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Additional Nitrogen Fertilization on Soybean via Soil and Leaf at the Reproductive Stage

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ABSTRACT

The ability of organic nitrogen fixation (BNF) to supply all the nutrient N in soybeans, considering high productivity, is questioned about the possible need to complement of this nutrient to the culture through other means of supplemental conventional fertilization. In this work the aim was to check the response of soybeans inoculated with bacteria of the genus Rhizobium and Bradyrhizobium, conditioned to supplementary nitrogen fertilization in the reproductive phase, in soil and in leaf. The treatments were the witness (without application of N), 30 and 60 kg of N ha-1, via soil and 2% solution of N, R1 and R 5.3 phases, in a randomized block design with 4 replications, using the soybean cv. Desafio, with the seeds inoculated with bacteria of the species Bradyrhizobium japonicum and Bradyrhizobium elkanii (strains Semia 587 and Semia 5019). The late application of N into R1 and R 5.3 provided increase in grain productivity of 478.6 kg ha-1 and 472.8 kg ha-1, respectively. The application, via soil, N in R1 features better harvest index, when compared to R 5.3. The late application of 2% solution of N, via foliar, provided no increase in productivity of soybean culture.

Keywords: Glycine max. Productivity. Biological fixation. Nitrogen fertilization.

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INTRODUCTION

Most part of the nitrogen demand of soybean is supplied mainly by biological fixation, by means of the symbiosis of plants with bacteria of the genus *Bradyrhizobium* being responsible for around 90% of N fixed in a natural way, the remainder of the nitrogen demand would be supplied by the soil by means of the organic matter mineralization. The majority of the nutrient assimilated by soybean is exported in the grain, and in accordance with Maehler et al. (2003), the period of greater demand occurs between the stages R1 (onset of flowering) and R6 (maximum volume of grains).

The application of N at sowing can bring loss in case of symbiosis (bacteria and plant), demonstrated in Hungria et al. (2006) e Zilli et al. (2010a) with application at sowing of 30 and 45 kg N ha^{-1}, respectively, reduced the nodulation and the contribution of BNF, in total of N accumulated in the aerial part by the soybean plants and in productivity.

Franchini et al. (2015) did not obtain response in soybean yield in the application of 30 kg/ha of N. Werner et al. (2016), working with 0 and 34 kg N ha-^{1} verified that the effect of nitrogen fertilization would be in the plant growth and does not increase productivity

With the increase of the soybean productivity in recent years, there are doubts as to whether the inoculation and soil organic matter would be able to meet all the plant’s demands. As the fertilization with N at sowing as potential prejudice nodulation, dazzles the late implementation efficiency of N would increase the productivity of the crop. As the fertilization with N in sowing stage may cause potential loss to nodulation, it is realized the late application of N, so that it is possible to obtain an increase in the crop productivity.

Mendes et al. (2008) studying the late supplementary fertilization with nitrogen in soybean under tillage observed that 50 kg ha^{-1} of N, in the form of ammonium sulphate applied in R5, showed higher yields than dealing only with inoculation. Bahry et al. (2014) and Bahry et al. (2013) working with N in the reproductive phase of soybean verified the positive influence of some components of yield, however, there was no increase in productivity.

Novo et al. (1999) using side dressing nitrogen, in the form of urea, found that there was a positive contribution to the increase of productivity of soybean, however these authors used soybeans in the period of mild winter. Fipke et al. (2016) working with application of 150 kg N ha^{-1} in side dressing in the reproductive phase observed increase of 300 kg of grain is in an area with co-inoculation of *Bradyrhizobium +Azospirillum brasilense* in soybean seed. Marcon et al. (2017) verified for the soybean crop that the application of liquid nitrogen via leaf, supplementary on the reproductive phase showed a positive effect on weight of one thousand grains.

In this work the aim was to check the response of soybeans inoculated with bacteria of the genus *Rhizobium* and *Bradyrhizobium,* the additional nitrogen fertilization on reproductive phase, via soil and via leaf.

MATERIALS AND METHODS

The experiment was carried out in a soil classified as dystrophic red latosol, according to the Brazilian System of Soil Classification in the following coordinates: Latitude 19°28'36.40"S and longitude 54°38'47.27"and altitude of 720 m in the municipality of São Gabriel do Oeste - MS. The region's climate is Aw according to the Köppen climate classification, with rains in summer and dry winter and annual average temperature of 25 ºC. The average rainfall in the region is 1800 mm annually.

The area has been used for agriculture since 1992 with the soybean crop in the period from September to March and the corn crop after soybean harvest. The system adopted was conventional until 200 and after tillage having the millet as cover crop.

The soil of the experimental area presented in the layer from 0 to 0.2 m pH in water of 5.42, phosphorus and potassium (Mehlich) 13.5 and 164.0 mg dm^{-3}, respectively, calcium 3.4 $cmol_+ dm^{-3}$, magnesium 1.9 $cmol_+ dm^{-3}$ Hydrogen 7.2 $cmol_+ dm^{-3}$, organic matter 37.6 g dm^{-3}, clay 560 g dm^{-3}, silt 8 g dm^{-3} and total sand 432 g dm^{-3}.

The treatments used were: Witness (without the application of N), 30 and 60 kg N ha^{-1} applied in phase R1 and R5.3, according to scale proposed by Sediyama, Silva and Borém (2015) via soil and leaf application with a solution of 2% of N in R1 and R5.3. It was used as a source of N the source urea with 45% of N, for both via soil and leaf. The application of urea via soil was done manually in the middle of the crop spacing. The leaf application was performed by dissolving 4.44 kg of urea in 100 liters of water and applied on the aerial part of the soybean with manual spray pressurized with CO_2 regulated in flow rate of 200 L ha^{-1}.

In the area before the choice of experimental plots, a basic fertilization was performed of 80 kg of P_2O_5 ha^{-1} and 80 kg de K_2O ha^{-1}, using as a source the granulated mixture of 00-20-20, broadcast application. After seeds of the cultivar Desafio were distributed, inoculated with bacteria of the species *Bradyrhizobium japonicum and Bradyrhizobium elkanii* (strains Semia 587 and Semia 5019), in furrows spaced 0.45 m with 18 seeds per linear meter, through mechanized seed drill.

After 10 days of sowing, the experimental plots were delimited and treatments distributed according to a completely randomized block design with 4 replications, and the plot consisted of 4 soybean lines spaced by 0.45 cm with 5 m of length (9 m^2). The useful plot was considered the two central rows with 1 m of border line in the ends totaling an area of 2.7 m^2.

The management was done normally throughout the experimental area, with application of herbicide, fungicide and insecticide as necessary. Anomaly was not observed during the experimental due to soil water deficit or injury caused by insects or diseases.

When the plant reached the stage R1 a collection of nodules was made of 1m linear in each plot, the withdrawal of the plants was made with care not to lose soil volume, a digging shovel was used for the withdrawal of plants and roots and placed in a sieve with a mesh of 3 mm and applied water to wash the soil volume. In the sieve the nodules were withdrawn that there were those who were adhered to the roots of the plants.

The nodules were counted and checked whether active or inactive by their internal staining. If the color was slightly red or violet it was considered active, if whitish or yellowish inactive. After that, they were placed in paper bags and dried in an forced air circulation oven at 65 ºC, in order to obtain the dry weight of the nodules.

The efficiency of the nodulation was obtained by the ratio of the numbers of nodules inactive by the total number of nodules and extrapolated to a percentage.

In the R2 stage (full flowering) 10 leaves of useful plot were removed from each plot (index leaf for nutritional diagnosis) randomly and one per plant of the 3rd node from the apex (diagnostic leaf) as described in Kuhiara et al. (2013) and determined the content of N, after sulfuric digestion, by the Kjeldahl method.

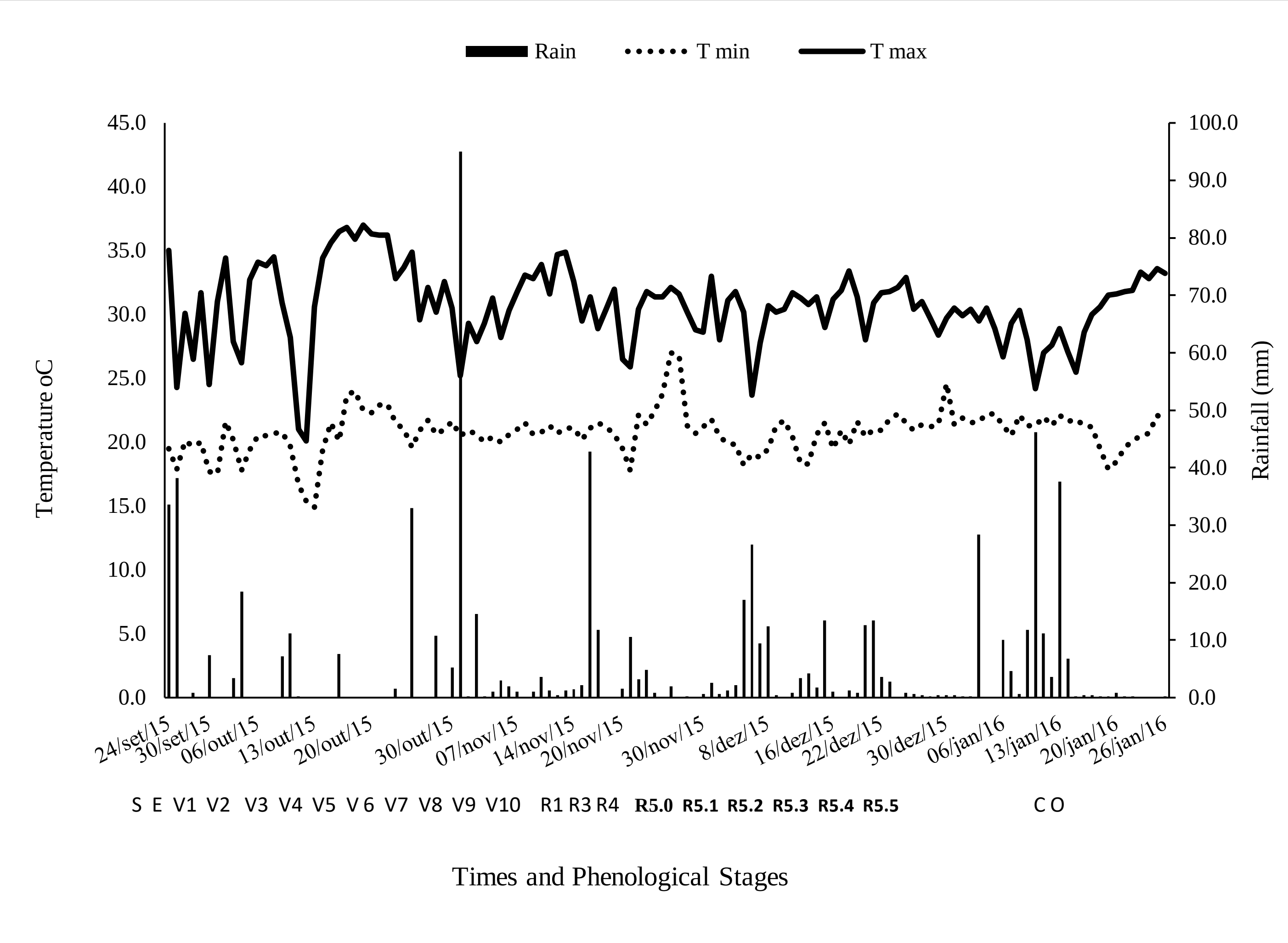
After the plants reach the state of physiological maturity (R8) the manual harvest was performed harvesting all the useful plot, cutting the plant close to the soil surface. In the collected material the insertion of the 1st pod, number of pods, number of grains per pod, weight of the whole plant were measured. After threshing of the grains was performed manually, and the mass of grains and 1000 grains 1000 randomly selected were measured. Both the masses evaluated were corrected to 13% moisture and the mass of the grains extrapolated to kg ha^{-1} to obtain the productivity.

The apparent harvest index was obtained by the ratio between the mass of grain and the sum of the mass of grains, stems and pods without the grains.

After the harvest ground sample was collected from each plot for analysis of soil fertility, according to Embrapa (2011), in the layer from 0 to 0.2 m.

In the experimental period, there was 683.60 mm of precipitation and maximum temperature reaching 37.0 ºC, with an average of 30.69° C and the minimum temperature of 14.90° C, with an average of 20.77 ºC (Figure 1), values obtained from the Meteorological Station of São Gabriel do Oeste of CEMTEC (2017).

Figure 1. Minimum and maximum temperatures, rainfall during the period from September 25th, 2015 to January 25th, 2016, from the meteorological station of São Gabriel do Oeste. (9.820 m from the experimental area). Source (CEMTEC, 2017).



(S=sowing, E=emergency, V1, V2...R1..R8 = phenological stages and CO= Harvest.)

The data were subjected to analysis of variance and the F test. After checking the homogeneity of variances and the data normality and when significant at 5% of probability the test was performed to separate the means by Waller-Duncan's. The computational Application Statistics Analyzes Systems (SAS) for statistical analyzes was used.

RESULTS AND DISCUSSION

On the occasion of the application of the treatments analysis was performed to check the effectiveness of inoculation carried out in soybean seeds. The average values of 54.12 total nodules, being 52.70 active and 4.46 non-active with efficiency of 91.47% and 0.34 g pl^{-1} of dry weight of nodules (Table 1).

Table 1. Average values of active, non-active nodules, efficiency, dry weight in the root system of soybean in two phenological stages and three forms of management of nitrogen fertilization.

Phenological Stage	N kg ha^{-1} and leaf	Number of active Nodules (pl)	Number of non-active Nodules (pl)	Number of total Nodules (pl)	Efficiency (%)	Dry weight of the nodules (g pl^{-1})
Witness	0	55.15 a	4.81 b	59.96 a	91.98 a	0.35 b
R1	30	54.73 a	3.78 b	58.51 a	93.53 a	0.45 a
R1	60	51.08 a	3.95 b	55.03 a	92.82 a	0.28 b
R1	2%	55.33 a	6.98 a	62.30 a	88.80 a	0.29 b
R5	30	58.10 a	3.78 b	61.88 a	93.90 a	0.33 b
R5	60	51.10 a	4.42 b	55.52 a	92.04 a	0.30 b
R5	2%	53.40 a	3.54 b	56.94 a	93.78 a	0.38 b
Mean		52.70	4.46	54.13	91.47	0.34
CV		22.47	26.54	23.12	18.42	32.45
F test		1.24^{ns}	3.15*	0.85^{ns}	1.44^{ns}	4.24*
DMS		5.22	1.82	7.55	8.67	0.18

^{ns} = not significant and * significant at 5% probability. Averages followed by the same letter at the column, differ among themselves, by the Waller-Duncan at 5% of probability.

The average value of 0.34 g pl^{-1} can be regarded as satisfactory for obtaining high productivities. Zilli et al. (2010b)) verified dry mass of nodules of 0.236 g pl^{-1} (average of years), lower values found in this study, it was enough to achieve productivity of 3,500.0 of soya grains ha^{-1}. The phosphorus in the soil before sowing of 3.5 mg dm^{-3}, may have influenced negatively the nodulation. Soares et al. (2016)) observed variation of 0.2 g to 1.4 g of dry mass of nodules influenced by phosphorus applied on the ground.

The nodulation of the crop in the experiment can be considered adequate, when compared to those obtained by Sartori et al. (2015) who observed values of 65.7 nodules per plant and 5.4 inactive nodules per plant, provided an efficiency of 92.2% and achieving productivities of 3,750.0 kg ha^{-1}.

Other highlights the efficiency of inoculation to be observed in the values of the leaf contents of the index leaf for nutritional status of the plant where there was a significant effect for N, P, K, Ca, Mg and S (Table 2). Another evidence on the inoculation efficiency can be observed in the values of foliar contents of the index leaf for the nutritional state of the plant where there was no significant effect for N, P, K, CA, MG, and S (Table 2). Comparing these values with those given by Kuhiara et al. (2013), it was found that N and K were appropriate and P, Ca, Mg and S were on average 10% below, demonstrating that the crop was in good nutritional status, before the application of nitrogen in the reproductive phase. Still considering the data of Kuhiara et al. (2013), the micronutrients showed significant differences, however, the values were above the plant's necessity, with the exception of Mn that was below the need, a fact attributed to high values of iron found in the leaves that may have influenced the absorption of Mn by competitive inhibition.

Table 2. Average values of the levels of nutrients in the leaf tissue of soybean in two phenological stages and three forms of management of nitrogen fertilization in soybean crop.

Phenological Stage	N kg ha^{-1} and leaf	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
		- - - - - - - - - - - - - g kg^{-1} - - - - - - - - - - - - -						- - - - - - - - - - - mg kg^{-1} - - - - - - - - - - -				
Test	0	41.35 a	2.51 a	20.00 a	7.52 a	2.45 a	1.61 a	437.25 b	27.64 a	37.44 b	64.51 a	91.83 a
R1	30	41.91 a	2.84 a	19.75 a	8.22 a	2.67 a	1.70 a	814.71 a	35.98 a	63.22 a	15.62 b	70.78 a
R1	60	35.06 a	2.62a	21.00 a	7.63 a	2.64 a	1.67 a	451.96 b	27.64 a	33.22 b	9.57 b	71.88 a
R1	2%	39.25 a	2.55 a	20.25 a	7.63 a	2.61 a	1.65 a	584.31 b	32.09 a	37.02 b	10.08 b	73.88 a
R5	30	42.33 a	2.38 a	18.75 a	7.12 a	2.51 a	1.63 a	525.49 b	25.41 a	29.69 b	9.07 b	79.87 a
R5	60	41.77 a	2.48 a	19.50 a	7.63 a	2.65 a	1.96 a	466.67 b	24.86 a	30.54 b	9.07 b	82.50 a
R5	2%	42.75 a	2.76 a	21.50 a	7.91 a	2.69 a	2.17 a	447.06 b	27.08 a	28.42 b	8.57 b	74.33 a
CV		9.51	11.24	10.15	12.42	9.37	10.17	15.77	13.22	12.47	10.56	11.24
F test		1.85^{ns}	1.25^{ns}	1.98^{ns}	2.05^{ns}	1.65^{ns}	2.16^{ns}	3.95*	1.73^{ns}	4.58**	7.85**	2.07^{ns}
DMS		9.32	0.71	2.83	1.34	0.41	0.62	215.04	11.67	12.36	17.54	23.77

^{ns} = not significant e* e ** respectively significant at 5% and 1% of probability. Averages followed by the same letter at the line, differ among themselves, by the Waller-Duncan at 5% of probability.

The biometry of the crop's aerial part, considered here as the insertion of the 1st pod, number of pods per plant, number of grains per pod, were not affected by treatments, probably because these parameters were already set before the stage R1 of culture (Table 3) and the plant genotype. Procópio et al. (2014), Ferreira Junior et al. (2010) and Rambo et al. (2004) comment on these soybean plants' biometrics, being more influenced by genotype and the interaction between the environment and not by nitrogen and plant population, confirmed in the work of Balbinot Júnior et al. (2015).

Table 3. Average values for insertion of the 1st pod, number of pods and grains per pod in the soybean plant in two phenological stages and three forms of management of nitrogen fertilization.

Phenological Stage	N kg ha^{-1} and leaf	Height Insertion of 1st pod (cm)	Number of pod (pl)	Number of grains per pod
Witness	0	13.50 a	37.63 a	2.42 a
R1	30	14.20 a	41.60 a	2.40 a
R1	60	11.38 a	38.23 a	2.30 a
R1	2%	14.63 a	39.73 a	2.33 a
R5	30	14.25 a	37.48 a	2.28 a
R5	60	12.63 a	44.15 a	2.50 a
R5	2%	13.00 a	42.90 a	2.28 a
CV		18.26	12.35	13.71
F test		1.44^{ns}	1.09^{ns}	1.22^{ns}
DMS		3.85	7.58	0.47

^{ns} = not significant Averages followed by the same letter at the line, differ among themselves, by the Waller-Duncan at 5% of probability.

The mass of 1000 grains was not affected by treatments and productivity was significant for the treatments with 60 kg N ha^{-1} applied in stages of R1 and R5.3. In the harvest index the treatment with 60 kg of N ha^{-1} in R1 was superior than the others, including the 60 kg N ha^{-1} in R5.3. Considering the via leaf treatments, i.e., application of a solution of 2% of N in R1 and R5.3, there was a significant effect in relation to witness for grain productivity and harvest index (Table 4).

Table 4. Average values for weight of 100 grains, and yield for the soybean crop in two phenological stages and three forms of management of nitrogen fertilization.

Phenological Stage	N kg ha^{-1} and leaf	Weight of 1000 grains (g)	Yield (kg ha^{-1})	Harvest index
Witness	0	145.19 a	3,740.8 b	0.482 c
R1	30	148.33 a	3,632.2 b	0.479 c
R1	60	150.14 a	4,219.4 a	0.498 a
R1	2%	152.46 a	3,762.5 b	0.480 c
R5	30	145.27 a	3,877.2 b	0.487 c
R5	60	145.95 a	4,213.6 a	0.493b
R5	2%	149.77 a	3,605.0 b	0.479 d
CV		17.64	15.43	9.37
F test		1.95 ^{ns}	6.47*	5.85*
DMS		9.72	297.92	0.003

^{ns} = not significant and * significant at 5% probability. Averages followed by the same letter at the line, differ among themselves, by the Waller-Duncan at 5% of probability.

For the grains mass these data are corroborated by those found by Duarte et al. (2016), Balbinot et al. (2015) and Rambo et al. (2004). The harvest index, as it is a ratio of commercial grain mass by the dry mass of the whole plant, is associated with the leaf area index which allows the interception of active photosynthetic radiation. Not always all the light intercepted by the leaf area is converted into biomass and consequently higher grain productivity (Silva et al. 2013). Thus, the harvest index expresses the efficiency of the crop in converting biomass into grains productivity, which in this study, the application showed the same tendency with the grains productivity, but not with the mass of 1000 grains, differentiating from the work of Petter et al. (2016) who observed a positive relationship of the harvest index with the productivity and weight of 1000 grains.

The application of 60 kg of N ha^{-1}, in both R1 and R5.3, although significant, resulted in an increase of 478.6 kg $ha^{-1}$and 472.8 kg ha^{-1}, respectively in R1 and R5.3, which should be considered the cost of the product and the application to verify the economic feasibility. Petter et al. (2012) verified average gains of 300 kg ha^{-1} with the late application of N in the soybean crop, the quantity considered by the authors as economically unfeasible, which must be taken into account in assessing both the management adopted as the climatic conditions which interfere with the response of the soybean crop, which are reflected in the productivity. Mendes et al. (2008), who observed the economic unfeasibility of N fertilization in soybean crop in Latosol in the Cerrado region.

The evaluation of soil fertility, held at the time immediately after harvest, showed that the levels of phosphorus (average of 3 mg dm^{-3}) were below the recommended levels (Table 5), when compared to the critical levels of P in the soil of Vieira et al. (2015) who recommend values of 8 mg dm^{-3}. This fact may have limited the response of the crop, especially in dose of 30 kg N ha^{-1} and in the application via leaf. The other parameters of soil fertility were considered suitable for the soybeans crops.

Table 5. Average values of chemical attributes in the soil, for the purpose of yield in soybean crop in two phenological stages and three forms of management of nitrogen fertilization.

Phenological Stage	N kg ha^{-1} and leaf	pH		P	K	Ca	Mg	H	MO
		water	CaCl2	- -mg dm^{-3}-- -		- - - - - - $cmol_+ dm^{-3}$ - - - - -			g dm^{-3}
Witness	0	5.47 a	4.57 a	3.23 a	170 a	3.50 a	1.85 a	6.81 a	40.38 a
R1	30	5.35 ab	4.45 ab	3.03 a	198 a	2.95 a	2.03 a	7.89 a	36.59 a
R1	60	5.30 b	4.43 ab	3.59 a	190 a	3.38 a	1.73 a	6.87 a	31.02 a
R1	2%	5.52 a	4.62 a	5.41 a	180 a	3.68 a	1.85 a	6.32 a	39.26 a
R5	30	5.27 b	4.41 ab	2.78 a	170 a	3.35 a	1.93 a	6.39 a	38.96 a
R5	60	5.23 b	4.34 b	3.31 a	118 a	3.30 a	2.28 a	9.54 a	37.11 a
R5	2%	5.46 a	4.56 a	3.58 a	128 a	3.48 a	1.75 a	6.42 a	40.08 a
CV		15.22	16.14	13.79	12.47	16.52	18.26	14.75	19.33
F test		3.73*	6.52*	1.77^{ns}	2.03^{ns}	1.94^{ns}	2.11^{ns}	1.67^{ns}	2.18^{ns}
DMS		0.21	0.19	2.67	75.2	0.86	0.83	3.74	9.84

^{ns} = not significant and * significant at 5% probability. Averages followed by the same letter at the line, differ among themselves, by the Waller-Duncan at 5% of probability.

CONCLUSIONS

The late application of N, via soil in R1 and in R5.3 provides an increase in grains productivity of the crop;

The application, via soil, of N in R1 presents better harvest index, when compared to R5.3.

The late application of N via leaf, did not provide increase in productivity of soybeans crop.

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