

**Forms of application of nitrogen in different stages of the development of soybean culture****Formas da aplicação de nitrogênio em diferentes estádios do desenvolvimento da cultura da soja**

DOI:10.34117/bjdv6n11-531

Recebimento dos originais:08/10/2020

Aceitação para publicação:25/11/2020

**José Roberto Rocha**

Engenheiro Agrônomo

Instituição: Universidade Federal de Mato Grosso – UFMT

Endereço: Universidade Federal de Mato Grosso, Sinop, MT, Brasil

Endereço: Av. Alexandre Ferronato, 1200 - Res. Cidade Jardim, Sinop - MT, 78550-728

E-mail: roberto-rocha@hotmail.com

**Marcos Trentin**

Engenheiro Agrônomo

Instituição: Universidade Federal de Mato Grosso – UFMT

Endereço: Universidade Federal de Mato Grosso, Sinop, MT, Brasil

Endereço: Av. Alexandre Ferronato, 1200 - Res. Cidade Jardim, Sinop - MT, 78550-728

E-mail: magtrentin@gmail.com

**Bruno Conceição de Veiga**

Mestrando em Agronomia – PPGA

Instituição: Universidade Federal de Mato Grosso – UFMT

Endereço: Universidade Federal de Mato Grosso, Cuiabá, MT, Brasil.

Endereço: Av. Fernando Correa da Costa, 2.367 – Bairro: Boa Esperança

CEP: 78060-900 - Cuiabá – MT

E-mail: brunoveiga\_@hotmail.com

**Cassiano Spaziani Pereira**

Doutor em Fitotecnia (Produção Vegetal)

Instituição: Universidade Federal de Mato Grosso – UFMT

Endereço: Universidade Federal de Mato Grosso, Sinop, MT, Brasil

Endereço: Av. Alexandre Ferronato, 1200 - Res. Cidade Jardim, Sinop - MT, 78550-728

E-mail: caspaziani@gmail.com

**Rafael Orlandini Bosqueiro**

Mestrando em Agronomia – PPGA

Instituição: Universidade Federal de Mato Grosso – UFMT

Endereço: Universidade Federal de Mato Grosso, Sinop, MT, Brasil

Endereço: Av. Alexandre Ferronato, 1200 - Res. Cidade Jardim, Sinop - MT, 78550-728

E-mail: rafaelbosqueiro@hotmail.com

**Ivan Vilela Andrade Fiorini**

Doutor em Agronomia (Fitotecnia)

Instituição: Universidade Federal de Mato Grosso – UFMT

Endereço: Universidade Federal de Mato Grosso, Sinop, MT, Brasil

Endereço: Av. Alexandre Ferronato, 1200 - Res. Cidade Jardim, Sinop - MT, 78550-728

E-mail: ivanvaf@yahoo.com.br

**Adriano Alves Silva**

Doutor em Agronomia (Fitotecnia)

Instituição: Centro Universitário de Formiga - UNIFOR

Endereço: UNIFOR- Centro Universitário de Formiga, Formiga, MG, Brasil

Endereço: R. Dr. Arnaldo Sena, 328, Formiga - MG, 35570-000

E-mail: adrianoas@msn.com

**Jussane Antunes Fogaça dos Santos**

Mestrando em Agronomia – PPGA

Instituição: Universidade Federal de Mato Grosso – UFMT

Endereço: Universidade Federal de Mato Grosso, Sinop, MT, Brasil

Endereço: Av. Alexandre Ferronato, 1200 - Res. Cidade Jardim, Sinop - MT, 78550-728

E-mail: jussaneantunes@hotmail.com

**ABSTRACT**

In Brazil, inoculation with *Bradyrhizobium japonicum* and *B. elkanii* strains is the main form of Nitrogen (N) supply to soybean, but there are reports of the use of mineral N as a complement to biological fixation. The objective was to evaluate the effect of different forms and times of N application on the development and yield of soybean. The treatments include a control without N application and in all other treatments 10 kg ha<sup>-1</sup> of N was applied, altering the time (V2, V4, R1 or R2) and the application form, namely, at sowing, broadcast on topdressing or by leaf spraying. The application of N provided an increase in leaf area, dry mass of shoot, number of nodules and dry mass of nodules in treatments with application of N at sowing and N at V2 on topdressing. For plant height, the treatment with N at V2 by leaf spraying was superior compared to control. The weight of one thousand seeds was not affected by N application. For grain yield the treatments with application of N by leaf spraying in V2 and R1 were highlighted. The greatest increase in grain yield on soybean is reached with leaf spraying at V2 stage.

**Keywords:** nodulation, foliar fertilization, phenological stages.

**RESUMO**

No Brasil, a inoculação com estirpes de *Bradyrhizobium japonicum* e *B. elkanii* é a principal forma de fornecimento de nitrogênio (N) à soja, porém existem relatos do uso de nitrogênio mineral como complemento a fixação biológica. Objetivou-se avaliar o efeito de diferentes formas e épocas de aplicação do N no desenvolvimento e produtividade da cultura da soja, no norte de Mato Grosso. O delineamento experimental foi blocos casualizados (DBC), com 10 tratamentos; testemunha sem aplicação de N e nos demais tratamentos a aplicação de 10 kg ha<sup>-1</sup> de N, alterando época e forma de aplicação: a lanço em cobertura na semeadura; em V2 a lanço em cobertura; V2 via foliar; V4 a lanço em cobertura; V4 via foliar; R1 a lanço em cobertura; R1 via foliar; R2 a lanço em cobertura e R2 via foliar. Utilizou-se a cultivar TMG 132 RR, inoculada com *Bradyrhizobium japonicum* e *B. elkanii*. A aplicação de nitrogênio proporcionou aumento da área foliar, massa seca de parte aérea, número de nódulos e massa seca de nódulos, nos tratamentos com aplicação de N na semeadura e N em V2 de cobertura. Para altura de plantas, o tratamento com N em V2 via foliar se destacou da testemunha. O peso de mil sementes não foi alterado pela aplicação de N. Para produtividade de grãos destacaram-se os tratamentos com aplicação de N via foliar em V2 e R1. A aplicação foliar de N em V2 proporciona a maior produtividade na cultura da soja.

**Palavras-chaves:** nodulação, adubação foliar, estádios fenológicos.

## 1 INTRODUCTION

Nitrogen (N) in living organisms has among various functions the structural amino acids constitution, proteins, nucleic acids and, especially in plants, it constitutes the chlorophyll molecule. In atmosphere, N<sub>2</sub> is a stable molecule and it is not easily found available for plants, which do not have the capacity to directly utilize it. In the soil, the organic matter acts as a supplier of soluble and absorbable forms of N by plants, because in the free form N is rapidly utilized by soil bacteria or leached due to its high solubility (Parente et al., 2015; Ferreira et al., 2016).

In Brazil, where the majority of soils have low rates of organic matter, soybean yield depends on N deriving from Biological Fixation of Nitrogen (BFN), through inoculation of soybean plant with strains of bacteria *Bradyrhizobium japonicum* and *B. elkanii*, the main form of N supply to soybean (Hungria et al., 2007). However, there are reports of mineral N as a complement to BFN.

In soybean, due to high protein levels in the grains, there is a high necessity of N (Sales et al., 2015). According to EMBRAPA (2011), each 1000 kg of soybean grains contains 51 kg of N, in the remaining parts of the plant there are more 32 kg of N, totaling 83 kg of N per ton of grains. With the increasing levels of yield of current cultivars, more demanding in N, N fertilization has been recommended as a complement to BFN, even because from R2 stage occurs a sharp reduction in the activity of rhizobacteria (Bahry et al., 2013; Parente et al., 2015). Thus, only BFN may not be able to supply N necessary to higher levels of yield, requiring complementation via mineral N (Sfredo and Oliveira, 2010; Bahry et al., 2013; Pierozan et al., 2015). Another factor of inoculation is that it is an energetically costly process for the plants and the supplying of mineral N has a lower energetic expenditure for the absorption than the BFN. However, there are controversies regarding this, being necessary the understanding of the effect of N fertilization in the plant physiology, aiming the balance between addition of mineral N and BFN (Hungria et al., 2007; Brito et al., 2011). In recent years many cultivars, even more early, have been launched being necessary to find forms of fostering crop yield by means of supplementation with mineral N, because current cultivars are more productive and more demanding in N (Sfredo and Oliveira, 2010; Pierozan et al., 2015; Ferreira et al., 2016). However, this mineral N should not compete with the process of BFN (Brito et al. 2011). Thus, identifying the best management for supplying mineral N in order to propel the development and production of the crop would contribute for broader environmental and economical sustainability of soybean crop. The aim of the paper was to verify the effect of different forms and times of N application on the development and yield of soybean.

## 2 MATERIAL AND METHODS

The experiment was conducted in a cultivated commercial area, under the system of no till for five years, between the months of October 2016 and February 2017, in Sinop (MT). The place of the

experiment is located on the latitude 11°57'05''S and longitude 55°23'51''W and altitude of 380 m with flat topography. The climate according to Koppen-Geiger is classified as Aw, having two well definite seasons, being one rainy between October and April and the other dry from May to September, with low annual temperature ranging between 24 to 27°C. The average annual rainfall is around 2,100 mm (Souza et al., 2013).

Climatological data in the period of the experiment, between the time period of Oct 29 2016 and Feb 23 2017, were obtained in the meteorological station of Embrapa Agrossilvipastoril in Sinop-MT. The rainfall accumulated in the period was 1,179.85 mm, a quite higher volume than the crop requirement, which oscillates between 450 to 800 mm per cycle (Embrapa, 2011). The soil on the location of the experiment is classified as Red Yellow Latosol (Embrapa, 2013).

This soil was sampled in the layer of 0 to 20 cm depth, and after chemical and physical analysis was performed. The chemical analysis of the soil showed the following results: pH (CaCl<sub>2</sub>) 5.40; O.M. 18.55 g dm<sup>-3</sup>; P (Melich) 6.07 mg dm<sup>-3</sup>; K 52.00 mg dm<sup>-3</sup>; Ca 2.84 mg dm<sup>-3</sup>; Mg 0.93 mg dm<sup>-3</sup>; S 4.00 mg dm<sup>-3</sup>; V = 57.2%; Ca/Mg relation 3.05; Ca/K 21.85; Mg/K 7.16. The values of micronutrients in mg dm<sup>-3</sup> were: Zn 5.51; Cu 0.44; Fe 199.16; Mn 11.25; B 0.15. The physical analysis of the soil showed the values: sand 497; silt 125 and clay 378, in g dm<sup>-3</sup>, respectively. With the results of soil analysis, it was observed that the saturation of basis was in accordance to the crop demands, not requiring liming in pre-planting. The fertilization of basis was broadcast with the application of 500 kg ha<sup>-1</sup> of the formulation NPK 00:18:18.

The experimental design was randomized complete blocks (RCBD), with four replications and ten treatments, totaling 40 plots. The treatments were: witness without N application (only inoculated with *Bradyrhizobium japonicum* and *B. elkanii*). For the other treatments, besides seed inoculation, it was applied 10 kg ha<sup>-1</sup> of N (Urea 45% of N), in different times (phenological stages) and forms of application: broadcast on topdressing at sowing; broadcast on topdressing in V2; leaf spraying in V2; broadcast on topdressing in V4; leaf spraying in V4; broadcast on topdressing in R1; leaf spraying in R1; broadcast on topdressing in R2 and leaf spraying in R2.

The trial plots were constituted of four lines with five meters long, totalizing 10 m<sup>2</sup>. It was considered as useful area of the plots for sampling the two central lines and four meters long, totaling 4 m<sup>2</sup>. As margins, half meter was discarded in each extremity of the plots and the two lateral lines. The sowed cultivar was the TMG 132 RR, which has determined type of growth, light brown heel color, tolerant to lodging, cycle of 118 to 122 days, high demand in soil fertility to express high yields and it is indicated for planting between Oct 14th and Nov 14th in the north of Mato Grosso state. 15 seeds were sowed per meter aiming to obtain a stand of 260.000 plants ha<sup>-1</sup>.

Before sowing, seed treatment were performed applying Fipronil insecticide, from pyrazole group, and the Piraclostrobina fungicide, from estrubirulinas group, and tiophanate-methyl from

benzimidazoles group, in the dosage of 2 ml kg<sup>-1</sup> of seed. In V3, in order to increase the nodulation and yield of soybean, it was applied via foliar cobalt and molybdenum based products, in the proportion of 5 g of Co and 42 g of Mo, without application in the seed, as recommended by Mata et al., 2011. It was also performed, before sowing, the inoculation of seeds with peaty inoculant for soybean, *Bradyrhizobium japonicum*, SEMIA 5079 and 5080 breeds, minimal concentration of rhizobium of 7 x 10<sup>9</sup> cells g<sup>-1</sup>, in the dosage of 200 g ha<sup>-1</sup>, and *Bradyrhizobium elkanii*, SEMIA 597 and 5019 breeds, in the liquid form, dosage of 200 ml ha<sup>-1</sup> with concentration of rhizobium of 5 x 10<sup>9</sup> cells/ml. The inoculants and the seeds were placed in polyethylene bags and the mixture was performed.

The cultural practices were performed according to the requirements of soybean. For the control of rusting it was applied the fungicide Estrobirulina and Triazole, active principles Trifloxistrobina and Prothioconazole, four times. The control of weeds in post-emergency occurred within the recommended period, from germination until thirty days after planting. Before the sowing and at 30 DAE it was performed the application of 1.5 kg ha<sup>-1</sup> of glyphosate with solution volume of 100 L ha<sup>-1</sup> employing a self-propelled sprayer.

The evaluations of chlorophyll content, plant height, leaf area, shoot dry mass, number of nodules and nodule dry mass were performed in the stage of full flowering (R2), when samples were collected from four plants per useful plot. Chlorophyll content was evaluated in three intact leaves, in the medium region of each plant, in the stage of full flowering. It was used a chlorophyll measurer brand ClorofiLOG (model CFL-1030), which estimates the chlorophyll content in indirect form, by means of dimensionless units. According to Argenta et al. (2001) it estimates with good precision the chlorophyll content of the leaves, being an efficient parameter for monitoring the N level. Plant height was obtained with the help of a measuring tape, measuring from the soil up to the apical meristem of the plants. After the field measurements the plants were cut close to the soil, conditioned in paper bags and taken to the laboratory of seeds of UFMT, campus Sinop. In the laboratory, it was determined the leaf area with the help of a leaf area integrator model LICOR (LI-3010). The shoot dry mass was obtained by forced air circulation with temperature of 65°C until constant weight. After weighing and obtaining the leaves dry mass, the samples of the aerial part of the plants were sent to a laboratory for the analysis of the foliar N content through the semi-micro Kjeldahl method. The number of nodules and the nodules dry mass was obtained through the removal of the root system from a pit of approximately 20x20x20 cm, taking care not to damage the root system. Washing and collection of the nodules was performed with the help of scissors, separating them from the roots, posteriorly counting the number of nodules of the samples and after conditioning them in identified paper bags and taken to green house with forced air circulation at 65°C until constant weight. After drying it was obtained the nodules dry mass, weighing the samples in a precision scale.

Harvest was performed manually on the day Feb 2nd 2017. After that the number of pods and the number of grains per pod of four plants from each useful plot were counted. On the harvest the grains, with approximately 180 g kg<sup>-1</sup> of water, were threshed mechanically in stationary motor thresher. After threshing the grains were cleaned and sieved manually and conditioned in paper bags identified. The humidity of the grains was then corrected to 130g kg<sup>-1</sup>of water. The initial water content of the grains was determined through direct method in greenhouse with forced air circulation at 105o C for 24 hours (BRASIL, 2009). After correction for humidity it was determined the weight of a thousand seeds and the grain yield in kg ha<sup>-1</sup>.The data obtained were submitted to analysis of variance (ANAVA), to the level of 5% of probability by F test, with the help of the statistical software SISVAR (Ferreira, 2011). The averages were compared through the Skott-Knott test at 5% probability.

### **3 RESULTS AND DISCUSSION**

It is observed that the treatments influenced significantly the morphology and production of dry mass and yield on soybean, but did not affected the N and chlorophyll content on the leaves (Table 1). The values of indirect readings of chlorophyll varied from 45.21 to 48.95 and resembled the ones obtained by other authors in soybean (Nogueira et al., 2010; Silva et al. 2011; Werner et al., 2016). According to those authors, probably the N absorbed by the plants was redistributed to production of vegetative, reproductive and nutritional structures, which may have occurred in this paper.

The N contents on the leaves varied between 33.03 to 39.36 g kg<sup>-1</sup> and according to Urano et al. (2007) the optimal range should vary between 36.80 to 47.80 g kg<sup>-1</sup>. So, although not having significant difference between the treatments, it is observed in the values obtained that the witness was the only treatment that reached values below the ideal range, according to those authors.

Table 1. Averages of analyzed variables in full flowering R2: chlorophyll total content (CLO) , concentration of N on leaves (CNL), leaf area (LA), plant height (PH), shoot dry mass (SDM), number of nodules (NN), nodules dry mass (NDM) under different forms and times of application of 10 kg ha<sup>-1</sup> of Nitrogen.

| Treatments                | Variable analyzed |                           |                      |        |                              |          |                              |
|---------------------------|-------------------|---------------------------|----------------------|--------|------------------------------|----------|------------------------------|
|                           | CLO (SPAD)        | CNL (g kg <sup>-1</sup> ) | LA (m <sup>2</sup> ) | PH (m) | SDM (g plant <sup>-1</sup> ) | NN       | NDM (g plant <sup>-1</sup> ) |
| Without N                 | 46.20 a           | 33.03 a                   | 1.57 b               | 0.77 b | 24.71 b                      | 86.25 c  | 4.4 a                        |
| N at sowing               | 46.55 a           | 36.36 a                   | 2.01 a               | 0.82 b | 36.06 a                      | 91.00 b  | 4.7 a                        |
| N V2 topdressing          | 46.85 a           | 35.72 a                   | 2.03 a               | 0.88 a | 36.59 a                      | 102.75 a | 4.7 a                        |
| N V2 leaf spraying        | 48.95 a           | 35.53 a                   | 1.61 b               | 0.91 a | 25.08 b                      | 67.00 d  | 3.2 c                        |
| N V4 topdressing          | 47.26 a           | 39.36 a                   | 1.27 b               | 0.82 b | 18.78 b                      | 85.50 c  | 3.3 c                        |
| N V4 leaf spraying        | 47.75 a           | 36.26 a                   | 1.52 b               | 0.88 a | 31.48 a                      | 75.50 c  | 3.7 b                        |
| N R1 topdressing          | 46.92 a           | 35.96 a                   | 1.51 b               | 0.90 a | 26.41 b                      | 65.25 d  | 2.9 c                        |
| N R1 leaf spraying        | 45.21 a           | 36.30 a                   | 1.48 b               | 0.86 a | 27.07 b                      | 108.25 a | 3.7 b                        |
| N R2 topdressing          | 45.77 a           | 33.80 a                   | 1.52 b               | 0.82 b | 25.01 b                      | 107.25 a | 4.5 a                        |
| N R2 leaf spraying        | 46.26 a           | 33.23 a                   | 1.38 b               | 0.82 b | 26.63 b                      | 81.25 c  | 4.1 a                        |
| Coefficient Variation (%) | 3.37              | 7.17                      | 18.76                | 4.41   | 17.23                        | 7.14     | 10.46                        |

\*The averages followed by the same letters in the columns do not differ from each other at the 5% probability level by the Scott-Knott test.



For the variable leaf area, it was observed the difference on the treatments with the application of N at sowing and on topdressing at V2 stage. The application of N at sowing and at V2 resulted in greater values of leaf area than the other treatments. The values found were of 2.01 and 2.03 m<sup>2</sup>, which surpassed the witness in 21.84 and 22.60%, respectively, higher than the witness without N, which obtained 1.57 m<sup>2</sup> and did not differ from the other treatments. The fact may be an indicative that a greater availability of N in the soil on the initial phases may have promoted greater growth of roots and vegetative development, without loss of nodulation.

For plant height the treatments that differ in relation to the witness were: N at V2 on topdressing, N at V2 by leaf spraying, N at V4 by leaf spraying, N at R1 on topdressing and N at R1 by leaf spraying. The applications of N on topdressing and via foliar on stages V2, V4 and on topdressing at R1 resulted in greater height of the plants with 0.91 m, an increase of 0.14 m in height in relation to the witness, which was 18.20% superior to the witness with 0.71 m. Silva et al. (2011) applying doses up to 40 kg ha<sup>-1</sup> of N in soybean at sowing have verified quadratic effect for plant height up to the doses of 22 kg ha<sup>-1</sup> of N reaching plant heights of 0.75 m on the second year of cultivation. Likewise, Franchini et al. (2015) applying 30 kg ha<sup>-1</sup> of N in soybean at sowing also verified greater height of plants. The results of this paper corroborated the ones from other authors, which the application of treatments at stages V2 to R1 resulted in greater height of plants.

The variable shoot dry mass differed from the witness on the treatments with application of N at sowing, at V2 on topdressing and at V4 by leaf spraying, with the respective values of 8.655, 8.737 and 7.552 ton ha<sup>-1</sup>. The treatment with N at V2 on topdressing stood out, obtaining a greater shoot dry mass of 8.737 ton ha<sup>-1</sup> with a 47% increase in relation to the witness, which reached 5.927 ton ha<sup>-1</sup>. Werner et al. (2016), in two agricultural years, found differences in total dry mass, just in one agricultural year on the treatments that received 45 kg ha<sup>-1</sup> of N applied on topdressing at V2 stage. It is verified on this paper that N available in the soil at sowing, at V2 and by leaf spraying at V2 stage provide a greater amount of shoot dry mass.

For the number of nodules per plant the values situated between 75.50 to 108.25. It was observed that the plants which received N at V2 stage on topdressing and at the R1 and R2 stages by leaf spraying showed greater number of nodules in relation to the witness and the other treatments. The application of N at R1 stage by leaf spraying showed greater number of nodules (108.25) among all treatments, addition of 44% in relation to the plants that did not receive any supplementation with N (witness).

The observed increase in the number of nodules due to N fertilization in some stages of development (V2, R1 and R2) is contrary to what other authors have verified. Viera Neto et al. (2008) and Nogueira et al. (2010) verified that plants fertilized with N showed reduction in the efficiency of



Bradyrhizobium colonization. In studies carried out by Stephens and Neyra (1983) it was verified that application of N in the form of  $\text{KNO}_3$  reduces nitrogenase in more than 50%. This occurs, because nitrate and nitrite accumulated to the nodular level inhibit N fixation due to reduction of availability of energy to bacteroides. However, it must be pointed out that in the present study the doses utilized was of just  $10 \text{ kg ha}^{-1}$  of N.

Parente et al. (2015) studying two soybean varieties which received 0, 10, 20, 30 and  $40 \text{ kg ha}^{-1}$  of N at sowing or at reproductive stage R1 (beginning of flowering), verified that the cultivar BRS Valiosa RR also showed greater number of nodules when fertilized at R1 stage in comparison to fertilization at sowing. N fertilization at R1, according to the authors, allowed the development of a greater number of nodules, result that corroborates with the one observed in this paper.

The nodules dry mass did not differ among the witness and the treatments that received N at sowing, N at V2 on topdressing, N at R2 on topdressing and N at R2 by leaf spraying. The treatments with N at V2 by leaf spraying, N at V4 on topdressing and by leaf spraying, N at R1 on topdressing and by leaf spraying, obtained lower nodules dry mass, differing from the others. Parente et al. (2015) applying doses of up to  $40 \text{ kg ha}^{-1}$  of N in two different cultivars of soybean verified that the nodules dry mass from the cultivar BRS Valiosa RR showed higher values at N fertilization at R1 than at sowing. This shows that N fertilization at R1 allows the increase of mass and volume of nodules, when compared to the application of N at sowing. The lower formation of nodules with N application may have occurred due to reduction of the efficiency of the bacteria Bradyrhizobium in the presence of mineral N (Vieira Neto et al., 2008). It is verified that application of N at vegetative stages from V4 to R1 reduced the nodules dry mass of soybean. On the other hand, the application of N at phenological stages from V2 to R1 promoted increase on plant height and shoot dry mass.

The treatments affected significantly the components of performance and yield of soybean (Table 2). The witness and the topdressing fertilization with N at V2, the leaf spraying at V4 and R1 resulted in increase on the number of pods (NP). The highest NP was observed with N at V2 on topdressing and outperformed in 18.25 pods per plant (39.2%) the lowest NP obtained R2 by leaf spraying. Silva et al. (2011) verified increase on NP in doses up to  $40 \text{ kg ha}^{-1}$  of N at sowing, however, without rise in grain yield.

Table 2. Means of the variables analyzed at the harvest in the reproductive period R8: number of pods (NP), number of grains per pod (NGP), weight of one thousand seeds (WTS) and grain yield (GY) under different forms and times of application of 10 kg ha<sup>-1</sup> of nitrogen.

| Treatments                | Variable analyzed |        |          |                           |
|---------------------------|-------------------|--------|----------|---------------------------|
|                           | NP                | NGP    | WTS (g)  | GY (kg ha <sup>-1</sup> ) |
| Without N                 | 58.25 a           | 2.07 b | 111.66 a | 3286.50 b                 |
| N at sowing               | 57.25 a           | 2.16 b | 109.74 b | 3472.23 b                 |
| N V2 topdressing          | 64.75 a           | 1.92 b | 114.58 a | 3621.73 b                 |
| N V2 leaf spraying        | 47.00 b           | 2.60 a | 116.49 a | 4136.00 a                 |
| N V4 topdressing          | 47.25 b           | 2.26 b | 108.66 b | 3504.18 b                 |
| N V4 leaf spraying        | 62.00 a           | 2.18 b | 108.91 b | 3739.31 b                 |
| N R1 topdressing          | 61.00 a           | 2.12 b | 112.49 a | 3610.06 b                 |
| N R1 leaf spraying        | 58.00 a           | 2.60 a | 112.49 a | 3838.50 a                 |
| N R2 topdressing          | 53.50 b           | 2.52 a | 109.41 b | 3633.43 b                 |
| N R2 leaf spraying        | 46.50 b           | 2.64 a | 109.74 b | 3558.25 b                 |
| Coefficient Variation (%) | 12.63             | 13.46  | 3.96     | 6.74                      |

\*The averages followed by the same letters in the columns do not differ from each other at the 5% probability level by the Scott-Knott test.

Petter et al. (2012) also determined increase on NP per plant, applying doses of 20 and 40 kg ha<sup>-1</sup> of N, at the phenological stage R1; and verified that the maximum agronomical efficiency was obtained with the doses of 29.8 kg ha<sup>-1</sup> of N, obtaining on average 49.3 pods per plant, an increase of 6.7% in relation to the witness. According to Hungria (2006) N is an element involved in the synthesis of chlorophylls and protein compounds, showing potential to increase the capacity of the plants in producing reproductive gems. In the present paper the doses of N applied on different stages did not increase the NP in relation to the witness.

For the variable number of grains per pod (NGP) it was verified difference in relation to the witness with the application of 10 kg ha<sup>-1</sup> of N. The fertilization by leaf spraying with N at V2, R1 and R2 and on topdressing at R2 resulted in gains on the number of grains per pod in relation to the other treatments. Those treatments presented average increase of 0.52 NGP in relation to the witness (Table 2). The greater value of NGP was obtained by leaf spraying of N at R2 (2.4), 12.75% superior to the witness with 2.07 grains per pod. It should be noted that independently from the crop stage, leaf spraying of N resulted in greater NGP.

In the present study the treatments with N at V2 and R1 by leaf spraying stood out obtaining on average 2.6 NGP, consequently it was obtained higher grain yield. Silva et al. (2011) did not verified difference on this variable applying doses between 10 and 40 kg ha<sup>-1</sup> of N with application on soybean at sowing, obtaining values between 1.73 to 1.93 grains per pod.

For the variable weight of one thousand seeds, it was verified that the application of N at V2 and R1 on topdressing and also by leaf spraying did not differ statistically from the witness. The treatment with application of N at V2 by leaf spraying presented higher weight of one thousand seeds, with value of 4.14% superior to the witness. The other treatments; N at sowing, N at V4 and R2 by leaf spraying and on topdressing, obtained a lower weight of one thousand seeds than the witness (Table 2).

Bernis and Viana (2015), with the application of 2 L ha<sup>-1</sup> (N 32%) at V3 and R1 stages by leaf spraying, found higher weight of a thousand seeds (WTS), differentiating from the witness without N, obtaining gain in weight of grains. It was stated in the present paper similar result to the previously mentioned, where the treatments with N at V2 on topdressing and by leaf spraying presented higher WTS. The increase of WTS may be related to higher accumulation of protein in grains, due to higher synthesis of amino acids occasioned by the applied N. Silva et al. (2011) did not obtain significant differences in mass of 100 seeds with the application of 0, 10, 20, 30 and 40 kg ha<sup>-1</sup> of N at sowing on soybean in two years of cultivation. The results of this paper corroborate with those authors, where the doses of N applied did not show statistical difference of WTS in relation to the witness.

The foliar fertilization at V2 and R1 resulted on higher yields. The fertilization in V2 reached the grain yield of 4,136 kg ha<sup>-1</sup> and surpassed the witness in 12.58%, what generated a gain of 849.5

kg ha<sup>-1</sup>., meaning 14.16 sacs ha<sup>-1</sup>. On the treatment with N in R1 foliar the gain was of 552 kg ha<sup>-1</sup>. On the treatment with N in R1 by leaf spraying, the gain was of 552 kg ha<sup>-1</sup>, meaning 9.2 sacs ha<sup>-1</sup>. Different results on the present paper were obtained by other authors, for example, Werner et al. (2016) applying doses of 45 kg ha<sup>-1</sup> of N, Silva et al. (2011) and Parente et al. (2015) applying doses up to 40 kg on topdressing and Franchini et al. (2015) applying 30 kg ha<sup>-1</sup> of N and Zilli et al. (2010) with the average of two years, verified that just inoculating the soybean produced the same than the N treatment.

In other works Hungria et al. (2007) found out a reduction on yield of soybean with the application of 50 kg ha<sup>-1</sup> of N. Petter et al. (2012), with applications of 80 kg ha<sup>-1</sup> and 160 kg ha<sup>-1</sup> of N via soil, verified reduction on grain yield. High doses of N, applied via soil or by leaves, according to these authors, may cause reduction of nodulation and thus low efficiency on BFN, besides toxicity on the leaves, with marked reduction on grain yield of soybean. In the present paper, the application of N obtained complementary results regarding BFN in all treatments with increase on grain yield, especially on the treatments V2 and R1, corroborating with Hungria (2006), who relates that the demand for N on soybean aiming high yields is not entirely supplied by BFN. The N is a nutrient that stimulates the absorption of other nutrients by the plant, fact verified in this paper when applied doses of 10 kg ha<sup>-1</sup> at sowing or by leaf spraying on the treatments.

According to Amado et al. (2010) the application of mineral N on the vegetative phase of soybean has been an alternative to complementation of BFN. The authors also relate that on the vegetative stages from V2 up to V5 the plants present higher demand of nutrients. Thus, a higher amount of nutrients are translocated to the grains, resulting in higher contents of proteins and weight on the seeds. However, the demand for nutrients on the reproductive stages, flowering to the fulfilling of grains, also continues high, fact that corroborate with this paper, which obtained significant increase on yield on treatments with N at V2 and R1.

#### **4 CONCLUSIONS**

The application of 10 kg ha<sup>-1</sup> of N at V2 by leaf spraying increases the plant height, the weight of a thousand seeds and the grain yield of soybean. The application of N at V2 and R1 stages by leaf spraying, as a complement to BFN, promotes increase on grain yield. The highest grain yield of soybean is obtained with N applied at V2 by leaf spraying.

**REFERENCES**

AMADO, T. J. C.; SCHLEINDWEIN, J. A.; FIORIN, J. E. Manejo do solo visando à obtenção de elevados rendimentos de soja sob sistema plantio direto. In: THOMAS, A. L.; COSTA, J. A. (Org.). Soja: manejo para alta produtividade de grãos. Porto Alegre: Evangraf, 2010.

ARGENTA, G.; SILVA, P.R.F.; BORTOLINI, C.G. Teor de clorofila na folha como indicador do nível de N em cereais. *Ciência Rural*, v. 31, n. 1, p. 715-722, 2001. DOI: <http://dx.doi.org/10.1590/S0103-84782001000400027>.

ALCANTARA, R. M. C. M.; REIS, V. M. Metabolismo do Carbono nos Nódulos. Seropédica: Embrapa Agrobiologia, 2008. (Documentos n. 253).

ALMEIDA, J. A. R. Eficiência da fixação biológica de nitrogênio na cultura da soja com aplicação de diferentes doses de molibdênio (Mo) e cobalto (Co). *Revista Trópica: Ciências Agrárias e Biológicas*, v. 5, n. 2 p. 17-24, 2011.

BAHRY, C. A.; VENSKE, E.; NARDINO, M.; FIN, S. S.; ZIMMER, P. D.; DE SOUZA, V. Q.; CARON, B. O. Características morfológicas e componentes de rendimento da soja submetida à adubação nitrogenada. *Agrarian*, v.6, n. 21, p. 281-288, 2013.

BRASIL. Ministério da Agricultura. Departamento Nacional de Produção Vegetal. Regras para análise de sementes. Brasília, 2009. 399p.

BERNIS D. J. E; VIANA O. H. Influência da aplicação de nitrogênio via foliar em diferentes estágios fenológicos da soja. *Revista Cultivando o Saber*, edição especial, p. 88-97. 2015.

BRITO, M. D. M. P.; MURAOKA, T.; SILVA, E. C. D. Contribuição da fixação biológica de nitrogênio, fertilizante nitrogenado e nitrogênio do solo no desenvolvimento de feijão e caupi. *Bragantia*, v.70, n.1, p.206-215, 2011. DOI: <http://dx.doi.org/10.1590/S0006-87052011000100027>.

EMBRAPA- EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA. Tecnologias de Produção de Soja: região central do Brasil 2012 e 2013. Londrina: Embrapa Soja, 2011. (Sistemas de Produção, n.15).

EMBRAPA- EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA (2013) Centro Nacional de Pesquisa de Solos. Sistema Brasileiro de Classificação de Solos. 3ª ed. Brasília, Embrapa, 353p.

FERREIRA, A. S., BALBINOT JUNIOR, A. A., WERNER, F., ZUCARELI, C., FRANCHINI, J. C.; DEBIASI, H. Plant density and mineral nitrogen fertilization influencing yield, yield components and concentration of oil and protein in soybean grains. *Bragantia*, v.75, n. 3, p. 362-370, 2016. DOI: <http://dx.doi.org/10.1590/1678-4499.479>.

FERREIRA, D. F. Sisvar: A computer statistical analysis system. *Ciência e Agrotecnologia*, v. 35, n. 1, p. 1039-1042, 2011. DOI: <http://dx.doi.org/10.1590/S1413-70542011000600001>.

FRANCHINI, J. C.; BALBINOT JUNIOR, A. A.; DEBIASI, H.; CONTE, O. Desempenho da soja em consequência de manejo de pastagem, época de dessecação e adubação nitrogenada. *Pesquisa Agropecuária Brasileira*, v.50, n.12, p.1131-1138, 2015. DOI: <http://dx.doi.org/10.1590/S0100-204X2015001200002>.

HUNGRIA, M.; CAMPO, R. J.; MENDES, I. C. A importância do processo de fixação biológica do nitrogênio para a cultura da soja: componente essencial para a competitividade do produto brasileiro. Londrina: Embrapa Soja, 2007. 82p. <<http://www.infoteca.cnptia.embrapa.br/handle/doc/468512>>. 20 Mai. 2016.

HUNGRIA, M.; FRANCHINI, J. C.; CAMPO, R. J.; CRISPINO, C. C.; MORAES, J. Z.; SIBALDELLI, R. N. R.; MENDES, I. C.; ARIHARA, J. Nitrogen nutrition of soybean in Brazil: contributions of biological N<sub>2</sub> fixation and of N fertilizer to grain yield. *Canadian Journal of Plant Science*, v.86, n.4, p.927-939, 2006. DOI: <http://dx.doi.org/10.4141/P05-098>.

MENDES, I. C.; REIS JUNIOR, F. B.; HUNGRIA, M.; SOUSA, D. M. G.; CAMPO, R. J. Adubação nitrogenada suplementar tardia em soja cultivada em latossolos do Cerrado. *Pesquisa Agropecuária Brasileira*, v. 43, n. 3, p. 1053-1060, 2008.

NOGUEIRA, P. D. M.; SENA JÚNIOR, D. G.; RAGAGNIN, V. A. Clorofila foliar e nodulação em soja adubada com nitrogênio em cobertura. *Global Science and Technology*, v. 3, p. 117-124, 2010.

PARENTE, T. DE L.; LAZARINI, E.; CAIONI, S.; PIVETTA, R. S.; SOUZA, L. G. M.; BOSSOLANI, J. W. Adubação nitrogenada em genótipos de soja associada à inoculação em semeadura direta no Cerrado. *Revista Brasileira de Ciências Agrárias*, v.10, n.2, p.249-255, 2015.

PETTER, F. A.; PACHECO, L. P.; NETO, F. A.; SANTOS, G. G. Respostas de cultivares de soja à adubação nitrogenada tardia em solos de cerrado. *Revista Caatinga*, v. 25, n.1, p. 67-72, 2012.

PIEROZAN, C.; FAVARIN, J. L.; DE ALMEIDA, R. E. M.; DE OLIVEIRA, S. M.; LAGO, B. C.; TRIVELIN, P. C. O. Uptake and allocation of nitrogen applied at low rates to soybean leaves. *Plant and Soil*, v. 39, n.1-2, p. 83-94, 2015.

SALES, P. V. G.; SALES, V. H. G.; PELUZIO, J. M.; AFFERRI, F. S.; SALES, A. C. R. C. Effect of pods' position on the protein content in soybean grains at low latitude. *Journal Bioenergy and Food Science*, v. 3, p. 216-221, 2016. DOI: <http://dx.doi.org/10.18067/jbfs.v3i4.102>.

SFREDO, G. J.; OLIVEIRA, M. C. N. de. Soja: molibdênio e cobalto. Londrina: Embrapa Soja, 2010. (Documentos/Embrapa Soja, ISSN 2176-2937; n. 322).

SILVA, A. F., CARVALHO, M. A. C., SCHONINGER, E. L., MONTEIRO, S., CAIONE, G.; SANTOS, P. A. Doses de inoculante e nitrogênio na semeadura da soja em área de primeiro cultivo. *Bioscience Journal*, v. 27, p. 404-412, 2011.

SOUZA, A. P.; MOTA, L. L.; ZAMADEI, T.; MARTIM, C. C.; ALMEIDA, F. T.; PAULINO, J. Classificação climática e balanço hídrico climatológico no estado de Mato Grosso. *Nativa*, v.1, p.34-43, 2013.

STEPHENS, B. D.; NEYRA, C. A. Nitrate e nitrite reduction in relation to nitrogenase activity in soybean nodules and *Rhizobium japonicum* bacteroids. *Plant Physiology*, vol. 71, p. 731-735, 1983. DOI: [http://dx.doi.org/0032-0889/83/71/731/05/\\$00.50/0](http://dx.doi.org/0032-0889/83/71/731/05/$00.50/0).

URANO, E. O. M.; KURIHARA, C.H.; MAEDA, S.; VITORINO, A. C. T.; GONÇALVES, M. C.; MARCHETTI, M. E. Determinação de teores ótimos de nutrientes em soja pelos métodos chance matemática, Sistema Integrado de Diagnose e Recomendação e Diagnose da Composição Nutricional. *Revista Brasileira de Ciência do Solo*, v. 31, p. 63-72, 2007. DOI: <http://dx.doi.org/10.1590/S0100-06832007000100007>.



VIEIRA NETO, S.A.; PIRES, F.R.; MENEZES, C.C.E.; MENEZES, J.F.S.; SILVA, A.G.; SILVA, G.P. & ASSIS, R.L. Formas de aplicação de inoculante e seus efeitos sobre a nodulação da soja. *Revista Brasileira de Ciência do Solo*, v. 32, p. 861-870, 2008. DOI: <http://dx.doi.org/10.1590/S0100-06832008000200040>.

WERNER, F.; BALBINOT JUNIOR, A. A.; FERREIRA, A. S.; SILVA, M. A. A.; DEBIASI, H. & FRANCHINI, J. C. Soybean growth affected by seeding rate and mineral nitrogen. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v.20, n.8, p.734-738, 2016. DOI: <http://dx.doi.org/10.1590/1807-1929/agriambi.v20n8p734-738>.

ZILLI, J. E. ; GIANLUPPI, V.; CAMPO, R. J.; ROUWS, J. R. C.; HUNGRIA, M. Inoculação da soja com *Bradyrhizobium* no sulco de semeadura alternativamente a inoculação de sementes. *Revista Brasileira de Ciências do Solo*, v. 34, p. 1875-1881, 2010. DOI: <http://dx.doi.org/10.1590/S0100-06832010000600011>.