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Macro and micronutrient contents in grain and soybean yield [*Glycine max* (L). Merr.] after foliar application of elemental sulfur

Teores de macro e micronutrientes no grão e produtividade de soja [*Glycine max* (L). Merr.] após aplicação de enxofre elementar foliar

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A B S T R A C T

Low levels of organic matter in soil may limit the soybean crop development due to the low availability of sulfur (S) and the high demand of this nutrient by plants. The foliar fertilization is an alternative to supplement plant request. This study aimed at assessing the effect of applying foliar sulfur fertilizer in the soybean at different time and doses. The treatments studied were: (1) 0.0 kg ha^{-1} of S foliar; (2) 0.5 kg ha^{-1} of foliar S in beginning bloom (R1) stage; (3) 0.5 kg ha^{-1} of foliar at beginning seed (R5.1) stage; (4) 0.5 kg ha^{-1} of foliar S in R1 and R5.1; (5) 1 kg ha^{-1} of foliar S in R1; (6) 1 kg ha^{-1} of S foliar in R5.1; (7) 1 kg ha^{-1} of foliar S in R1 and R5.1 using randomized block design with 4 replicates. The variables analyzed were: one thousand grain mass, yield and the macro and micronutrient contents in the grains. The soybean yield increased 614 kg ha^{-1} with the application of foliar sulfur in relation to the control. The dose that obtained the best results was 0.5 kg ha^{-1} applied into the R3 or R5.1 stage. The use of elemental sulfur via foliar may be an alternative of sulfur supplementation.

Keywords: sulfur fertilizers, supplementary fertilization, mineral nutrition.

R E S U M O

Baixos teores de matéria orgânica no solo podem limitar o desenvolvimento da soja, devido a baixa disponibilidade de enxofre (S) no solo e pela alta demanda deste nutriente pelas plantas. Uma alternativa para complementar esta necessidade é a aplicação S foliar. O objetivo foi avaliar o efeito da aplicação de fertilizante foliar à base de enxofre na cultura da soja, em diferentes épocas e doses. Os tratamentos foram: (1) 0,0 kg ha^{-1} de S foliar; (2) 0,5 kg ha^{-1} de S foliar no estágio de início do florescimento (R1); (3) 0,5 kg ha^{-1} foliar no estágio de início do enchimento de grãos (R5.1); (4) 0,5 kg ha^{-1} de S foliar nos estádios R1 e R5.1; (5) 1 kg ha^{-1} de S foliar no estádio R1; (6) 1 kg ha^{-1} de S foliar no estádio R5.1; (7) 1 kg ha^{-1} de S foliar nos estádios R1 e R5.1, delineados em blocos ao acaso com 4 repetições. As variáveis respostas foram: massa de mil grãos, produtividade e teores de macro e micronutrientes nos grãos. A produtividade dos grãos aumentou 614 kg ha^{-1} com aplicação de S foliar em relação à testemunha. A dose que obteve os melhores resultados foi de 0,5 kg ha^{-1} aplicado nos estádios R3 ou R5.1. A utilização de enxofre elementar via foliar pode ser uma alternativa de complementação.

Palavras-chave: fertilizantes sulfatados, adubação suplementar, nutrição mineral.

INTRODUCTION

The soils from tropical regions, such as the Oxisols have a low natural fertility as feature. The supply of nutrients via soil is one of the main practices that provide the crop yields currently achieved (Prochnow *et al*., 2010). However, the application of fertilizers is one of the most expensive practices for crop production due to price volatility and relative impacts in crop profitability (Huang *et al*., 2009).

Sulfur (S) is one of the nutrients that have most demanded attention in the last years, which limits the crop production (Vitti *et al*., 2007, Rezende *et al*., 2009; Stipp and Casarin, 2010). The reasons for the increase in S fertilization responses by the crops are the low soil content (Kaiser and Kim, 2013), mainly in areas of soybean production (Argentina, Brazil and United States of America), reduced atmospheric inputs, reduced organic matter (OM) (Salvagiotti *et al*., 2012), the non-replacement of this nutrient through fertilization and the higher yields currently obtained by soybean crop (Stipp and Casarin, 2010). For soybean crop, S is required in similar amounts of phosphorus (P) and magnesium (Mg), considered substantial quantities that should be supplemented for a better plant development (Rezende *et al*., 2009). Although under tropical conditions S is applied in smaller quantities (Moreira *et al.,* 2018).

The average of S content in the soils from tropical regions is between 5 and 10 mg dm^{-3} (Sfredo and Lantmann, 2007). About 90% of this S is in the organic form that is gradually mineralized to SO_4^{2-} and reaches the roots by mass flow to be absorbed by the roots predominantly in this form (Stipp and Casarin, 2010). Since the OM is the main source of S for plants, its soil content depends directly of the S mineralization rates (Horowitz and Meurer, 2006).

Considering the plants, S concentration ranges between 1.0 to 3.0 g kg^{-1}. Furthermore, S is an essential nutrient found in amino acids, such as methionine and cysteine, which act in the structure and metabolism of the plants (Takahashi *et al.,* 2011). Thus, S has influence in fundamental processes for the plant growth and development, such as photosynthesis, cellular respiration, resistance to water deficit, biological nitrogen fixation, root development, among others (Marschner, 2012; Corsi *et al*., 2007).

The most used sources of S in agriculture are single superphosphate (12% S), ammonium sulphate (24% S) and phosphogypsum (15-18% S). The first two are the most used as fertilizers, and the phosphogypsum as a soil conditioner. Over the last decade, the use of elemental S via soil and foliar application has been increasing (Vitti *et al.,* 2007; Broch *et al*., 2011).

Among the alternatives of S supplying to plants, the foliar application can be used as a complement to fertilization applied on soil (Vitti *et al*., 2007; Rezende *et al*., 2009). Boaretto *et al*. (1986) applied S via foliar in soybean crop and showed that 50% of S absorption occurs after 16 hours, and the element translocation begins only 8 hours after application. Rezende *et al*. (2009) evaluated the efficiency of S via foliar application in soybean crop and obtained 641 kg ha^{-1} of yield increase with a fertilizer containing 26% elemental S and a density of 1.16 kg L^{-1}, at the dose of 2.0 L ha^{-1}, applied in soybean at beginning Development (R3) (Fehr *et al*., 1971), when compared to the control treatment, without the application of sulfur via foliar. Vitti *et al*., (2007) did not find any soybean yield difference applying 20 kg ha^{-1} of S on soil or 6 kg ha^{-1} via foliar. Despite this fact and based on the total soluble protein content, the efficiency of S foliar application was higher than via soil application.

However, according to Vitti *et al*. (2006), there is a lack of scientific evidence supporting to recommend S via foliar. Therefore, there are not enough studies addressing S fertilization, whether via soil or foliar for soybean crop; even regional fertilization manuals do not have well established guidelines regarding the management of this element.

The hypothesis of this study is that the supply of elemental S complementary via foliar results in metabolic and physiological modifications in soybean plants, which positively changes the chemical composition (increasing ions content) and grain weight, as well as the crop yield. Thus, an experiment was installed under field conditions, with the purpose of assessing the agronomic efficiency of using elemental S in homogeneous suspension applied via foliar in order to define the best time and dose application.

MATERIAL AND METHODS

The present study was carried out in Terra Boa city located in the north central region of Paraná State-Brazil, at a latitude of 23º76′22″ S and longitude of 52º73′64″ W. The soil of the experimental area was classified as an Oxisol (Bhering and Santos, 2008), and the climate as Cfa (Alvares *et al*., 2013). The area selected for the experiment has been cultivated with no-tillage seeding system for more than 10 years, with soybean and maize as summer and winter crops, respectively.

The soil samples were collected in the 0.0-0.2 m depth for chemical characterization and particle size distribution (Table 1). The sowing fertilization was performed based on the crop nutrient extraction and soil analysis. It was applied 200 kg ha^{-1} of the NPK formulation 03-21-00 at sowing and 80 kg ha^{-1} of coated-KCl at the V4 stage (fourth node formation). The sowing was carried out on October 22^{nd}, 2015, with a no-tillage seed drill. The seeding depth was 0.03 m and the fertilizer deposited 0.05 m below and next to the seeds. The seeds were inoculated with *Bradyrhizobium japonicum*. The phytosanitary and weed control of the crop was carried out according to its necessity.

The plots consisted of six of 5.0 m length rows, spaced 0.45 m apart, corresponding to 13.5 m^2 of total area. The useful area had 4.05 m^2, corresponding to the three central rows, with a border of 1.0 m at each end of the rows. The treatments were delineated in complete randomized blocks with 7 treatments and 4 replicates. The treatments evaluated are described in Table 2, the phenological stages application were R1 (beginning bloom) and R5.1 (beginning seed). It was used a commercial product with 50% elemental S, density of 1.5 kg L^{-1} at 25°C, and 1.3% mineral oil, in homogeneous suspension. The applications were carried out with a coastal sprayer pressurized with CO_2, with a 6-nozzle bar spaced at 0.5 m, XR 110/02 tips, adjusted with a constant pressure of 2.8 kgf cm^{-2} for applying a volume of 200 L ha^{-1}.

Table 1 - Chemical characteristics and particle distribution of the soil at 0-0.2 m depth before the experiment implementation

pH $CaCl_2$	Al	H+Al	P	$S\text{-}SO_4^{2-}$	OC	OM
	---$cmol_c$ dm^{-3}---		----mg dm^{-3}----		g dm^{-3}	%
5.48	0.00	3.82	8.20	6.01	16.45	2.84

Ca	Mg	K	SB	CEC	Clay	Silt	Sand
---------------$cmol_c$ dm^{-3}-------------					-------g kg^{-1}------		
6.05	2.25	0.56	8.87	12.69	688	124	188

Al: Aluminium; H+Al: Hidrogen + Aluminium; P: Phosphorus; S: Sulfur; OC: Organic carbon; OM: Organic matter [(OCx1.724)/10]; Ca: Calcium; Mg: Magnesium; K: Potassium; SB: Sum of bases (Ca+Mg+K); CEC: Cation exchange capacity (SB+H+Al). Extractors: Melich 1 1:10 – P e K; KCl 1M 1:10 – Al, Ca e Mg; Calcium acetate 1M pH 7.2 1:15 – H+Al; Phosphate monocalcium in acetic acid 2M – Sulfur.

Table 2 - Description of the dose and time of foliar elemental S application in soybean plants

Treatments	Doses (kg ha^{-1})	Phenological application stage*
1 – Control	-	-
2 - Foliar sulfur	0.5	R1
3 - Foliar sulfur	0.5	R5.1
4 - Foliar sulfur	0.5 + 0.5	R1 + R5.1
5 - Foliar sulfur	1.0	R1
6 - Foliar sulfur	1.0	R5.1
7 - Foliar sulfur	1.0 + 1.0	R1 + R5.1

*Phenological scale based on Fehr *et al*. (1971). R1= Beginning bloom; R5.1 = Beginning seed.

The soybean yield (Y) obtained from the harvest and manual track of the plants found in the useful area of each experimental plot are expressed in kg ha^{-1}. The one thousand grain mass (TGM) in grams was measured by counting the samples of 100 grains in quadruplicate separated from the grains harvested for evaluating Y. The values of Y and TGM were corrected for a humidity of 13%.

The macro and micronutrients content in the grains were determined in the samples collected from the total of grains used for assessing the Y. The nitrogen (N) contents were determined by means of complete digestion in concentrated H_2SO_4 and subsequent distillation by using the micro-Kjeldahl method. In order to obtain the total contents of the nutrients P, S, calcium (Ca), Mg, potassium (K), iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) of the grains, a digestion with nitric-perchloric solution was performed.

S was determined by applying the turbidimetry method with barium sulphate. Ca, Mg, Fe, Cu, Zn and Mn were determined by using the atomic absorption spectrophotometry in an AA 240FS Agilent with air:acetylene mixture, being used for the determining Ca and Mg lanthanum oxide solution for the suppression of interferents. K was determined by using a flame photometer. P was determined by metavanadate colorimetry. B was extracted by applying the incineration method with extraction by HCl 0.1 M and determined by curcumin colorimetry. All these analyses were performed according to Malavolta *et al*. (1997).

The results were analyzed by using the SAS program. First, the data were submitted to the error normality analysis and homogeneity of the variances to verify the basic assumptions of the statistics. Subsequently, the results were submitted to analysis of variance at 5% of probability, and the analysis of orthogonal contrasts (C), according to Banzatto and Kronka (2006). The contrasts were elaborated as it follows: C1: without S (T1) vs. with S (T2 + T3 + T4 + T5 + T6 + T7); C2: one application of S (T2 + T3 + T5 + T6) vs. two applications of S (T4 + T7); C3: one application of 0.5 kg ha^{-1} (T2 + T3) vs. one application of 1 kg ha^{-1} (T5 + T6); C4: two applications of 0.5 kg ha^{-1} (T4) vs. two applications of 1.0 kg ha^{-1} (T7); C5: 0.5 kg ha^{-1} in R.1 (T2) vs. 1.0 kg ha^{-1} in R.5.1 (T3); C6: 1.0 kg ha^{-1} in R.1 (T5) vs.1.0 kg ha^{-1} in R.5.1 (T6).

RESULTS AND DISCUSSION

The S foliar application did not change the macronutrient contents in soybean grains (Table 3). These results were expected for K, Ca and Mg, since there is low relation among S with these elements in the plant regarding the nutrient translocation (Marschner, 2012). However, according to Malavolta (2006) the P content may be related to the S content and vice versa, and the proteins cause this interaction, a fact that was not seen for the grain content in the present study. One of the results expected of this study was based on the increase of N and S in the grains after applying S, since the greater amount of S is associated with N in the constitution of amino acids, such as methionine, cystine and cysteine, which, in turn, are structural components of proteins (Choudhary *et al*., 2014). This authors showed that 60 mg kg^{-1} of S applied in soil increased soybean protein content reaching 38%, however this fact was not corroborated in the present research. On average, the soybean grains have 40% protein (Moraes *et al*., 2006).

The results found agree with those of Vitti *et al*. (2007), who concluded that the foliar application of elemental S did not influence the S content in the grains. This may occur because, in some plant species the phenological stage have determinant effects in the redistribution of all the elements in phloem (Fernández *et al*., 2013).

Table 3 - Macronutrients content in soybean grains as a result of elemental foliar S application in different doses and soybean stages (n=4)

Treatment	Dose	Stage	N^{ns}	P^{ns}	K^{ns}	Ca^{ns}	Mg^{ns}	S^{ns}
					g kg^{-1}			
1	-	-	67.99	4.92	16.80	1.99	1.93	2.43
2	0.5	R1	68.26	4.97	16.81	2.06	1.95	2.43
3	0.5	R5.1	69.00	5.02	16.48	1.99	1.95	2.17
4	0.5 + 0.5	R1 + R5.1	66.53	4.94	16.41	2.21	1.90	2.29
5	1.0	R1	69.73	5.04	16.50	2.09	1.98	2.46
6	1.0	R5.1	69.06	4.97	16.69	1.96	1.99	2.50
7	1.0 + 1.0	R1 + R5.1	67.75	5.01	16.80	1.98	1.96	2.33
Average			68.33	4.98	16.64	2.04	1.95	2.37
CV(%)			2.46	3.21	3.53	7.82	2.17	10.16

ns: not significant at 5% probability. CV= Coefficient of variation.

The micronutrient contents in the grains did not significantly change in the present study. The interaction between S and micronutrients is still little investigated, which may either change or not the contents in the plants, varying according to the species, source of fertilizers, dose, period and application form, thus, further studies addressing this theme are necessary.

In spite of this, the results reinforce the data obtained by Fiorini (2011) who worked with S and micronutrients in the maize crop (*Zea mays* L.) and did not find significant relationships among the variables assessed. However, Viégas *et al*. (2013), when studying the mineral composition of the long pepper (*Piper hispidinervum* DC) found that by omitting the sulfur supply via nutrient solution, the foliar content of B, Mn and Zn decreased.

Table 4 - Micronutrients content in soybean grains as a result of the elemental foliar S application in different doses and soybean stages (n=4)

Treatment	Dose	Stage	B^{ns}	Cu^{ns}	Fe^{ns}	Mn^{ns}	Zn^{ns}
				---------------------mg kg^{-1}----------------			
1	-	-	1.93	16.8	67.9	4.9	1.99
2	0.5	R1	1.95	16.8	68.2	4.9	2.06
3	0.5	R5.1	1.95	16.5	69.0	5.0	1.99
4	0.5 + 0.5	R1 + R5.1	1.90	16.4	66.5	4.9	2.21
5	1.0	R1	1.98	16.5	69.7	5.0	2.09
6	1.0	R5.1	1.99	16.7	69.1	4.9	1.96
7	1.0 + 1.0	R1 + R5.1	1.96	16.8	67.7	5.0	1.98
Average			1.95	16.64	68.33	4.98	2.04
CV(%)			11.7	6.6	66.3	13.6	5.4

ns: not significant at 5% probability. CV= Coefficient of variation.

Table 5 - One thousand grain mass (TGM) and soybean yield (Y) means as a result of applying the elemental foliar S in different doses and soybean stages

Treatment	Dose	Stage	TGM^{ns}	Y*
			g	kg ha^{-1}
1	-	-	184.8	2990
2	0.5	R1	186.8	3666
3	0.5	R5.1	184.3	3879
4	0.5 + 0.5	R1 + R5.1	187.8	3613
5	1.0	R1	179.5	3474
6	1.0	R5.1	183.3	3534
7	1.0 + 1.0	R1 + R5.1	187.0	3460
Average			184.8	3517
CV(%)			6.3	13.64

ns: not significant at 5% probability; * significant at 5% de probability.CV= Coefficient of variation.

In Table 5, data on variance analysis are shown, and no significance was found for the TGM variable, thus, it is concluded that the treatments assessed did not influence this variable response in the experimental conditions of the present study.

According to Pimentel-Gomes (1985) the coefficients of variation (CV) are classified as low when less than 10%; medium between 10% and 20%; high between 20% and 30%, and very high when higher than 30%. Despite the non-significance of the data, it was obtained a CV considered low for 9 variables, medium for 3, and high for only one variable, showing that the experimental and analytical controls were rigid.

Considering soybean yield, there was a significant response as a result of the treatments tested (Table 5). Thus, the unfolding in orthogonal contrasts were carried out at a significance level of 5% (Table 6).

When comparing the yield average of the control (2990 kg ha^{-1}) with the other treatments (3604 kg ha^{-1}) with the foliar application of S, which is represented by C1 (Table 6), there were significant statistical differences. These data are in accordance with the Rezende *et al*. (2009), which showed a yield increase of up to 32% in relation to the control, with the application in R3 (beginning pod) of products with 26% and 56% of S and density of 1.16 and 1.43 kg L^{-1}, respectively. The soil S contents of the present study are mean contents and, nevertheless, the treatments with the S foliar application differed and surpassed the control in productivity.

According to Fernández *et al*. (2013), among the situations in which foliar fertilization is applicable, there are conditions that limit the solubility of nutrients in the soil, making the foliar application an excellent nutritional management tool. Therefore, the oxidation of elemental S when applied to the soil is influenced by some factors such as soil texture, pH, aeration, temperature, nutrient availability and microbiological populations (Degryse *et al.* 2016), among others, which are difficult to control. Therefore, the application of S via foliar is an excellent alternative to supply the plant, a fact that may have been determinant for C1 yield difference (Table 6).

Vitti *et al*. (2007), when comparing the efficiency of the use of elemental S via soil and via foliar, in a soil with low S content, did not find yield differences for the treatments analyzed. Sfredo *et al*. (2003) carried out researches in four sites in Brazil, during five consecutive crops (1998/99 to 2002/03) and concluded that the yield obtained with foliar S fertilization was equal to the best S dose applied to the soil. Both studies corroborate the yield data obtained and show that the foliar application may be an alternative for applying S with the purposes of supplementation.

When analyzing C2 between one or two foliar S applications there were no significant differences. When the specific doses of 0.5 kg ha^{-1} or 1.0 kg ha^{-1} were analyzed in one application, there was a statistically significant difference (C3), in which the dose of 0.5 kg ha^{-1} differed and overcame the dose of 1.0 kg ha^{-1} (3773 and 3504 kg ha^{-1}, respectively).

Table 6 - Summary of the orthogonal contrasts analysis and significance for the parameter referred to as soybean yield (Y)

Contrast	V1 *vs* V2	V1	V2	Meaningfulness
		kg ha^{-1}		
C1	Without S vs others	2990	3604	*
C2	1 application vs 2 applications	3638	3574	ns
C3	0.5 kg vs 1.0 kg (1 application)	3773	3504	*
C4	0.5 kg vs 1.0 kg (2 applications)	3613	3460	ns
C5	0.5 kg R1 vs 0.5 kg R5.1	3666	3879	ns
C6	1.0 kg R1 vs 1 kg R5.1	3474	3534	ns

ns: not significant at 5% probability; * significant at 5% de probability.

These results show that the supply of high amounts of S may result in negative responses, since the mechanism of S assimilation is under a strict control, in which, high concentrations of reduced sulfuric components are hardly ever found. Thus, the application of high doses of S does not necessarily result in a high concentration of products from the S reduction process in the cells, since the excess of this ion is directed to the vacuole and later assimilated (Marschner, 2012).

Bender *et al*. (2015) evaluated macro and micronutrient accumulation in soybean cultivars and observed the maximum accumulation rate of S varied between R2 (full bloom) and R4 (full pod) stages, evidencing to be the higher demanding period of the S by the plant. Zobiole *et al*. (2008) showed that the maximum daily accumulation of S for soybean occurred 74 and 73 days after emergence (DAE), respectively, corresponding to the beginning of the R5 stage. On the other hand, the beginning of the exponential accumulation of nutrients was between R1 and R3 stages (40 to 60 DAE) in both studies; and it can be inferred that this is the ideal moment for foliar S supplementation. Despite this, no statistical differences were found for the application periods (C5 and C6), thus, it is suggested that the application can be performed in both R1 and R5.1 stages of the soybean crop.

CONCLUSIONS

Although soil fertilization is the preferred method for supplying S to plants, leaf application of S was efficient in providing this nutrient to soybean. However, the application of elemental S via foliar did not promote significant differences in accumulation/export of either macronutrients or micronutrients in soybean grains. On the other hand, the foliar application of elemental foliar S promoted an increase of yield, and the best dose to be applied was 0.5 kg ha^{-1}, applied once in the R1 or R5.1 stage. Thus, the foliar fertilization of S may be an alternative to increase the availability of the element due to its easier application and distribution, in addition to a consequent increase of soybean yield.

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