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Application of nitrogen fertilizer in high-demand stages of soybean and its effects on yield performance

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Abstract

The typical nitrogen supply recommendation for soybeans is the application of inoculant with no additional required supplementation via fertilization. However, the adoption of no-till farming, the release of high-yielding cultivars and recent studies concerning soybean response to late application of nitrogen have sparked doubts about the possible benefits of nitrogen fertilization. Recent studies using nitrogen fertilization of soybeans show no increase in yield, due to efficient biological fixation (BNF); however, the effects of different application times have not been widely studied. In this context, the aim of this study was to investigate different methods and times of nitrogen fertilization of soybeans. Using the recommended supplements, along with a search of the scientific literature on the same theme, nine treatments were evaluated. These consisted of the application of single and split doses of nitrogen, at 20 kg.ha^{-1} and 40 kg.ha^{-1}, during sowing and reproductive stage R4. The nitrogen source used was urea (45% nitrogen). It was applied in adequate environmental conditions, at levels typically found in rain forecasts, and were incorporated into the soil to avoid losses by volatilization. The split application of nitrogen (at sowing + stage R4) provided an increase in yield, reaching a 47% difference in treatment 6 in relation to control, not taking into account supplementation costs. Furthermore, the variables related to plant architecture displayed no significant differences.

Keywords: *Glycine max*, topdressing, nitrogen supplementation.

Introduction

Soybean (*Glycine max* L.) is a nodulating plant. In addition to physical anchorage functions, absorption of water and mineral elements in solution, roots can establish a symbiotic relationship with bacterial species capable of fixing molecular nitrogen (N_2) from the soil and air (Vilalba et al., 2014). Biological nitrogen fixation (BNF) is a process in which nitrogen is captured from the atmosphere, and is converted into nitrogen compounds such as amino acids and allantoic acid (ureide) (Unkovicht, 2008). The current recommendation for soybean cultivation is the use of inoculants without supplementation with nitrogen fertilizers. Hungria et al. (1997) and Mendes et al. (2003) stated that nitrogen supplementation at sowing, as a boost to soybean growth, is unnecessary, in both conventional and no-till farming systems. According to Embrapa (2001), the application of nitrogen fertilizer at sowing or topdressing in any stage of the soybean cycle, whether in an unconventional or no-till farming system, reduces nodulation and the efficiency of biological nitrogen fixation. Nevertheless, since topdressing fertilizer formulation containing nitrogen ischeaper than nitrogen-free formulations, it may be applied, assuring that up to 20 kg N ha^{-1} is supplied. However, considering the advances in no-till farming systems, release of high-yielding cultivars, and the results of studies evaluating the soybean response to nitrogen supplementation at sowing (Starling et al., 1998 Starling et al., 2000; Taylor et al., 2005); high doses of N(Ray et al., 2006),the need for nitrogen supplementation through fertilizers in the late pre-blooming and early grain filling stages (Brevedan et al., 1978; Parente, 2014; Mendes et al., 2008;. Wesley et al., 1998) is puzzling researchers (Lamond and Wesley, 2001). The extra supplementation could be filling the crop need for nitrogen, or providing nitrogen in high-demand stages, when biological nitrogen fixation is not sufficient. The first root nodules on soybean are initiated from infections in the primary root and its branches. Since the plant demands reduced doses of nitrogen, the nodules are small and few in the early growth stages. It should be pointed out that during these early stages, a nitrogen deficiency may be present due to N immobilization (Nunes et al. 2003), particularly in the succession of grasses (Vargas et al., 2005) and reduced BNF at this stage. In addition, to attain good nodulation, the initial availability of N in the soil cannot be too high or too low(Eaglesham et al., 1983). In the early development stages of the soybean cycle, the supply of nitrogen can satisfy crop demand without affecting nodulation, especially in soils with nitrogen deficiency and/or areas previously cultivated with grasses, since it is applied in reduced doses up to 20 kg ha^{-1} of N (Embrapa, 2011). From the expansion of the 3rd or 4th trefoil (V4 to V5), there is an increase in dry mass and the number of nodes. Also, the intensification of BNF arises in dry matter of the shoots and roots. The BNF reaches its first peak in full blooming (stages R1 and R2), and then decays due to the change of source-drain. The second peak of the BNF occurs during grain filling (R5 stage) (Camara, 2000).

In the subsequent stages to flowering, the soybean demand for nitrogen increases, as a consequence of nitrogen accumulation in the grains (Watanabe et al., 1986). However, alongside it, a reduction in BNF occurs due to competition for photoassimilates between the plant and rhizobia (Zapata et al., 1987; Shibles, 1998).

The N is redistributed from leaves to grains, reducing chlorophyll and rubisco content, resulting in photosynthesis reduction. This process is known as "programmed senescence", and limits soybean yield (Sinclair and De Wit, 1975). Thus, nitrogen supplementation between the BNF peak and its low may be necessary (Pausch et al. 1996). Nitrogen fixation by leguminous plants is a process with several interactions between plant and bacteria, in which elevated concentrations of N in the soil can inhibit the symbiotic process. Thus, levels of nitrogen that undermine this process are unwanted. Yinbo et al. (1997) and Gan et al. (2002) reported that the application of nitrogen during the period of high demand by the crop did not affect BNF, and an increase in yield was observed.

Numerous studies have investigated nitrogen fertilizer in soybean culture. Indeed, most studies do not present an increase in yield, e.g. Aratani et al. (2008), Bahry (2011), Ham et al. (1978), Koutroubas et al. (1998), Schmitt et al. (2001), Bodrero et al. (2004). However, the effects of different application times, and especially the combination of N application in stages of high demand or low BNF, have not been widely studied.

In this context, the aim of this study was to evaluate the effects of low and medium levels of nitrogen fertilization with respect to soybean culture, applied at different times, in two locations under no-till farming.

Results and Discussion

Experiment I

No statistical difference was observed between treatments for height, number of seeds per pod (two and three seeds) or total number of pods (Table 1). Aratani et al. (2008) and Bahry (2011), studying the effects of different rates and times of N application, both in vegetative and reproductive stages, in a no-till farming system found no differences for the same variables, as the crop demand was met. However, regarding yield and the hundred grains mass (HGM), significant statistical differences were observed between treatments (Table 2). The treatmentsT6 (20 kg ha^{-1} at sowing + 20 kg ha^{-1} in R4), T8 (40 kg ha^{-1} at sowing + 20 kg ha^{-1} in R4), T9 (40 kg ha^{-1} at sowing + 40 kg ha^{-1} in R4) and particularly treatment 7 (20 kg ha^{-1} at sowing + 40 kg ha^{-1} in R4) had the highest averages of HGW and yield. Similar results were observed by Parente (2014); even though there was an inoculation at sowing, the rates used were similar, but with shorter intervals (0,10,20,40 kg ha^{-1}), and there were only two application times (sowing, start of blooming). The treatments with a combination of rates and times provided the highest means. For example, the treatments T3 and T5, consisting of 40 kg ha^{-1} of N applied at sowing and R4, respectively, had a lower average than T6 (40 kg ha^{-1} split at sowing and R4). Therefore, the split application was more effective than the same rate applied once. Treatment T7 (20 kg ha^{-1} at sowing + 40 kg ha^{-1} in R4) had the highest average, even higher than other treatments with split nitrogen application. We observed, therefore, that treatments T8 and T9, with the application of 40 kg ha^{-1} at sowing, were not as effective as the application of 20 kg ha^{-1} of nitrogen at sowing, as compared toT7. This might mean that the N rate of 40 kg ha^{-1} at sowing could have restricted BNF, while the N rate of 20 kg ha^{-1} did not.

Another observation is that T7, with 40 kg ha^{-1} in R4, provided greater yield than T6, with 20 kg ha^{-1} of nitrogen. This suggests that supplementation of 40 kg ha^{-1} of N in R4 is better than 20 kg ha^{-1}.

Experiment II

In experiment II, the statistical analysis for height and number of pods showed no difference (Table 1), as in experiment I. These results corroborate with those obtained by Costa et al. (2011), in which the nitrogen rates (0,80,160,240 kg ha^{-1}) and application times (sowing and early blooming) did not affect the same variables, as the inoculation met crop demand and the nitrogen overdose applied had negative effects onthe culture.

The analysis of HGW and yield were also statistically different for a second time (Table 2). The treatments T7 (20 kg ha^{-1} sowing + 40 kg ha^{-1} R4) and especially T6 (20 kg ha^{-1} + 20 kg ha^{1}) provided the greatest averages, as in experiment I. However, contrasting experiment I, these treatments did not differ statistically, which suggests equivalence when applying 20 or 40 kg $ha^{-1}$of nitrogen in the R4 stage.

In experiment II, the application of split doses of nitrogen fertilizer delivered the best results. Among the treatments with split fertilization (sowing and R4 stage), those with a sowing rate of 40 kg ha^{-1} of nitrogen (T8 and T9) had a lower yield than treatments with 20 kg ha^{-1} (T6 and T7). Once again, this indicates that nitrogen supply at sowing can damage BNF.

The mineral forms of nitrogen, NO_3^- and NH_4^+, present in the soil affect BNF, inhibiting nodule formation and/or causing senescence of nodules already formed (Bottomley and Myrold, 2007). Consequently, the single as well as split fertilizations with 40 kg ha^{-1} at sowing damaged BNF, and resulted in insufficient nitrogen uptake, triggering reduced yields (Fancelli, 2012).

In contrast with this study, Hungary et al. (2006) found that the application of nitrogen fertilizer at sowing, associated with topdressing fertilization in stages R2 and R4, reduced nodule weight, resulting in less biologically fixated nitrogen, and lower yield. These results might be a consequence of

Table 1. Averages obtained for height and number of pods (R7).

Experiment 1					Experiment 2			
Treatments	Height	Pods R7	Pods R7	Pods R7	Height	Pods R7	Pods R7	Pods R7
Sowing+R4	(cm)	2 grains	3 grains	totaly	(cm)	2 grains	3 grains	Totaly
Sowing 0 $kgha^{-1}$	93.7 a	10.66 a	57.33 a	68.00 a	54.1 a	10.50 a	23.33 a	33.63 a
Sowing 20 $kgha^{-1}$	96.7 a	8.83 a	60.83 a	69.66 a	54.5 a	9.25 a	24.16 a	33.41 a
Sowing 40 $kgha^{-1}$	96.5 a	11.66 a	65.16 a	76.33 a	55.3 a	10.33 a	24.66 a	35.00 a
20 $kgha^{-1}$	95.1 a	10.08 a	64.66 a	74.75 a	55.6 a	11.66 a	23.66 a	35.33 a
40 $kgha^{-1}$	98.1* a	11.50 a	63.75 a	75.25 a	56.6 a	12.16* a	22.00 a	34.16 a
20 $kgha^{-1}$ + 20 $kgha^{-1}$	97.1 a	12.50 a	71.50* a	84.00* a	56.5 a	11.16 a	24.50 a	35.66 a
20 $kgha^{-1}$ + 40 $kgha^{-1}$	94.3 a	11.83 a	63.83 a	75.66 a	56.3 a	11.33 a	26.66* a	38.00* a
40 $kgha^{-1}$ + 20 $kgha^{-1}$	92.6 a	14.16* a	59.33 a	73.50 a	57.3* a	10.66 a	25.33 a	36.00 a
40 $kgha^{-1}$ + 40 $kgha^{-1}$	91.6 a	12.16 a	59.33 a	71.50 a	56.6 a	9.16 a	25.66 a	34.83 a
Pr>F (Test F)	11.15	578	24.88	26.27	5.12	4.08	8.06	26.27
C.V. (%)	619	26.74	20.92	18.68	484	20.16	17.44	14.60
Mean	95.14	11.43	62.86	74.30	55.92	10.70	24.44	35.14

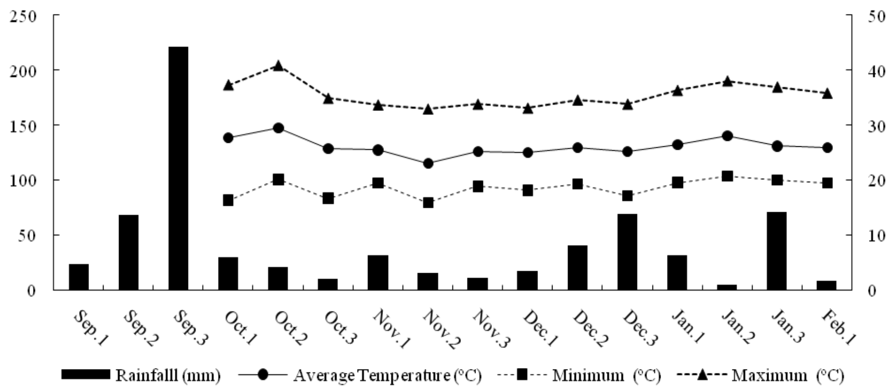

Fig 1. Rainfall and air temperature in the 2014/2015 harvest

Table 2. Averages obtained for yield and mass of a hundred grains.

Treatments	Experiment 1		Experiment 2	
(Seeding +R4)	Yield (kg ha^{-1})	Mass of 100 grains (g)	Yield (kg ha^{-1})	Mass of 100 grains (g)
0 $kgha^{-1}$	2471 e	17.28 e	1876 e	13.14 e
20 $kgha^{-1}$	2525 e	18.20 de	2221 d	13.45 d
40 $kgha^{-1}$	2739 d	18.54 cde	2302 d	13.53 d
20 $kgha^{-1}$	2708 d	18.27 de	2591 c	13.65 cd
40 $kgha^{-1}$	2899 c	18.56 cde	2805 de	13.82 bc
20 $kgha^{-1}$ + 20 $kgha^{-1}$	3172 c	18.54 cde	3364* a	14.06* a
20 $kgha^{-1}$ + 40 $kgha^{-1}$	3368* a	18.94* a	3195 cd	13.97 cd
40 $kgha^{-1}$ + 20 $kgha^{-1}$	3173 c	18.69 de	2995 de	13.97 cd
40 $kgha^{-1}$ + 40 $kgha^{-1}$	3072 c	18.85 b	2917 c	13.94 cd
Pr>F (Test F)	136.91	0.42	228.73	0.236
C.V. (%)	2.49	1.20	4.48	0.91
Mean	2903.52	18.43	2696.71	13.73

Table 3. Results of soil analysis.

Area 1										
C	P	K^+	Ca^{2+}	Mg^{2+}	$H^+ + Al^{3+}$	Al^{3+}	SB	T	V	pH
g dm^{-3}	mg dm^{-3}	------------------------ $cmol_c dm^{-3}$ ------------------------							%	$CaCl_2$
12.38	7.40	0.35	3.43	0.95	3.69	0.0	4.73	8.42	65.18	5.10
Area 2										
C	P	K^+	Ca^{2+}	Mg^{2+}	$H^+ + Al^{3+}$	Al^{3+}	SB	T	V	pH
g dm^{-3}	mg dm^{-3}	------------------------ $cmol_c dm^{-3}$ ------------------------							%	$CaCl_2$
12.99	12.30	0.15	2.21	0.41	3.18	0.00	2.77	5.95	56.55	5.00

Table 4. Treatments used in experiments I and II. Varying rates and nitrogen application time in soybeans. Harvest of 2014/2015 - Palotina / Pr.

Treatments	Rates(N $kgha^{-1}$)	Aplication Time
T1	0 $kgha^{-1}$	Attestant
T2	20 $kgha^{-1}$	Sowing
T3	40 $kgha^{-1}$	Sowing
T4	20 $kgha^{-1}$	R4
T5	40 $kgha^{-1}$	R4
T6	20 $kgha^{-1}$ + 20 $kgha^{-1}$	Sowing + R4
T7	20 $kgha^{-1}$ + 40 $kgha^{-1}$	Sowing + R4
T8	40 $kgha^{-1}$ + 20 $kgha^{-1}$	Sowing + R4
T9	40 $kgha^{-1}$ + 40 $kgha^{-1}$	Sowing + R4

the high doses of nitrogen employed in the study: 200 kg ha^{-1} of nitrogen split in two application times (half in sowing and half in the R2 stage). In the same study, the authors evaluated the effects of single application of 30 and 50 kg ha^{-1} of nitrogen fertilizer at sowing and phenological stages R2 and R4, and observed an inhibition of nitrogen fixation.

Therefore, we can determine the best rates of mineral nitrogen fertilizer to supply crop demand in critical stages. First, nitrogen fertilization at sowing supplies early soybean growth demands, once there is a delay in establishment of the symbiotic relationship between plant and nitrogen-fixing bacteria (Fancelli, 2012).However, supplementation in phenological stage R4 is strategic, because the upcoming begging seed stage promotes translocation of nutrients and water to the seeds (Oliveira et al. 2013), requiring the plant to absorb more nitrogen. So, in either stage, nitrogen supplementation is required, but fertilizations over 40 kg of N/ha can compromise and somehow inhibit the symbiotic relationship with nitrogen-fixing bacteria and should be avoided.

We can also relate these results with nodulation variation during the soybean phenological cycle, as reported by Hall (2014). Nodulation starts after sowing, with a certain delay, and reaches a peak in early reproductive stages. Later in R2 stages, nodulation plummets and is maintained untilR5. Therefore, the supplementary nitrogen fertilization with urea employed in this study is in accordance with the soybean high-demand stages.

The results of this study indicate that the application of mineral nitrogen, especially at sowing, in doses over 20kg ha^{-1}, can inhibit biological nitrogen fixation. We can also affirm that the split application supplemented plant demand for nitrogen, as observed by an increase in yield, but more studies are required to properly confirm this statement.

Considering this a current, controversial topic, with commercial interests, it is not appropriate to claim that positive results were attained without losses to BNF. However, the big picture is that nitrogen fertilization in adequate times and rates may positively affect soybean yield and profitability.

Materials and methods

Crop description

The study was carried out in two sites located in Linha Palmital, City of Palotina, in Western Paraná. The geographical coordinates of experiment site I are Latitude24^{0} 15’ 26’’, and Longitude 53^{0} 55’ 40’’, with an altitude of 345 m, while experimental site II is located at Latitude 24^{0} 13’ 30’’, Longitude 53^{0} 55’ 60’’ and altitude 336 m. The soil was classified as Eutroferric Red Oxisol for both sites.

Although the experimental sites are closely located, they present chemically different characteristics. Experimental site soil analyses are displayed in Table 3. Maximum and minimum air temperature and rainfall data were collected daily throughout the experiment. The climate information is depicted in 10-day periods in Figure 1. The same figure depicts all information collected during the soybean cycle in experiment I, from sowing, on September 23rd, 2014, through topdressing nitrogen fertilization on November 30th, until harvest on January 14th, 2015. The sowing, topdressing fertilization and harvest in experimental site II happened on October 6th, December 12th, 2014, and January 2nd, 2015, respectively.

Plant materials

The cultivars sowed in experiment I were MONSOY 6210 IPRO, either with indeterminate growth habit and short cycle (maturity group 6.2). In experiment II, the conventional soybean cultivar BRS 283 was picked up, which also presents indeterminate growth habit and a short cycle (maturity group 6.5). In both experimental sites, the space between rows was 0.5 meters, resulting in a population of 320,000 plants per hectare. The fertilization during sowing consisted of 270 kg ha^{-1} of the formulation 00-20-20 (N-P-K), totaling 54 kg of both K_2O and P_2O_5.

Experimental design

The randomized block design was employed with six repetitions. The plots had six five-meter-long lines, spaced 50 centimeters between rows. The treatments consisted of the application of different nitrogen rates during sowing and/or pod formation (R4 stage), as shown in Table 4. The applications occurred in appropriate environmental conditions, reflecting those of a rain forecast, which is associated with fertilizer incorporation into the soil, preventing losses by volatilization. The application of nitrogen fertilizer in Experiment I at sowing was made on 09/23/2014, and the application in the R4 stage on 11/30/2014. In experiment II, the dates for sowing andR4 stage application were 06/10/2011and 12/12/2014, respectively.

In both experiments the plant height, number of pods (considering pods with two and three grains), yield and mass of 100 grains were evaluated.

The evaluations were performed at the full maturity stage (R8). The measurement of plant height and number of pods was performed on five randomly selected plants in the useful area of each plot. The yield evaluation consisted of harvesting two linear meters in the useful area of the plot, which accounted for around 30 plants. Grain samples of these plants were used to estimate the hundred grain weight (HGW). Yield and HGW in each plot were standardized at 13% grain moisture.

Results were submitted to normality and homogeneity test of variances, and later to variance analysis by F-test at a 5% probability using Statistical Analysis System Windows 9.2 version (SAS Inst., 2008). If rejecting the null hypothesis, mean comparisons were performed using the Tukey test $p \leq 0.05$ for the times and sites, and regression analysis for the rates.

Conclusion

Nitrogen fertilization times and rates did not affect structural characteristics of plants, such as plant height and number of pods. However, in relation to the hundred grains weight and yield, nitrogen fertilization in two stages of high demand, with doses up to 20 kg ha-1, saw significant increases in both areas and with both cultivars, being a little more evident for the transgenic cultivate, which is considered more modern. Thus, the results indicate that a reduction in fertilizer application may be beneficial for a crop; however, more specific studies should be conducted to quantify the effects on BNF.

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