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Bioregulator and foliar calcium supplementation in soya (*Glycine max* L.)

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Flower and pod abortions in large quantities are commonly observed in soybean plants. In normal conditions of crop cultivation, flower abortion is commonly above 60%. Among the strategies that may promote great flower and pod set in soybean, there is the use of plant growth regulators and foliar applications of calcium. Thus, this study aimed to evaluate the effects of different doses of plant growth regulator and the calcium sprayed on the agronomic performance of soybean plants. Five doses of systemic bioregulator (cytokinin) and five doses of calcium carbonate ($CaCO_3$) were applied in a 5 x 5 factorial arrangement with four replications field implemented in randomized block design. Soybean plant biometrics, the calcium content in different plant parts and crop yield were recorded. None of the treatments had a significant effect on the characteristics observed. The result was probably due to adequate conditions that prevailed during the soybean cropping cycle. In fact, flower and pod abortions happen when plants experience water stress during the flowering period. This lack of any treatment effect indicated the importance of regular and trustful monitoring of crop important variables to prevent routine applications of growth regulator and calcium.

Key words: *Glycine max*, cytokinin, calcium carbonate, plant biometrics, crop yield.

INTRODUCTION

Currently, Brazil is the world's second-largest producer of soybeans, with an estimate of 215 million tones for the 2018/2019 harvest (CONAB, 2019), and there is perspective to overtake the USA soybean production in the next years becoming the largest soybean producer in the world. The grain yield for this crop is defined by components such as the number of pods per plant, or per area, the number of grains per pod and the weight of

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grains and factors that are directly dependent on the number of flowers per plant (Caverzan et al., 2018). However, the abortion of large quantities of flowers and pods are commonly observed in soya.

Flowers set in *Glycine max* is controlled by relations among hormonal, anatomical and nutrition factors, as well as by the availability of photoassimilates (Liu et al., 2004). In normal conditions of cultivation, the flower abortion reaches up to 60-65% (He et al., 2019), and these losses are strongly induced by nutritional imbalances, water deficiency, extremes of temperature and light intensity. Fageria et al. (2006) also indicated that the period of up to three days after anthesis, when the initial processes of cell division are occurring, as the most sensitive period for soybean flower fixation. In this period, abiotic stresses, especially the water deficit, can significantly increase the number of flowers and pods aborted.

Plant nutrition is important to reduce the effects flower and pod abortion, especially the availability of nutrients such as calcium (Ca), which is very important to the process of cell wall synthesis, pollen germination and growth of the pollen tube (Fioreze et al., 2018). Its deficiency causes abortion of flowers and pods, directly influencing on yield components and on the soybean productivity (Faquim, 2005; Fageria et al., 2014). Also, Ca has low mobility in plant and hydric stress periods during flowering decreases the Ca availability to reproductive tissues increasing flower and pod abortion.

Another important factor is the supply of plant hormones, such as cytokinin (Basuchaudhuri, 2016). Cytokinin is a plant hormone that is directly involved in the photosynthetic metabolism and the processes of cell division and differentiation, being determinant for the proper development of the reproductive structures (Fiorenze, 2013). Bioregulators are products that present plant hormones and/or mineral nutrients have been used to inhibit, promote or modify morphophysiological processes in plants with the aim of increasing agricultural production.

Although there are studies on factors that can promote a greater fixation of the reproductive structures in plants, the information is scarce to support decision-making by use, concentration, specific environmental conditions and time of application of bioregulators in the soybean crop. Therefore, the objective of this work was to evaluate the effects of different doses of bioregulator including cytokinin and doses of foliar calcium carbonate on the agronomic performance of soybean crop.

MATERIALS AND METHODS

The present study was conducted in the experimental area of the Federal Institute of Education, Science and Technology of Triângulo Mineiro (Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro - IFTM), Campus Uberaba, latitude 19° 39' 19" S and longitude 47° 57' 27" W, in the municipality of Uberaba, Minas Gerais state, Brazil. The soil of the area is characterized as dystrophic Red Latosol, with a medium texture. The climate of the region according to the classification of Köppen type is Aw (tropical, hot and humid summer, with cold dry winter) (Beck et al., 2018). The monthly rainfall recorded in the experimental area during the conduction of the experiment varied from 0 to more than 60 mm (Figure 1). In this period, total precipitation was about 1023.1 mm and average temperature about 23.4°C. The chemical analysis of the soil in the experimental area is presented in Table 1. At planting, 230 kg ha^{-1} of 08-28-16 fertilizer was applied in the planting furrow. The side-dress fertilization was performed ten days after sown by applying 400 ml ha^{-1} Supa Bor (10% B w/v) and 17 kg ha^{-1} of manganese sulfate. At 15 days after sown, 150 kg ha^{-1} of ammonium sulfate was applied (CFSEMG, 1999).

Seeds treatment consisted of 100 ml of Dermacor® (chlorantraniliprole - 625 g L^{-1}) for each 100 kg of seeds, plus graphite to facilitate the sowing. During the experiment applications of fungicide, insecticide and herbicide were performed. The products used were two applications of Elatus® (azoxystrobin - 300 g kg^{-1} + benzovindiflupir 150 g kg^{-1}) at the dose of 300 g ha^{-1}, 45 days after sown and 20 days after the first application. In the same fungicide spray, insecticide Connect® (imidacloprid 100 g L^{-1}) at the dose of 2 L ha^{-1}. The herbicide application was performed soon after sowing with Crucial® (isopropylamine salt of glyphosate 400.8 g L^{-1} + potassium salt of glyphosate 297.75 g L^{-1}) at a dose of 3 L ha^{-1}, plus Podium® (fenoxaprope-P-ethyl alcohol 110 g L^{-1}) at a dose of 0.8 L ha^{-1}.

A 5x5 factorial arrangement was adopted for the five doses of systemic bioregulator (0, 0.5, 1, 1.5, 2 L ha^{-1}) commercialized as Veritas®, and five doses of calcium carbonate ($CaCO_3$) (0, 1, 2, 3, 4 L ha^{-1}) commercial name Cal Super® (41% w/v), with four replications. Combinations were allocated to plots in a randomized block design. Veritas® is a systemic bioregulator of the diphenyl-urea chemical group (Bayer, 2018) that includes Zn (12 g L^{-1}), N (48 g L^{-1}), and Ca (60 g L^{-1}) readily available for plant absorption.

Each experimental unit was composed of 16 planting rows spaced by 0.5 m with 4 m long, totaling 32 m^2 in each portion of a total of 40 plots. The useful area consisted of the 14 central lines, being harvested the central 2 m of each line (280,000 plants per hectare). The sowing was mechanically performed with a sowing machine and tractor in the conventional system (tillage) along with the fertilizer. The cultivar sown was Monsoy 6410, which has indeterminate growth and medium cycle ranging between 108 to 135 days depending on the region. The application of Cal Super® and Veritas® was performed when the plants reached R1 phenological stage (beginning of flowering, one flower opened in the main stem), and were applied with the aid of a backpack sprayer, using a spray volume equivalent to 400 L ha^{-1}, according to manufacturer recommendations.

When plants reached R6 phenological stage (full seed, green seed fully filling the pod cavity in one of the four uppermost nodes), the height of first pod insertion (measured with a ruler with millimetric scale), number of grains per pod and number of pods per plant in 10 plants of each plot were recorded. The dry mass of plants (shoot), leaves, stems and pods were collected from the same 10 plants. Leaf samples were washed, dried in a forced air circulation oven at 65°C until constant weight, then ground for nitrogen, phosphorus, potassium, calcium, magnesium and sulfur contents analyses according to the methodology described by Bataglia et al. (1983).

Crop harvest was performed when plants reached R8 phenological stage that is when 95% of the pods are mature (brown color). Each plot was threshed using mechanical thresher, for subsequent grain weight determination and correction to 13% moisture. The average weight of 1000 grains and grain moisture were determined from three samples of 1000 grains taken from the total grain produced in the useful plot.

Grain ash was determined by calcination of the sample in the

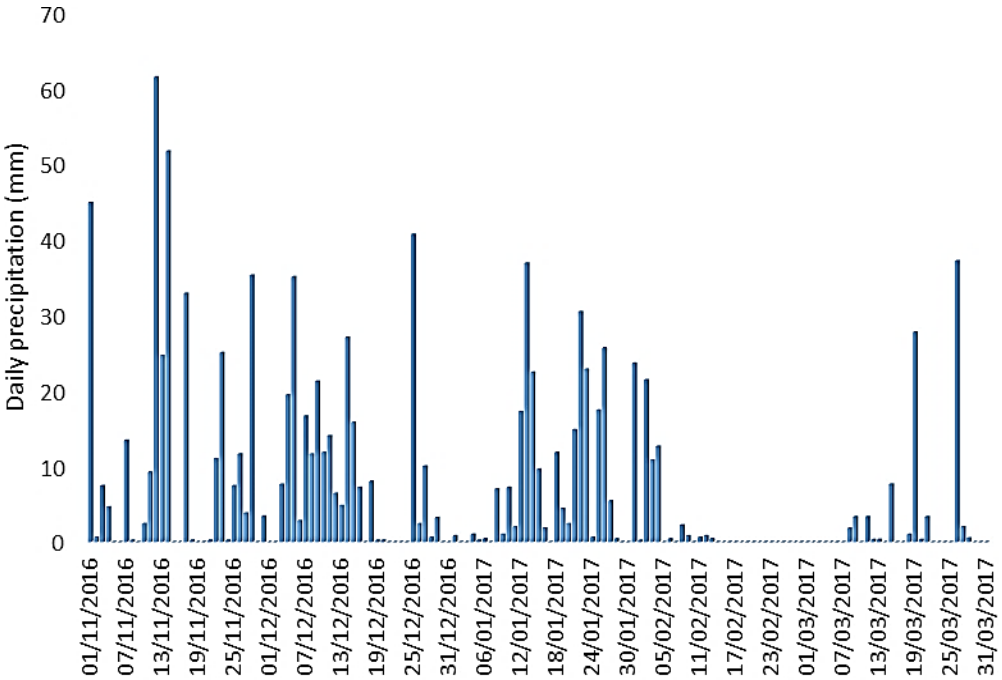

Figure 1. Dayly rain precipitation observed during field experiment.
Source: Meteorological station in the IFTM Campus Uberaba.

Table 1. Soil chemical characterization at 0 to 0.2 m soil depth.

pH (H_2O)	Ca	Mg	Al	P	K	S	H+Al	CEC	V	O.M.
---1:2.5---	-----$cmol_c$ dm^{-3}-----			------mg dm^{-3}------			-$cmol_c$ dm^{-3}-		%	g kg^{-1}
6.3	1.9	0.5	0	40.1	50.8	5	1.7	4.23	60	10

pH in H_2O, Ca, Mg, Al = KCl solution (1 mol L^{-1}); P, K = 0.05 mol L^{-1} HCl + H_2SO_4 0.0125 mol L^{-1}; S = 0.01 mol L^{-1} CaH_2PO_4; P available = extractor Mehlich-1; H + Al = SMP buffer solution (pH 7.5); CEC = cation exchange capacity at pH 7; V = saturation of bases; O.M. = soil organic matter (colorimetric method). Methodology source: Embrapa (2017).

oven at 550°C, until obtaining clear ash. Crude protein was obtained by the Kjeldahl method through the determination of the nitrogen in soybean following the methodology described by the Association of Official Analytical Chemistry (AOAC, 1990). Lipids content was determined using the Soxhlet gravimetric method - based on the quantity of material solubilized by ether - was used. Fibers were quantified by sample digestion (AOAC, 1990). Carbohydrate content was determined by the method proposed by Vannucchi (1990), using the formula: total carbohydrates = 100 - (% moisture + % ash + % protein + % lipids + % fiber).

The data collected underwent an analysis of variance for each of the parameters considered in the study, and multiple regression analysis was performed when significant differences were detected among treatments for a given variable.

RESULTS AND DISCUSSION

The summary of the analysis of variance for the agronomic characteristics presented no significant interaction between the application of Ca and bioregulator for any of the variables considered, as well as no effects of each Ca or bioregulator (Tables 2 and 3).

Klahold (2005) evaluated the effect of bioregulator (Stimulate®) applied in seed treatment, in foliar spraying and in combination of both and found grater averages of dry mass for leaf (11.68 g), stem (16.26 g), above-ground plant (shoot) (37.32 g) and whole plant (shoot + roots = 48.36 g). The lower averages of the variables reported in the present study - compared to Klahold (2005) study - can be related to cultivar differences between the studies and to the scarcity of rainfall during the initial phase of the experiment of the present study, despite the good rainfall distribution during the soybean flowering period.

Soybean reproductive stages, such as the beginning of the flowering stage (R1), the beginning of the filling of pods (R5), full-filling of pods (R6) and the germination phase are greatly affected by water deficiency (Pereira-

Table 2. Analysis of variance for agronomic characteristics recorded in function of doses of plant growth regulator and of calcium foliar-applied in soybean crop at R1 phenological stage (beginning of flowering).

Biorregulator (L ha^{-1}) [B]	Dry mass (g)				First pod Insertion (cm)
	Plant	Leaf	Stem	Pod	
0	18.17	3.28	3.28	11.70	11.79
0.5	19.25	3.23	3.91	12.05	11.33
1.0	19.79	3.45	3.80	12.55	11.41
1.5	19.72	3.39	3.91	12.45	11.63
2.0	19.80	3.44	4.13	12.35	12.16
F value	1.069^{ns}	0.189^{ns}	1.635^{ns}	0.543^{ns}	1.520^{ns}
Calcium (L ha^{-1}) [C]					
0	18.53	3.18	3.70	11.65	11.54
1.0	19.33	3.11	3.89	12.35	11.87
2.0	19.10	3.38	3.95	11.80	11.98
3.0	19.87	3.63	3.63	12.75	11.51
4.0	19.90	3.48	3.86	12.55	11.42
F value	0.723^{ns}	0.882^{ns}	0.3^{ns}	1.032^{ns}	0.834^{ns}
Interaction B x C	1.52^{ns}	1.430^{ns}	0.78^{ns}	1.213^{ns}	0.779^{ns}
Mean	19.34	3.36	3.80	12.22	11.67
C.V. (%)	15.55	30.31	29.36	17.17	10.32

^{ns} = non-significant at 5% of probability level.

Table 3. Summary of the analysis of variance of agronomic characteristics evaluated in function of doses of plant growth regulator and calcium foliar-applied in the soybean plants.

Biorregulator (L ha^{-1}) [B]	Pods per plant	Grains per pod	Grain humidity (%)	100 mass (g)	Productivity (Mg ha^{-1})
0	42.8	2.21	6.90	12.57	6.00
0.5	39.8	2.18	6.20	12.43	5.97
1.0	41.3	2.21	6.50	12.48	5.85
1.5	44.7	2.23	6.65	12.16	6.19
2.0	43.9	2.19	7.00	12.46	6.06
F teste	1.865^{ns}	0.246^{ns}	1.019^{ns}	1.347^{ns}	0.109^{ns}
Calcium (L ha^{-1}) [C]					
0	42.4	2.30	6.50	12.36	5.39
1.0	42.3	2.20	6.40	12.33	5.67
2.0	43.6	2.20	7.05	12.28	6.34
3.0	42.0	2.16	6.30	12.45	6.01
4.0	42.2	2.17	6.95	12.68	6.68
F teste	0.205^{ns}	1.731^{ns}	1.081^{ns}	1.434^{ns}	1.888^{ns}
Interaction B x C	0.829^{ns}	0.854^{ns}	0.780^{ns}	0.804^{ns}	1.233^{ns}
Average	42.49	2.20	6.65	12.4	6.02
C.V. (%)	15.22	8.41	21.33	4.83	27.95

^{ns} = non-significant at 5% of probability by the F test.

Flores and Justino, 2019). When hydric stress occurs at flowering triggers the abortion of flowers (Queiroz, 2014). In the present study, the good rain distribution during the flowering stage may prevent the occurrence of difference among the treatments for these variables.

In a controlled environment, soybean plants submitted to water deficit stress and shading at the flowering stage were treated with Ca, alone or in combination with cytokinin, and presented superior values of relative water content in soybean leaves at the end of the first water

Table 4. Calcium content and accumulation in different soybean parts as a function of doses of plant growth regulator and calcium foliar-applied in soybean plants.

Biorregulator (Lha^{-1}) [B]	Calcium accumulated						
	Leaf	Pod	Stem	Leaf	Pod	Stem	Total
	.....g kg^{-1}.....			.....mg pl^{-1}.....			
0	14.36	6,10	4.50	47.22	72.04	15.02	135.01
0.5	14.30	6.00	4.46	46.86	67.51	17.08	130.22
1.0	14.22	6.00	4.14	49.53	68.74	15.42	133.69
1.5	14.10	5.80	4.10	47.87	68.28	16.06	132.22
2.0	14.69	5.51	4.18	50.98	67.15	17.42	135.56
Teste F	0.263^{ns}	0.930^{ns}	0.409^{ns}	0.214^{ns}	0.329^{ns}	0.421^{ns}	0.102^{ns}
Calcium (L ha^{-1}) [C]							
0	13.84	5.70	4.53	44.47	66.27	16.97	128.39
1.0	14.01	6.20	4.35	44.35	73.53	16.58	134.46
2.0	14.25	5.64	4.69	48.00	67.86	18.07	133.93
3.0	14.64	5.99	3.89	53.34	68.27	14.18	135.81
4.0	14.96	5.86	3.93	52.59	67.50	15.21	133.97
F teste	1.115^{ns}	0.808^{ns}	1.427^{ns}	1.326^{ns}	0.705^{ns}	0.916^{ns}	0.314^{ns}
Interaction B x C	1.102^{ns}	1.383^{ns}	0.651^{ns}	1.334^{ns}	1.009^{ns}	0.588^{ns}	1.204^{ns}
Average	14.33	5.89	4.28	48.51	68.71	16.20	133.35
C.V. (%)	13.42	19.03	31.01	34.2	21.68	43.89	16.76

^{ns} = non-significant at 5% of probability by the F test.

deficiency cycle (p>0.05) (Fiorenze, 2013). The authors observed that the effects of Ca and cytokinin on the maintenance of water content seem to occur in an isolated manner and not jointly since the effect of the combined application was not superior to the effect of the application alone. The authors also reported an average number of pods of about 37.7, with the application of the bioregulator in R2 soybean stage. In the present study, the average number of pods per plant was 42.5 (Table 3).

The summary of the analysis of variance for the contents and accumulation of Ca is presented in Table 4. The variables of content and accumulation of Ca were similar among treatments and no significant effect was observed for the interaction between the factors (Ca or bioregulator). The Ca accumulation in leaf, pod and stem were 48.51, 68.71 and 16.20 mg plant^{-1}, respectively, summing 133.35 mg plant^{-1} of Ca. Thus, the largest part of the Ca absorbed by the plant is accumulated in pods (51.52%), followed by leaves, which accumulated 36.37% of the total Ca in the plant.

Other studies found no beneficial results from the Ca application to soybean (Ben et al., 1993; Fioreze et al., 2018). However, it is noteworthy that the quantities of Ca applied in the present study are very small when compared to the nutritional requirement of the soybean plant, being expected only a flag effect of the application of Ca, since the quantities applied would probably not be able to change the leaf Ca content.

The control of the abscission of the reproductive structures in the soybean plant has sulfate to the availability of photoassimilates and nutrients, especially Ca, and the concentration of endogenous plant hormones, such as the cytokinin (Liu et al., 2004). These characteristics are also highly influenced by the environment of cultivation where the abortion of the reproductive structures can be observed in stressful conditions, such as water deficit, high temperatures or low light intensity, conditions hardly manipulated.

The summary of the analysis of variance for nutritional variables evaluated is presented in Table 5. There was no significant difference for the interaction between Ca foliar-applied and the bioregulator for none of the variables assessed, as there were no effects of the factors evaluated in isolation. However, Vieira and Castro (2001), evaluate the composition of soybean cultivars and found greater averages than those found in the present study for lipids (23%), proteins (40%) and carbohydrates (32%), and lower averages for ash (5.75%) and fiber (5.41%).

Considering the results obtained in the present study, it is possible to conclude that the factors not controlled in this field experiment, such as rainfall, temperature and luminosity must have created very adequate conditions for soybean cultivation. The reduced environmental stresses influenced the effectiveness of the bioregulator, as well as the application of Ca foliar. Therefore, further studies should be conducted, especially under controlled conditions, so the studied factors can be better understand under bioregulator and calcium influences. Also, the results found highlight the importance of careful

Table 5. Summary of the analysis of variance of nutritional compounds in soybean seeds.

Bioregulator (L ha^{-1}) [B]	Protein (%)	Ash (%)	Lipid (%)	Fiber (%)	Carbohydrate (%)
0	32.97	6.9	17.78	15.34	21.82
0.5	35.19	6.25	17.45	13.78	21.89
1.0	33.13	6.5	17.93	13.64	23.33
1.5	34.62	6.7	17.01	15.78	20.39
2.0	35.86	6.9	16.94	13.53	21.86
F teste	0.103^{ns}	0.604^{ns}	0.868^{ns}	0.697^{ns}	0.874^{ns}
Calcium (L ha^{-1}) [C]					
0	34.38	6.55	18.56	14.93	20.31
1.0	34.12	6.5	18.49	12.94	22.38
2.0	34.87	7.05	17.15	13	22.59
3.0	33.86	6.35	16	14.61	24.21
4.0	3.53	6.8	16.92	16.6	19.8
F teste	0.944^{ns}	0.608^{ns}	0.116^{ns}	0.345^{ns}	0.463^{ns}
Interaction B x C	0.268^{ns}	0.919^{ns}	0.487^{ns}	0.922^{ns}	0.572^{ns}
Average	34.35	6.65	17.42	14.41	21.86
C.V. (%)	11.68	22.5	20.28	44.2	38.57

^{ns} = non-significant at 5% of probability by the F test.

crop monitoring for appropriated decisions related to the application, or not, of inputs during the soybean crop cycle.

Conclusion

Under good conditions for soybean productions, no differences were observed among treatments including the application, or not, of bioregulator and/or Ca foliar. The lack of treatment effect indicated the importance of regular and trustful crop monitoring to prevent routine applications of growth regulator and calcium.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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