) soybean cultivars (P98Y12, M9144 and M9056) under two herbicidal treatments (1080 g e. a. ha^{-1} and 1800 g e. a. ha^{-1}) and control. The cultivars remained in two soil moisture conditions (optimal and deficit). Additionally, conventional soybean cultivar (M-soy 9350) underwent moisture treatments with four replications. We measured plant height, root volume, root and shoot dry mass in the beginning of pod formation (R_3). We also carried out a chemical analysis of leaf tissue to determine macro and micronutrient accumulations. Glyphosate application reduced the content of macro and micro elements (N, P, K^+, Ca^{2+}, Mg^{2+}, B, Mn^{2+}, Zn^{2+} and Fe^{2+}), which was more significant at higher herbicide doses. Soybean cultivars had different behaviors regarding morphophysiological responses to glyphosate application and soil moisture conditions. The RR® cultivars are most sensitive to water stress when glyphosate is used.

Keywords: *Glycine max* (L.) Merrill; EPSPs inhibitors; mineral nutrition; Roundup Ready.
Abbreviations: RR_Roundup Ready; EPSP_5-Enolpyruvylshikimate-3-Phosphate Synthase; ATP_Adenosine Triphosphate; N-P_2O5-K_2O_ Fertilizer; DAE_ Days After Emergence; FC_Field Capacity; RDM_Root System Dry Mass; SDM_Shoot Dry Mass; N _Nitrogen; P_Phosphorus; K_ Potassium; Ca_ Calcium; Mg_ Magnesium; B_Boro; Mn_Manganese; Fe_ Iron; Zn_zinc; Cu_Copper.

Introduction

The rise of biotechnology has provided significant enhancement on glyphosate resistance for some soybean cultivars (RR®) through genetic engineering. These cultivars have been standing out in agricultural scenario and have been planted over an increasing area that solely in Brazil exceeds 29 million hectares (Céleres, 2014), what makes glyphosate one of the most demanded herbicide worldwide. Even if there are glyphosate resistant plants, this herbicide has recognized efficacy advantages as being a non-selective, systemic, post-emergence herbicide characterized by a broad-spectrum weed control (Rodrigues and Almeida, 2005; Ferreira et al., 2006), as well as being easily translocated throughout the plant (Shaner, 2009). Non-metabolized glyphosate is translocated within the plant in RR® transgenic soybean and reaches the soil in its original form (Prata and Lavaronti, 2000), and/ or in aminomethylphosphonic acid form (AMPA) (Toni et al., 2006). Glyphosate acts by inhibiting the synthesis of aromatic amino acids (phenylalanine, tyrosine and tryptophan) and interferes with secondary metabolism. Even though these transgenic plants have a key enzyme (5-enolpyruvylshikimate-3-phosphate synthase - EPSP) to synthetize amino acids insensitive to the herbicide, the process can cause stress and/ or promotes phytotoxic effect that affects water use efficiency, photosynthesis and nutrient absorption (Zablotowicz and Reddy, 2007; Albrecht et al., 2011). Therefore, such effects are evidenced in a different way for these soybean cultivars, acting directly in leaf tissue or indirectly by reducing microbial activity in soil. Generally, direct damage of glyphosate to leaves is the “yellow flashing”, which does not necessarily cause yield reductions. This effect may be associated with glyphosate complexation with some nutrients (Coutinho and Mazo, 2005) that reduces momentarily their availability for cellular metabolic reactions. However, this plant sensitivity is variable among RR cultivars and usually does not remain more than seven days after herbicide application (Petter et al., 2007). On the other hand, glyphosate impacts may go beyond a single chlorosis, but they can also include physiological metabolism issues as well as reduction of photosynthesis rate, stomatal conductance and transpiration (Zobiole et al., 2010a). Yet in soil, root-exudated glyphosate may have a direct effect on microbial activity (Zuffo et al., 2014) and consequently promoting leaf deficiency of certain nutrients. The absorption of these nutrients is reduced since some of them as manganese (Mn^{2+}), iron (Fe^{2+}) and nitrogen (N) require oxidation and reduction reactions performed by soil microorganisms to become available (Albrecht et al., 2011). Furthermore, the herbicide molecule is able to complex with metals such as Fe^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+}, making them unavailable to plants (Coutinho and Mazo, 2005). Moreover, the uptake of soil nutrients is variable among the cultivars (Cavalieri et al., 2012); therefore, nutrient accumulations in shoot tissue might be influenced with a greater or lesser level by glyphosate use, even for RR cultivars. Soybean physiological responses range closely related to the intrinsic characteristics of RR cultivars, as well as to soil and weather conditions (Zablotowicz and Reddy, 2004). Given the above, glyphosate effects could occur in greater intensity under certain weather conditions such as drought. Such statement is reinforced by field observations that reported substantial yield reductions of RR cultivars compared to conventional ones as water deficit intensifies.

In brief, based on the hypothesis that glyphosate application in RR soybean cultivars under water stress may impair the plant growth and mineral nutrition; we aimed at evaluating the morphophysiological responses and macro and micronutrient accumulations in such plants at optimal moisture and drought conditions in a Cerrado region (savannah-like environment).

Results and Discussion

Morphological traits

Table 2 shows that all tested cultivars tended to have reduced height of plants with the application of glyphosate and water deficit. Even so, P98Y12RR and M9056RR were the only to present significant effects, which were more pronounced under water stress conditions. Similarly, Zobiole et al. (2010a) reported similar reductions in plant height with increasing doses of glyphosate. P98Y12RR plants treated with 1,800 g e. a. ha^{-1} glyphosate had height reductions of 19.2% and 34.7% under optimal moisture and water deficit, respectively. It clearly shows the sensitivity of RR cultivars to glyphosate compared to conventional ones under water stress. It can be attributed to intrinsic characteristics of the plant such as capacity to form AMPA from glyphosate degradation (Reddy et al., 2004). This is responsible for the stress degree caused by glyphosate in RR® cultivars, thereby promoting phytotoxic effect by nutritional imbalance or reducing water use efficiency, and therefore compromising photosynthesis (Zobiole et al., 2010b; Zobiole et al., 2010c; Albrecht et al., 2011).

In addition, glyphosate decreased root volume and dry mass (RDM) as well as shoot dry mass (SDM), especially in water stress conditions (Tables 3, 4 and 5). Thus, it is noteworthy to observe the effects of soil water stress, when analyzing such parameters. The M9056RR cultivar had average reductions of 55%, 57% and 45%, for root volume, RDM and SDM, respectively, compared to treatments in soil under optimal moisture conditions. These findings corroborate with Salinet (2009) and Zobiole et al. (2010c), who had also observed RDM and SDM reductions in RR cultivars because of glyphosate application under water deficit conditions. On the other hand, the conventional cultivar (M-soy 9350) remained stable and did not present any significant difference in its morphophysiological traits as rhizosphere volume, RDM and SDM under both water conditions. This result indicate greater phenotypic plasticity or pre-existing plasticity unchanged regardless glyphosate application. Komatsu et al. (2010) stated that plasticity is a plant ability to adapt to environmental and crop management conditions by changing its morphological features and yield components. Such characteristic may be impaired by glyphosate application probably because RR plants spend higher amounts of energy to overcome herbicide impacts.

Furthermore, morphological and physiological traits suffer from consequences brought by glyphosate application derived from changes in shikimic acid biosynthesis. This is because the incorporation of a gene which encodes a glyphosate-insensitive EPSP synthase does not prevent EPSP synthase inhibition from non-transgenic (Duke et al., 2003), also from reductions of nutrient uptake and use efficiency, as found in this study and in several others (Santos et al., 2007; Zobiole et al., 2010ac; Zobiole et al., 2012a; Serra et al., 2011). According to Reddy et al. (2004), it is possible that glyphosate acts in the shikimic acid pathway and might induce changes in biosynthesis of compounds in leaf metabolism, which directly or indirectly affects intermediates and/ or activity of enzymes within the Calvin cycle. This way, once plant biomass production depends on photosynthesis energy to synthesize carbon compounds (Behling et al., 2012), reductions in CO_2 assimilation decrease biomass and carbohydrate accumulation (Magalhães Filho et al., 2008).

Macronutrient accumulations

We observed that macro and micronutrients such as N, P, K^+, Ca^{2+}, Mg^{2+} presented reduced amounts by glyphosate application, which is intensified with increasing doses (Supplementary Fig. 1 to 5). The same behavior was similar in both moisture conditions. On the other hand and as already expected, water stress condition had greater reductions than those influenced by glyphosate, especially for nutrients that have diffusion as main transport mechanism in soil (e.g. P and K). Regardless cultivar, leaf N accumulation decreased with glyphosate use, mainly for plants treated with 1800 g e. a. ha^{-1} under water deficit (Supplementary Fig. 1). These results are similar to those reported by Serra et al. (2011) and Zobiole et al. (2012a), who also found reductions in N content caused by glyphosate application. Glyphosate effect on leaf nitrogen accumulation is associated to the sensitivity of EPSP existing in N_2-fixing bacteria (*Bradyrhizobium* sp.), once as highlighted by Oliveira Júnior et al. (2008) and Zuffo et al. (2014), glyphosate can interfere on bacterial nodulation. These authors confirmed a decline in the number of nodules after the application of increasing doses of glyphosate. Zobiole et al. (2010a) reported that glyphosate promoted significant lessening in photosynthetic parameters like chlorophyll, photosynthesis rate, transpiration and stomatal conductance, ensuing in less phytomass production. Therefore, when reducing leaf N accumulations, there was lower production of assimilates, since nitrogen is a major constituent of chlorophyll molecule. Consequently, plants submitted to glyphosate application have their development impaired derived from a decreased production of photoassimilates, hence shoot and root partitioning, as observed in this study (Tables 2 to 5).

P accumulation was significantly reduced with glyphosate application in both moisture conditions for all RR cultivars (Supplementary Fig. 2). Zobiole et al. (2012a) had similar results in RR soybean. There were also isolated effect of soil moisture with drastic reduction in P accumulation under deficit condition. As previously reported, soil moisture effect is predictable, since P movement along soil profile is compromised by the scarcity of water; and contrarily

Table 1. Chemical composition of the soil depth (0-0.20 m) collected from the experimental area prior to the installation of the experiment in the city of Bom Jesus, Brazilian State of Piaui (crop season 2011/2012).

pH	P (Mehlich)	K	Ca	Mg	Al	H + Al	M.O.[1]
$CaCl_2$	—— mg dm^{-3} ——		——— cmol$_c$ dm^3 ———				g dm^{-3}
5.8	47.0	74.0	2.1	1.0	3.1	3.1	15
V[2]	CTC[3]	Fe	B	Mn	Zn	Cu	S
%	cmol$_c$$dm^{-3}$		——— mg dm^{-3} ———				
51.49	6.39	88.7	0.24	7.3	2.5	0.2	2.8

1O.M.: organic matter; 2V%: base saturation; 3CEC: cation-exchange capacity at pH 7.

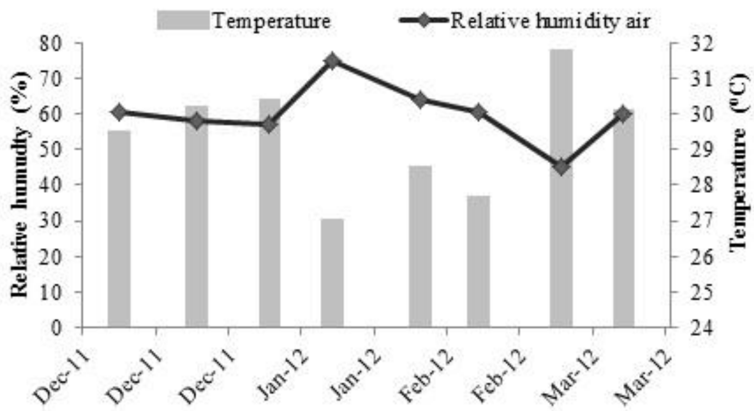

Fig 1. Relative moisture and average air temperature in the city of Bom Jesus. space of Piaui (crop season 2011/2012). during the conduction of experiment (data from INMET - Bom Jesus weather bureau).

Table 2. Plant height of RR® and conventional soybean cultivars, which received various glyphosate doses under suitable conditions and water stress, in Bom Jesus, State of Piauí, Brazil, in the 2011/2012 season.

Plant height (cm)						
Glyphosate	P98Y12RR			M9144RR		
	Without deficit	With deficit	Average	Without deficit	With deficit	Average
0 g e. a. ha^{-1}	134.50 Aa	108.50 Ab	121.15 A	128.00 Aa	96.75 Ab	112.37 A
1080 g e. a. ha^{-1}	109.75 Ba	93.00 Bb	101.37 B	110.50 Aa	90.25 Aa	100.37 A
1800 g e. a. ha^{-1}	97.25 Ba	63.50 Cb	80.37 C	103.50 Aa	86.50 Aa	95.00 A
Average	113.83 a	88.33 b	101.08	114.00 a	91.16 b	102.58
Glyphosate	M9056RR			M-Soy 9350*		
	Without deficit	With deficit	Average	Without deficit	With deficit	Average
0 g e. a. ha^{-1}	92.75 Aa	73.35 Ab	83.00 A	77.50 a	65.12 a	71.31
1080 g e. a. ha^{-1}	90.50 Aa	71.50 Ab	81.00 A			
1800 g e. a. ha^{-1}	75.00 Ba	65.75 Aa	70.37 B			
Average	86.08 a	70.16 b	78.12			

Means followed by the same uppercase letter in the column and lowercase in the line do not differ from each other significantly by the Tukey test at 5% probability. *Conventional soybean cultivar has not received glyphosate application..

Table 3. Root volume of RR® and conventional soybean cultivars, which received various glyphosate doses under suitable conditions and water stress, in Bom Jesus, State of Piauí, Brazil, in the 2011/2012 season.

Root volume (cm³)						
Glyphosate	P98Y12RR			M9144RR		
	Without deficit	With deficit	Average	Without deficit	With deficit	Average
0 g e. a. ha^{-1}	70.35 Aa	36.75 Ab	53.55 A	47.50 Aa	40.00 Aa	43.75 A
1080 g e. a. ha^{-1}	52.50 Ba	34.00 Ab	43.25 B	42.50 Aa	30.00 ABb	36.25 AB
1800 g e. a. ha^{-1}	40.25 Ca	26.25 Bb	33.25 C	37.50 Aa	21.75 Bb	29.62 B
Average	54.36 a	32.33 b	43.35	42.50 a	30.58 b	36.54
Glyphosate	M9056RR			M-Soy 9350*		
	Without deficit	With deficit	Average	Without deficit	With deficit	Average
0 g e. a. ha^{-1}	70.00 Aa	31.75 Ab	50.87 A	61.25 a	51.75 a	56.37
1080 g e. a. ha^{-1}	66.75 ABa	28.75 Ab	47.75 AB			
1800 g e. a. ha^{-1}	51.50 Ba	25.00 Bb	38.25 B			
Average	62.75a	28.50 b	45.62			

Means followed by the same uppercase letter in the column and lowercase in the line do not differ from each other significantly by the Tukey test at 5% probability. *Conventional soybean cultivar has not received glyphosate application..

Table 4. Dry mass root of RR® and conventional soybean cultivars, which received various glyphosate doses under suitable conditions and water stress, in Bom Jesus, State of Piauí, Brazil, in the 2011/2012 season.

Dry mass root (g $plant^{-1}$)						
Glyphosate	P98Y12RR			M9144RR		
	Without deficit	With deficit	Average	Without deficit	With deficit	Average
0 g e. a. ha^{-1}	11.15 Aa	3.97 Ab	7.56 A	6.66 Aa	4.60 Ab	5.63 A
1080 g e. a. ha^{-1}	5.92 Ba	3.55 Ab	4.73 B	5.85 Aa	3.90 ABb	4.87 AB
1800 g e. a. ha^{-1}	3.52 Ca	3.47 Aa	3.50 B	5.42 Aa	2.86 Bb	4.14 B
Average	6.86 a	3.66 b	5.26	5.98 a	3.78 b	4.88
Glyphosate	M9056RR			M-Soy 9350*		
	Without deficit	With deficit	Average	Without deficit	With deficit	Average
0 g e. a. ha^{-1}	17.17 Aa	5.93 Ab	11.55 A	7.20 a	5.13 a	6.16
1080 g e. a. ha^{-1}	11.42 Ba	5.57 Ab	8.50 B			
1800 g e. a. ha^{-1}	7.10 Ca	4.32 Bb	5.71 C			
Average	11.90 a	5.27 b	8.58			

Means followed by the same uppercase letter in the column and lowercase in the line do not differ from each other significantly by the Tukey test at 5% probability. *Conventional soybean cultivar has not received glyphosate application..

Table 5. Dry mass root of RR® and conventional soybean cultivars, which received various glyphosate doses under suitable conditions and water stress, in Bom Jesus, State of Piauí, Brazil, in the 2011/2012 season.

Dry mass shoot (g $plant^{-1}$)						
Glyphosate	P98Y12RR			M9144RR		
	Without deficit	With deficit	Average	Without deficit	With deficit	Average
0 g e. a. ha^{-1}	13.05 Aa	8.35 Ab	10.70 A	17.96 Aa	11.23 Ab	14.59 A
1080 g e. a. ha^{-1}	12.37 Aa	8.00 Ab	10.18 A	12.32 Ba	9.95 ABb	10.19 B
1800 g e. a. ha^{-1}	7.90 Ba	7.45 Aa	7.67 B	11.86 Ba	8.52 Bb	11.13 B
Average	11.10 a	7.93 b	9.52	14.05 a	9.90 b	11.97
Glyphosate	M9056RR			M-Soy 9350*		
	Without deficit	With deficit	Average	Without deficit	With deficit	Average
0 g e. a. ha^{-1}	18.02 Aa	6.77 Ab	12.42 A	13.37 a	10.92 a	12.14
1080 g e. a. ha^{-1}	9.20 Ba	6.82 Ab	8.05 B			
1800 g e. a. ha^{-1}	9.30 Ba	6.80 Ab	7.98 B			
Average	12.17 a	6.80 b	9.48			

Means followed by the same uppercase letter in the column and lowercase in the line do not differ from each other significantly by the Tukey test at 5% probability. *Conventional soybean cultivar has not received glyphosate application.

increasing this element adsorption by absence of water film on the surface of clay (Prado, 2008). P forms part of many important compounds in plant cells, mainly of energetic compounds like adenosine triphosphate (ATP) (Taiz and Zeiger, 2009). Then, lower accumulations of P in soybeans affect their growth as observed in this study (Tables 2 to 5). Concerning K^+, Ca^{2+} and Mg^{2+}, glyphosate application at both doses of 1,080 and 1,800 g e. a. ha^{-1} promoted a significant decrease on leaf accumulation of these nutrients, which was only observed under optimal soil moisture (Supplementary Fig. 3, 4 and 5). These outcomes match those found by Zobiole et al. (2010b) and Zobiole et al. (2012ab), who also identified reductions on the accumulation of K^+, Mg^{2+} and Ca^{2+} against glyphosate use. As for P, the reductions in K^+, Ca^{2+} and Mg^{2+} were proportionally greater in function of soil moisture condition. Moreover, the lowest values were observed under deficient moisture condition. It can be explained by the fact that K^+, Ca^{2+} and Mg^{2+} translocate within the soil profile by mass flow to reach roots; therefore, in water stress conditions their absorption might be impaired. This explains the lower accumulation of these nutrients in leaves. On the other hand, in moist soils, such reductions can be related to glyphosate exudation by roots. If the molecule exuded intact, it may have occurred an ionic dissociation in soil solution due to high pH (5.8). It would contribute with glyphosate to form complexes with Ca and Mg, similarly as in spray solutions when pH is high and water has great concentrations of Ca^{2+} and Mg^{2+} (Petter et al., 2012).

Micronutrient accumulations

For all RR tested cultivars, the accumulation of micronutrient as iron (Fe^{2+}), boron (B), zinc (Zn^{2+}), copper (Cu^{2+}) and manganese (Mn^{2+}) showed reductions for treatments receiving glyphosate (Supplementary Fig. 6 to 10), being higher in moist soil. However, Zn^{2+} and Fe^{2+} accumulations decreased in drought condition for M9144RR (Supplementary Fig. 9 and 10), as well as in B for M9144RR and M9056RR. Gordon (2007), Johal and Huber (2009) observed the same behavior for Fe^{2+}, Zn^{2+} and Mn^{2+} with glyphosate application. Regarding metal ions, a likely explanation for micronutrient depletion would be related to the formation of complexes of Fe^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+} with glyphosate. As reported previously, it may be related to how this herbicide molecule is exuded from root system and its subsequent ionic dissociation. The ability of glyphosate to form chelates with metal ions within plants or in the soil solution varies with cultivar, once the compounds excreted via roots are unique (Castro and Yamada, 2007). Some studies such as Coutinho and Mazo (2005), Gordon et al. (2007), and Zobiole 2012b also demonstrated that glyphosate induces macro and micronutrient deficiency due to the formation of insoluble metal complexes that affect these elements' absorption and transport (Eker, et al., 2006). Furthermore, Mn^{2+} availability is narrowly associated with microbial activity and reactions of oxidation and reduction in the soil, which could be adversely affected by glyphosate application, as reported by Zuffo et al. (2014).

In that sense, we concluded that such reductions of the evaluated parameters should be compensated to fulfill the nutritional requirements of these RR cultivars in order to achieve a maximum physiological efficiency and that it can be influenced by adverse conditions. Even though we have not approached soybean yield in this study (once plants were destroyed for data sampling), it can be inferred that the glyphosate effects as also seen in some other researches (Albrecht et al., 2010; Zadinello et al., 2012) are able to compromise yield capacity, since the parameters evaluated in this study have close correlation with production capacity. Therefore, the use of glyphosate can result in decreased soybean yield and these effects are highly variable among the used RR cultivar, besides of being most strong in drought conditions. In conclusion of this study, the hypothesis that glyphosate has different effects on morphological and physiological characteristics and can provide (directly or indirectly) reductions in nutrient accumulations for the RR soybean cultivars is supported. This fact is even more relevant when the morphophysiological data is compared to conventional cultivar data (M-Soy 9350), as it notoriously showed an improved morphoagronomic performance, primarily under drought conditions. In this way, our findings are able to confirm the assertion of some producers, technicians and researchers that report a superior agronomic performance of conventional soybean cultivars compared to RR at a field level and mainly under adverse conditions.

Materials and Methods

Study area

The experiment was conducted under greenhouse conditions in the experimental area of the Federal University of Piaui – UFPI (Universidade Federal do Piauí) in the city of Bom Jesus – PI, Brazil. The area is located at 09º04'28" South latitude, 44º21'31" West longitude and 277 m average altitude. Evaluations were carried during the crop season of 2011/2012. The local climate is classified as Aw, according to the Köppen global climate classification, with two well-defined seasons, a dry period from May to September and a rainy one from October to April. The climatic data were collected at the weather bureau of the National Institute of Meteorology (INMET), about 200 m far from the experimental site, and they are displayed in Fig. 1. The soil used in the experiment was classified as Dystrophic Yellow Latosol. The soil had the following granulometric constitution: 640, 80, and 280 g kg^{-1} of sand, silt, and clay, respectively. The chemical composition of the soil used in the experiment is displayed in Table 1.

Experimental design

The experimental design adopted consisted of randomized blocks, in which three cultivars Roundup Ready (RR) soybean cultivars (P98Y12, M9144 and M9056) were submitted two herbicidal treatments [glyphosate (1080 g e. a. ha^{-1} and 1800 g e. a. ha^{-1}) and control] and two soil moisture conditions (adequate moisture and water deficit), in addition to one further conventional soybean cultivar (M-soy 9350), which were subjected only to soil moisture treatments, with 4 replications. The experiment did not aim to compare the cultivars with each other, since each one has intrinsic characteristics which may interfere with the results. This way, the experiment was analyzed as a double factorial (glyphosate × moisture condition), aiming to verify the glyphosate effect and the soil moisture conditions, as well as the behavior of various cultivars with regard to the treatments.

Experimental management

The seeds were treated with pyraclostrobin + thiophanate methyl + fipronil at a 2 mL commercial product (c. p.) kg^{-1} dose of seed, and inoculated with Bradyrhizobium japonicum, at the dose of 3 mL c.p. kg^{-1} of seed. The fertilization conducted was 2 g of formulation N-P_2O_5-K_2O 04-24-12 per pot, what is equivalent to 500 kg ha^{-1} of the fertilization. Sowing was conducted on 20 December, 2011, in pots with an 8 dm^{-3} capacity and at a 2 to 3 cm sowing depth, placing five seeds per pot, with subsequent thinning, leaving only the most vigorous plant. The application of glyphosate (Roundup Ready, 480 g e. a. L^{-1}) was performed at 25 days after emergence (DAE) of crop (three trifoliate leaves), using a pressurized backpack sprayer with CO_2, connected to the bar with four XR 110.02 spraying nozzles at a constant 2 kgf cm^{-2} pressure, applying a spray volume equivalent to 125 L ha^{-1}. The environmental conditions at the time of treatment application were: 28°C average temperature, 78% relative humidity and wind speed ranging from 3 to 8 km h^{-1}. Soil moisture control was determined as follows: a) the weight of pots in field capacity (FC) was measured using a methodology adapted from Bonfim-Silva et al. (2011), where the pots were saturated with water, left at rest for 12 h, in order to drain the excess water and weight determined; b) then, with the FC value, the appropriate moisture condition (80% of FC), and the soil water deficit (30% of FC) was calculated. In all treatments the soil was kept with 80% moisture of FC up to 25 DAE. After this treatment period, the water deficit treatments were applied which lasted until 55 DAE. During this period, the plants were monitored to keep moisture around 30% of FC and avoid they entered a permanente wilting point at the onset of pod formation (R_3).

Morphological and nutritional analysis

Plant height was measured at the beginning of pod formation (R_3) (from the surface of the soil to the tip of the plant) using a ruler. Root volume (cm³) was determined by water displacement using a graduated cylinder; in other words, by placing roots into a beaker containing 100 ml water, after being washed and dried. Then, before-and-after immersion difference of volume was calculated using the equivalence of 1 mL equals to 1 cm^3, as stated by Basso (1999). Afterwards, leaf samples were collected and washed under deionized water. Then, shoot, leaves and roots were placed in an oven with forced air circulation at 60 °C to be dried up to reach constant weight. These materials were weighed separately to determine the respective dry mass (g) for them. The dried leaves were ground in a Wiley mill and chemical analysis were carried out following the methodology described by Sarruge and Haag (1974) to determine the contents of macro and micronutrient.

Statistical analysis

After collecting and tabulating data, the variance analysis was performed, and the averages of significant variables were grouped according to the Tukey test at 5% significance level ($p \leq 0.05$), using the statistical software Sisvar 4.1 (Ferreira, 2011).

Conclusions

Regarding morphophysiological traits, soybean RR® cultivars showed different behaviors against glyphosate application and soil moisture conditions. Glyphosate application promoted morphophysiological changes as reductions of plant height, root volume, root and shoot dry mass, which were more intense under water deficit conditions. The herbicide reduces macro and micronutrient accumulations in RR® cultivars, being proportionally stronger in under optimal moisture condition of soil.

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