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Defoliation and nitrogen fertilization on the physiological quality of soybean seeds[1]

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ABSTRACT - Stress caused by defoliation can compromise the physiological quality of soybean seeds. However, there is evidence that the application of nitrogen (N) can be an alternative to reduce the qualitative losses in seeds caused by defoliating insects. The objective of this study was to evaluate the effectiveness of topdressing N fertilization in improving the physiological quality of soybean seeds from plants subjected to artificial defoliation. The experimental design was a randomized block arranged in a 2×4 factorial scheme with four replicates. The treatments consisted of two levels of defoliation (33 and 66%) and four doses of N (0, 50, 100, and 150 kg ha^{-1}), using urea (45% N), applied two days after defoliation. Artificial defoliation was performed during the R_3 stage by removing one (33%) and two leaflets (66%) from each trifoliate leaf under field conditions. At stage $R_{5.1}$, leaf area was quantified, and after harvesting, germination, emergence, length, total dry mass, electrical conductivity, total protein content, mass of 1000 seeds, and tetrazolium (vigor and viability) tests were carried out. It was concluded that increasing the defoliation from 33 to 66% impaired the physiological quality of soybean seeds. The use of nitrogen fertilization for coverage after defoliation at the beginning of the reproductive phase cannot minimize qualitative losses in seeds.

Key words: *Glycine max* L. Merrill. Vigor. Germination. Nitrogen. Photosynthetic area.

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INTRODUCTION

Despite the economic importance of the soybean crop (*Glycine max* L. Merrill), seed productivity and quality are limited by several biotic and abiotic factors. The maximum crop yield is determined by the ability of plants to intercept solar radiation through the leaf area index (LAI) and convert this light radiation into dry matter through photosynthesis (ZANON *et al*., 2015).

Defoliation can reduce transpiration, photosynthesis, and the ability of plants to compensate for nutritional deficiencies, water loss, and other abiotic factors that can influence plant production (OWEN *et al*., 2013). However, if defoliation is not drastic, the plant is still capable of performing sufficient photosynthesis to guarantee energy production; therefore, productivity is not affected.

Under field conditions, insects and defoliating mites can cause stress to plants, reducing the photosynthetically active area, and consequently compromising productivity (BAHRY *et al*., 2013; SANGOI *et al*., 2014). Some studies have reported a reduction in soybean productivity caused by defoliation (CERA *et al*., 2016; GOBBI; CASIMIRO, 2019; SANTOS *et al*., 2018); whereas others have claimed that it has no influence on grain yield (ALVES; BELLETTINI; BELLETTINI, 2020; SOUZA *et al*., 2014). However, no studies have been conducted on the influence of defoliation on the physiological quality of soybean seeds.

However, the use of nitrogen fertilization in coverage can be a management strategy aimed at reducing qualitative losses in seeds caused by defoliating insects. Nitrogen (N) stands out for its importance in plant metabolism and controlling plant development because of its participation as a constituent of molecules such as proteins, coenzymes, nucleic acids, and other enzymes (PRADO, 2008). Thus, adequate plant nutrition is mainly reflected in the size and weight of the seeds (MARCOS-FILHO, 2015) as well as the formation of the embryo and reserve organs to obtain seeds with better physiological and sanitary qualities. However, studies have shown that the application of nitrogen fertilizers does not improve grain yield or protein content (KORBER *et al*., 2017; ZUFFO *et al*., 2018, 2019, 2020, 2021).

The amount of N fixed by soybeans is not sufficient to replace the N exported from the field by seeds, or is close to neutral if N in the soil is included (SALVAGIOTTI *et al*., 2008). In contrast, defoliation causes a reduction in the leaf area and inhibits the production of photoassimilates, as photosynthesis occurs in this structure. N promotes root formation, photosynthesis, production, and translocation of photoassimilates and increases the growth rate between leaves and roots (TAIZ *et al*., 2017). Thus, nitrogen in coverage can compensate for the damage caused by defoliation during the reproductive stage of soybean plants by maintaining or increasing the physiological quality of seeds.

Therefore, the objective of this study was to evaluate whether nitrogen fertilization in coverage benefits the physiological quality of soybean seeds from plants with different levels of artificial defoliation.

MATERIAL AND METHODS

The experimental design was a randomized block arranged in a 2 × 4 factorial scheme with four replicates. The treatments consisted of two levels of defoliation (33 and 66%) and four doses of N (0, 50, 100, and 150 kg ha^{-1}), using urea (45% N), applied two days after defoliation. Defoliation was performed at stage R3 (legume, 5 mm knife) with scissors, removing one leaflet (33%) or two leaflets (66%) from each trifoliate leaf of the plants under field conditions. Each experimental unit consisted of three rows spaced 0.45 m apart and 5 m long, totaling 6.75 m^2. The central line was considered, with 1.0 m discarded from each end, making up an area of 1.35 m^2.

The experiment was carried out in the experimental area of the Federal University of Mato Grosso do Sul, in Chapadão do Sul, northern region of the state of Mato Grosso do Sul (MS), Brazil (18°46'17.9 South; 52°37'25,0" West and average altitude of 810 m), during the 2018/2019 harvest. The region's climate, according to the Koppen classification, is tropical rainy (Aw), with rainy summers and dry winters, and with a precipitation, average temperature, and annual relative humidity of 1,261 mm, 23.97 °C, and 64.23%, respectively. The precipitation data for the experiments are shown in Figure 1.

The soil in the experimental area was classified as oxisol based on the Brazilian Soil Classification System (SANTOS, 2018). Before starting the experiment, the soil was sampled in the 0–0.2 m layer, and the main chemical properties are shown in Table 1.

Soil acidity correction was carried out with the surface application of limestone (CaO:29%; MgO:20%; PRNT:90.1%; PN:101.5%) to increase the base saturation of the soil to 60%. Liming was performed 60 days before the implementation of the experiment (0.4 kg ha^{-1}).

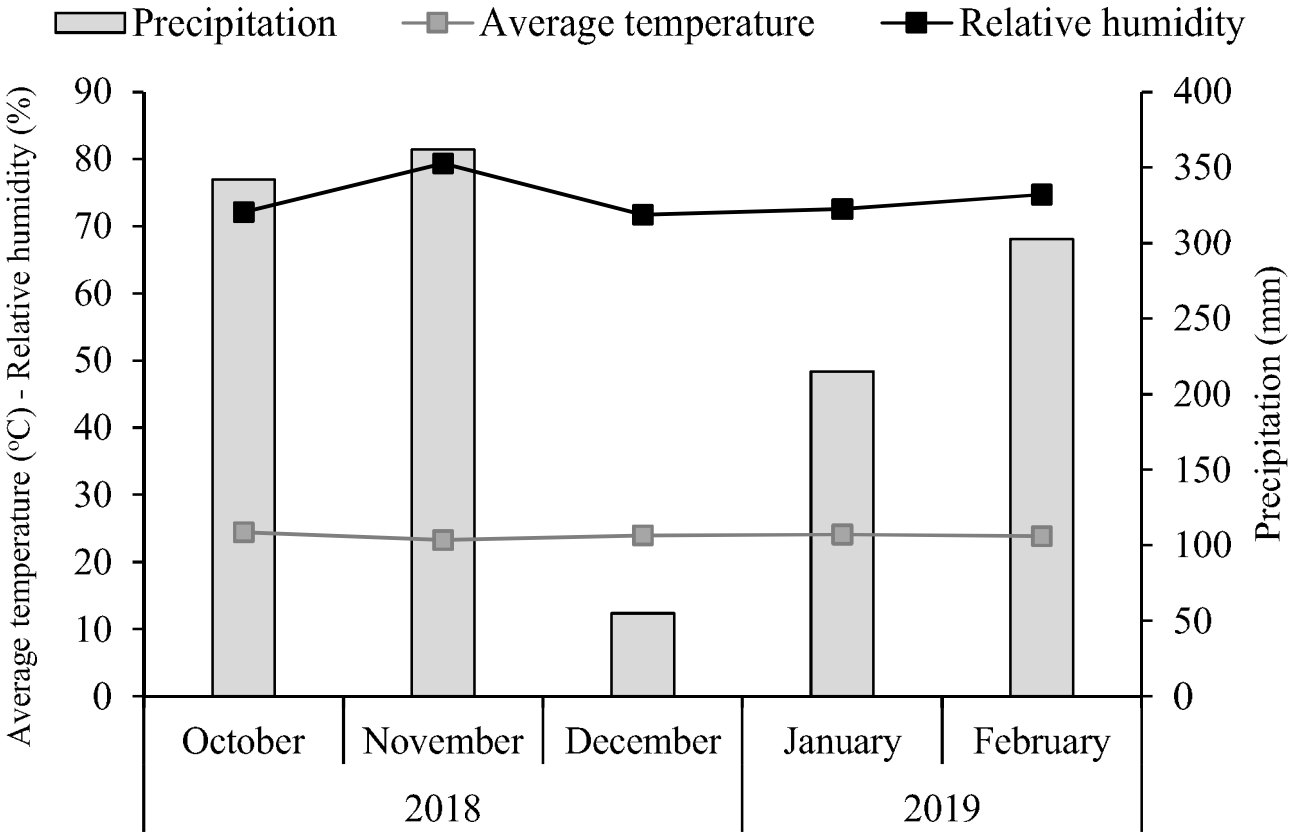
The area was prepared by desiccation using glyphosate (720 g ha^{-1} i. a.) + haloxyfop-P-methyl (63 g ha^{-1} i. a.) and the experiment was conducted after 10 d in a no-tillage system (SPD). The soybean cultivar used was BRASMAX BONÛS IPRO (indeterminate growth habit, cycle of 114 to 121 days, maturation group 7.9), mechanically sown on October 4, 2018, by means of a seeder-fertilizer, with

furrower mechanism rod type (machete), for SPD, at a depth of approximately 3.0 cm, with a spacing of 0.45 cm and 13 seeds per meter, to reach a final stand of 240,000 to 280,000 plants per hectare.

The base fertilization consisted of 150 kg ha^{-1} MAP (11% ammonia-N and 52% P2O5). In the topdressing fertilization, 100 kg ha^{-1} of K2O was used, whose source was potassium chloride 40 days after emergence, at which time the application of foliar fertilization of Actilase ZM products (Zn 50, 22 g L^{-1}; S 41.65 g L^{-1}; Mn 30.01 g L^{-1}) and Racine (Mo 108.75 g L^{-1}; Co 10.88 g L^{-1}; Total carbon 123.25 g L^{-1}) at doses of 1 L ha^{-1} and 120 mL ha^{-1}, respectively.

Soybean seeds were treated with pyraclostrobin + methyl thiophanate + fipronil at a dose of 2 mL p.c. kg^{-1} and were inoculated with Bradyrhizobium japonicum, using a commercial liquid inoculant containing SEMIA 5079 and SEMIA 5080 strains (minimum concentration of 7.2 x 109 viable cells per mL), at a dose of 150 mL for 50 kg of seeds. The inoculant was dissolved in a solution containing 2 ml p.c. kg^{-1} of Protege®TS seeds. To enhance soybean nodulation, the seeds also received micronutrients using commercial fertilizer containing Mo (10%), Co (1%), S (1%), Ca (1%), and Fe (0.2%) at a dose of 120 mL per ha^{-1}.

Figure 1 - Monthly averages of temperature, relative humidity, and accumulated rainfall occurred in Chapadão do Sul-MS in the 2018/19 harvest, during the soybean cycle



Source: National Institute of Meteorology (INMET)

Table 1 - Main chemical properties of the soil used in the experiment

pH	OM	$P_{Mehlich}^{-1}$	H+Al	Al^{3+}	Ca^{2+}	Mg^{2+}	K^{+}	CEC	V
$CaCl_2$	g dm^{-3}	mg dm^{-3}	---------- $cmol_c$ dm^{-3} ----------						%
4.3	22.8	12.8	5.7	0.37	2.20	0.40	0.27	8.6	33.5

OM: Organic matter. CEC: Cation exchange capacity at pH 7.0. V: Base saturation

For the management of weeds in the soybean crop, glyphosate herbicides were applied, using the commercial product Roundup Ready® (480 i.a. g L^{-1}), at a dose of 2 L ha-1, and haloxyfop-p-methyl (120 i.a. g L^{-1}) at a dose of 0.5 L ha^{-1} of the commercial product Verdict R® at pre-sowing (10 days before sowing) and post-emergence (30 days after sowing).

For disease control, preventive fungicide applications were carried out after the R2 stage (full flowering) at intervals of 15 days, totaling three applications. The fungicides used were mancozeb (750 g kg^{-1}) from the commercial product Mancozeb Nortox®, at a dose of 3 kg ha^{-1}, pyraclostrobin (100 g L^{-1}), and benzovindiflupyr (50 g L^{-1}) at a dose of 0,9 L of the commercial product Verssarya® per hectare, and azoxystrobin (200 g L^{-1}) + cyproconazole (80 g L^{-1}) at a dose of 300 mL of the commercial product Priori Xtra® per hectare at a volume of 200 L ha^{-1}.

Pest control was performed when necessary using the insecticide imidacloprid (100 g L^{-1}) + beta-cyfluthrin (12.5 g L^{-1}), at a dose of 1 L ha^{-1} of the commercial product Connect®, with a spray volume was 200 L ha^{-1}, and the contact insecticides cypermethrin (250 g L^{-1}) from the commercial product Cypermethrin Nortox® 250 EC and chlorpyrifos (480 g L^{-1}) from the commercial product Lorsban® 480 BR at dosages of 120 and 250 mL ha^{-1}, respectively.

At stage R5.1, five plants were collected per plot and the leaf area (cm^2) was evaluated using an electronic leaf area device model Li-Cor, L1-3100®. Harvesting was performed manually (stage R8) by mechanical threshing using a combined Wintersteiger Classic plot. Subsequently, the seeds were stored in a cold chamber at 17 ± 1 ºC for 30 days and, after, the weight of 1,000 seeds was determined (BRASIL, 2009), as well as the crude protein content using the Kjeldahl method. To determine the water content (BRASIL, 2009) and carry out physiological quality tests, the seeds were passed through sieves with round holes (hole diameter of 5.5 mm) to standardize the size.

In the germination test, four replicates of 50 seeds were equally distributed on three paper towels moistened with distilled water at a proportion of 2.5 × the mass of the dry substrate. The blotting paper sheets were rolled up together with the seeds and placed in plastic bags at 25 ± 1 ºC. The evaluation was carried out eight days after the installation of the test and the results obtained were expressed as a percentage of normal seedlings (BRASIL, 2009).

Emergence was conducted with four replications of 25 seeds for each treatment, sown in Styrofoam trays 2 cm deep, containing Click® commercial substrate. Wetting was performed daily, and the evaluation was conducted on the tenth day after the installation of the test. The emergence results were expressed as a percentage of the number of normal seedlings that emerged.

Seedling length was measured with four repetitions of 20 seeds distributed on a line drawn in the upper third of the paper towel moistened with distilled water at a proportion of 2.5 × the mass of the dry substrate. The paper towel sheets were rolled up together with the seeds, packed in plastic bags, and placed in a germinator at 25 ± 2 ºC for five days in the dark. Then, with the aid of a millimeter ruler, the total length of the normal seedlings was measured and the results were expressed in centimeters (cm $seedling^{-1}$).

After evaluating the normal seedlings in the total seedling length test, the plant material was packed in kraft paper bags and placed inside a forced air circulation oven at 65 °C for 72 h for the determination of dry mass. Soon after, the material was weighed on an analytical scale and the results were expressed in grams (g $seedling^{-1}$).

For the electrical conductivity test, four replicates of 25 seeds for each treatment were placed in 200 mL plastic cups and weighed on an analytical scale; subsequently, 75 mL of distilled water was added to each container and placed in a germination chamber at 25 ± 2 ºC for 24 h. Then, the solution was stirred and the electrical conductivity was measured using a conductivity meter. The results were expressed in μS cm^{-1} g^{-1}.

The tetrazolium test was performed using two replicates of 50 seeds per treatment. Seed preconditioning was performed in paper towel envelopes previously moistened at 25 ºC for 16 h. Subsequently, they were transferred to plastic cups with a 2,3,5-triphenyl-tetrazolium solution and colored in a dark chamber at 35 ºC for 3 h. After this process, the seeds were washed under running water, and each seed was examined individually to determine the location and extent of internal and/or external damage, cut longitudinally along the embryonic axis, and the coating was removed. The vigor and viability results are expressed as percentages (FRANÇA-NETO *et al*., 1998).

The results were subjected to analysis of variance. Defoliation levels were compared using Fisher-Snedecor's F test and N doses were compared using polynomial regression, both at a 5% probability.

RESULTS AND DISCUSSION

The 66% defoliation reduced the leaf area by 33%, even 15 days after the application of this treatment. According to Taiz *et al*. (2017), in leaves, chlorophyll carries out photosynthesis and produces photoassimilates. Thus, reducing the number of leaves

due to defoliation culminated in a reduction in the production of phototassimilates and, therefore, in the formation of new leaves. However, different N doses did not affect this variable.

The water content of the seeds between treatments ranged from 8.6 to 11.3%, which is within the range recommended by Marcos-Filho (2015), which is a maximum of 3%. This factor is important for the execution of the tests, and the uniformity of the water content of the seed is essential for obtaining consistent results.

In Table 2 it was verified that the results of germination, emergence, length, and total dry mass were significant only for defoliation. Defoliation at 33% obtained better results for the analyzed variables, and the application of N was not efficient in compensating for the damages caused by this practice, and the results were not significant (Table 2).

Greater defoliation damage causes a lower photosynthetic rate and translocation to the drains, resulting in reduced germination, emergence, length, and total dry mass of soybean seedlings. When the photosynthetic area of a plant is reduced during the reproductive stage, the availability of photoassimilates for seed filling decreases. In addition, the energy expenditure for the plant to regrow, form pods, and fill them is not met by nitrogen fertilization, generating seeds with a low capacity to emerge and resist stress in the growth and development of seedlings (DAN *et al*., 2010).

The results prove that the correct use of inoculants with bacteria that fix N, together with the application of Co and Mo in the seeds or leaves, promotes adequate N nutrition without the need to apply mineral N. Thus, only the N fixed by *Bradyrhizobium*-soybean symbiosis and fertilization of some micronutrients carried out until the end of the vegetative phase is sufficient for the plant to complete its cycle without promoting losses in seed quality (WERNER *et al*., 2016; ZUFFO *et al*., 2020).

It is assumed that even with the use of a cultivar with an indeterminate growth habit to carry out this experiment, aiming at the natural ability to recover from damage caused by defoliation, the plant was not able to finish its cycle without affecting the quality of the seeds, which negatively interfered with the analyzed variables.

In Table 3 it was verified that the results of electrical conductivity and protein were not significant. For mass of a thousand seeds, vigor, and viability, the results were significant for the levels of defoliation and N doses, but the interaction was not significant (Table 3).

Electrical conductivity (Table 3) was not affected by the treatments, indicating that the seeds had ideal concentrations of non-structural carbohydrates in their cell walls (PALACIO *et al*., 2011). This was probably due to fertilization with nutrients in the implantation and vegetative stages of the culture, while the plant was not suffering from the stress caused by defoliation.

The increase in defoliation caused a decreased in the mass of a thousand seeds (Table 3). Bahry *et al*. (2013) reported that the reduction in the leaf area of plants due to defoliation resulted in a lower production of photoassimilates for filling pods due to the probable abortion of plant structures due to the lack of assimilates. Glier *et al*. (2015) evaluated different levels of defoliation in the vegetative and reproductive stages of two soybean cultivars and observed a reduction in the mass of a thousand grains when defoliation was performed at stage R5, regardless of the level of defoliation applied.

Table 2 - Average values of leaf area (LA), germination (G), emergence (E), total length (TL), and total dry mass (TDM) of soybean seedlings as functions of defoliation levels associated with the application of N doses

Defoliation (D)	LA (cm^2)	G (%)	E (%)	TL (cm seedling $^{-1}$)	TDM (g seedling^{-1})
33	1449.09 a	88.75 a	81.75 a	16.40 a	135.86 a
66	1027.55 b	82.75 b	74.50 b	14.96 b	116.67 b
Doses of N (N)					
0	1236.90	87.50	77.00	15.26	115.78
50	1295.42	86.75	79.50	16.53	129.05
100	1314.62	83.25	77.50	15.07	137.65
150	1220.87	85.50	78.50	15.86	136.39
F (D)	18.16**	6.53*	6.48*	10.03*	5.85*
F (N)	0.77^{ns}	0.62^{ns}	0.15^{ns}	2.10^{ns}	1.63^{ns}
F (D*N)	1.19^{ns}	0.55^{ns}	0.70^{ns}	2.44^{ns}	0.65^{ns}
CV (%)	19.57	7.74	10.31	8.20	17.76

ns: not significant; ** and *: significant at 1% and 5% probability by the F Test, respectively ($p < 0.05$); CV: coefficient of variation

A maximum dose of 77.65 kg ha^{-1} N caused a greater increase in this parameter (Figure 2A), which may be related to the greater synthesis of amino acids, chlorophyll, and enzymes caused by the application of N (PEREIRA *et al*., 2018). This was verified by Marcon *et al*. (2017), who found that the mass of thousand seeds was greater when nitrogen was applied, regardless of the source or reproductive stage.

For vigor and viability (tetrazolium) (Table 3), a high level of defoliation had a negative effect, directly influencing seed quality, possibly owing to a reduction in photoassimilates. However, the maximum dose of 68.75 kg ha^{-1} increased the vigor of tetrazolium (Figure 2B). N is responsible for several reactions in plants, in addition to being a part of the chlorophyll molecule, which is directly related to photosynthetic activity and, consequently, greater plant performance, showing that the addition of this element interferes decisively with the physiological and agronomic performance of plants (BARBOSA *et al*., 2016). For viability (tetrazolium), data were adjusted using a third-degree equation.

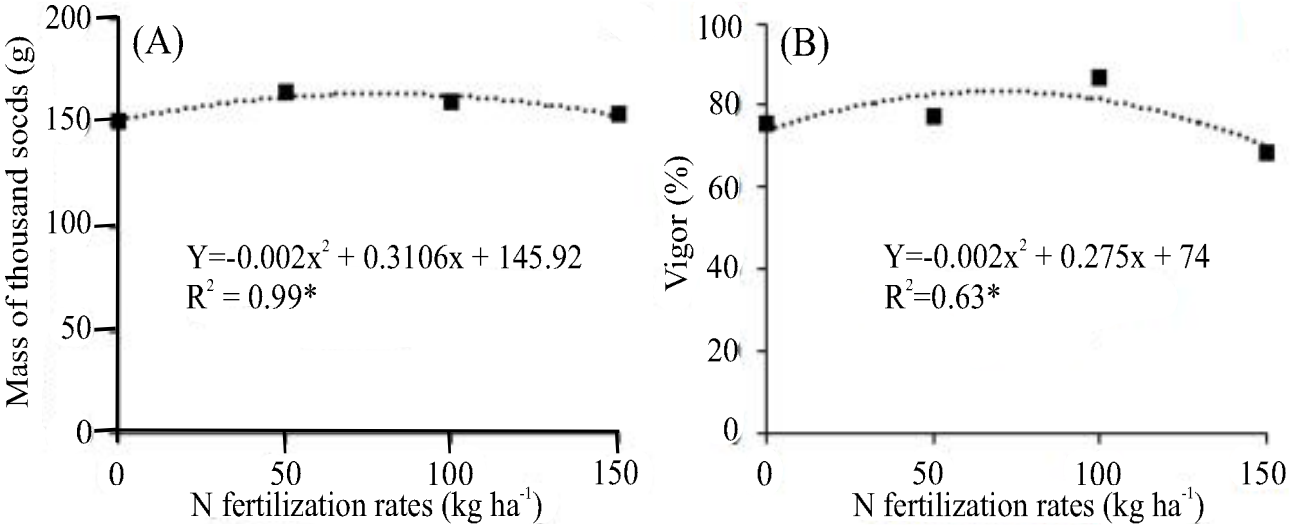
In general, an increase in defoliation during the reproductive stage affected most of the analyzed

Table 3 - Average values of electrical conductivity (EC), total protein content (PT), mass of a thousand seeds (MTS), vigor (VIG) and viability (VIA) of soybean seeds according to the levels of defoliation associated with the application of doses of N in coverage

Defoliation (D)	EC (µS cm^{-1} g^{-1})	TPC (%)	MMS (g)	VIG (%)	VIA (%)
33	85.82	35.88	163.22 a	79.50 a	89.75 a
66	85.08	34.06	152.63 b	74.50 b	83.00 b
Doses of N (N)					
0	87.74	35.58	150.96	75.75	87.50
50	93.75	34.20	165.30	77.50	83.50
100	86.93	34.29	160.82	86.75	92.00
150	89.99	35.80	154.63	68.50	82.50
F (D)	0.01^{ns}	2.53^{ns}	9.84*	6.25*	23.02*
F (N)	0.68^{ns}	0.52^{ns}	3.57*	16.04*	9.46*
F (D*N)	0.79^{ns}	0.05^{ns}	0.96^{ns}	1.87^{ns}	1.57^{ns}
CV (%)	22.48	9.38	7.74	7.35	4.61

ns: not significant; ** and *: significant at 1% and 5% probability by the F Test, respectively ($p < 0.05$); CV: coefficient of variation

Figure 2 - Mean values of mass of one thousand seeds (A) and vigor (tetrazolium) (B) of soybean seeds subjected to defoliation levels and application of N doses in topdressing



variables. This is because the reproductive period of soybeans is the most sensitive to defoliation owing to the high demand for water, nutrients, and photoassimilates that increase during the seed formation period to meet the growing accumulation of dry mass and the biosynthesis of oil and protein (HEIFFIG *et al*., 2006).

However, despite the benefits of nitrogen fertilization as a topdressing, it does not have the capacity to minimize the stress suffered by defoliation, causing irreversible damage to the plant when it is provoked in the reproductive stage.

CONCLUSIONS

1. The increase in defoliation from 33 to 66% impairs the physiological quality of soybean seeds;
2. The use of nitrogen fertilization for coverage after intense defoliation at the beginning of the reproductive phase cannot minimize qualitative losses in seeds.

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