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EFFECT OF SOY PRODUCTION COMPONENTS SUBMITTED TO DIFFERENT DOSES OF INOCULANT COMPOUND CONTAINING: *Bradyrhizobium e Azospirillum brasilense*

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Abstract: Brazil is now the world's leading producer of soybeans, and this is due, in addition to its economic importance, to the benefits provided by biological nitrogen fixation. For the maximization of its results, studies of new technologies are carried out, being the co-inoculation (*Bradyrhizobium* + *Azospirillum brasilense*) one of them. The present work aimed to evaluate, under direct sowing in sugarcane straw, the efficiency of co-inoculation in soybean in the sowing furrow with the use of the commercial compound inoculant recommended for co-inoculation in different doses of the product in production components. The experiment was installed in an area of sugarcane reform in the municipality of Arranha-SP. The experimental plot consisted of seven lines of 15 m in length, and the spacing between lines of 0.45 m and the cultivar used was HO IGUAÇU (64HO133) IPRO. The experimental design was a randomized block consisting of seven treatments with five replications, in a total of 35 experimental plots. Some parameters were evaluated in R8: grain yield, number of pods per square meter, average number of grains per pod, thousand grain mass and nitrogen content accumulated in the grains. For the conditions of the present work, taking into account the results, it is recommended to use the compound inoculant for co-inoculation in the sowing furrow at the highest doses of the treatments tested.
Keywords: Biological nitrogen fixation, co-inoculation in soybean, *Glycine max,* sugarcane reform.

INTRODUCTION

Soybean stands out as one of the most important crops in the world. In the 2019/20 harvest, Brazil produced about 124 million tons, making it the world's largest producer of the grain (CONAB, 2021). On the other hand, sugarcane is also one of the main Brazilian crops, with the state of São Paulo being the main producer with 51.05% (5,172.5 thousand hectares) of productive area (CONAB, 2018).

Since the 1980s, crop rotation has been an advantageous and sustainable option, especially when carried out with green manures or legume crops (SOARES, 2014). In order to improve land use in the interval between sugarcane plantations, some crops can be used in rotation, which in addition to increasing soil fertility, generates income at a time when the land is not being used (CHIARADIA et al., 2009).

Among the most required species in this rotation system are grain-producing crops. Of these, the most used are soybeans, sunflowers, beans and peanuts. Soybean represents a great option, since it has a short cycle (up to 130 days), and can occupy the area in periods that coincide with the rainy season in the region, usually October to March in the case of the State of São Paulo, guaranteeing a good productivity and possibility of profit from the sale of grains (LIMA et al., 2019).

In the cultivation of sugarcane, the use of oilseeds such as soybean stands out for the rapid decomposition of plant residues, due to the lower C/N ratio, providing nutrients for the main crop, such as the supply of nitrogen, since it has the ability to fix atmospheric nitrogen in its root system through the symbiosis established with bacteria of the genus *Bradyrhizobium* (AITA; GIACOMINI, 2003; AITA et al., 2004). Tanimoto and Bolonhezi (2002) confirm the viability of this system for rotation with the soybean crop, which presented, for an average of six harvests, higher yields than the conventional system combined with a 30% reduction in production cost.

Thus, commercial inoculants based on *Bradyrhizobium* in soybean have been routinely used in this crop, either applied via seed treatment or in the sowing furrow. Thus, all the nitrogen that the crop needs is obtained by biological nitrogen fixation, without the need to purchase chemical nitrogen fertilizers. In terms of technological innovations, the application of inoculant via the sowing furrow offers the advantage of optimizing the system, since its application takes place simultaneously with the sowing operation, which avoids direct and harmful contact between the biological fraction and the formulations. chemicals (fungicides and insecticides), just by using appropriate equipment (PASTORE, 2016), which is coupled to the seeder-fertilizer.

On the other hand, Brazilian research is also looking for new technologies to maximize the gains obtained with soybean inoculation, since commercial cultivars that are released annually tend to be increasingly productive, thus requiring a greater amount of nutrients, which nitrogen is one of the most demanded. Thus, today there is the possibility of using co-inoculation or mixed inoculation in soybeans. This practice consists of adding an inoculant containing bacteria of the genus *Bradyrhizobium* with inoculant for grasses bacteria-based: *Azospirillum brasilense*. To carry out this practice, the soybean farmer applies the two inoculants, each containing a genus of these aforementioned bacteria. It is important to point out that in the market there is only one biological input registered by MAPA that contains these two genera of bacteria in the same product.

The mechanisms of action of *Bradyrhizobium* and from *Azospirillum* are different. In the case of the latter, the benefits come beyond biological nitrogen fixation, the synthesis of plant growth phytohormones (SPAEPEN; VANDERLEYDEN, 2015). Thus, the action of this bacterium provides a more voluminous root system, consequently helping to produce a greater number of nodules, which provides better absorption and/or use of water and nutrients, and greater resistance to water stress is also observed (FUKAMI et al. , 2017; MARQUES et al., 2017). Regarding nutrients, greater vigor is observed in plants (ARDAKANI et al., 2011).

Thus, the objective of the present work was to evaluate, under field conditions, under direct sowing in sugarcane straw, the efficiency of soybean co-inoculation in the sowing furrow with the use of the compound inoculant with *Bradyrhizobium and Azospirillum* in different doses of the product, in the production components.

MATERIAL AND METHODS

PLACE OF CONDUCTING THE EXPERIMENT

The experiment was installed under field conditions, in an area of sugarcane renewal, on December 4, 2020, belonging to Usina Colombo, located in the municipality of Ariranha -SP. It is located at latitude 21º11'16" south and longitude 48º47'13" west, at an altitude of 595 meters.

Due to its location, Köeppen (1948) classified the climate of the region of the municipality of Ariranha as a hot climate with dry winter (Cwa) with maximum and minimum temperatures between 37º and 10 C, with the normal rainy season from September to March, with an average annual rainfall of 1,255 mm. The region of the municipality of Ariranha is at an altitude varying between 500 and 600 meters, as portrayed in the Topographic Chart of Catanduva.

Otter's predominant soil is the eutrophic red-yellow Podzoic - Pve1 and Pve4, classified by clay of low activity, abrupt, moderate A, sandy/medium texture and gently undulating relief.

TREATMENTS AND EXPERIMENTAL DESIGN

The treatments tested, as well as the doses of inoculants used in the present work are described in Table 1.

The description of the inoculants used in this experiment follows below:

a) MASTERFIX® L GRAMÍNEAS (standard inoculant): liquid inoculant for grasses, registered (MAPA Reg. - N° SP 003718-4.000082) and produced by Stoller do Brasil Ltda, having the bacteria as guarantee: *Azospirillum brasilense* (strains AbV5 and AbV6), in the concentration of 2 x 10^8 UFC/ml.

b) MASTERFIX L DUAL FORCE®: liquid inoculant for soybean cultivation, registered (MAPA Registration - N° SP

Treatments
T1 - Control (without mineral nitrogen fertilization and without inoculation) T2 – MASTERFIX® L SOJA (800 mL.ha^{-1}) – Inoculation in the planting furrow
T3 – MASTERFIX® L SOJA (800 mL.ha^{-1}) + MASTERFIX® L GRAMÍNEAS (200 ml.ha^{-1}) – Co-inoculation in the planting furrow
T4 - MASTERFIX L DUAL FORCE® (800 mL.ha^{-1}) – Co-inoculation in the planting furrow T5 - MASTERFIX L DUAL FORCE® (1000 mL.ha^{-1}) – Co-inoculation in the planting furrow T6 - MASTERFIX L DUAL FORCE® (1200 mL.ha^{-1}) – Co-inoculation in the planting furrow T7 – MASTERFIX L DUAL FORCE®- (1500 mL.ha^{-1}) – Co-inoculation in the planting furrow

Table 1. Treatments and doses of inoculants used in the soybean co-inoculation test using a compound-based inoculant *Bradyrhizobium* and *Azospirillum brasilense*, installed in an area of sugarcane renovation. Agricultural Year 2020/21.. Ariranha-SP.

003718-4.000002) and produced by Stoller do Brasil Ltda, having as a guarantee the presence of two species of bacteria, which are: *Bradyrhizobium japonicum* (strain Semia 5079), in the concentration of 1 x 10^9 UFC/ml and *Azospirillum brasilense* (strains AbV5 and AbV6), at a concentration of 1 x 107 CFU/ml.

c) MASTERFIX® L SOJA (standard inoculant): liquid inoculant for soybeans registered and produced by Stoller do Brasil Ltda, having as a guarantee containing a minimum concentration of 5 x 10^9 UFC ml^{-1} of *Bradyrhizobium elkanii* (Cepa Semia 5019) and *Bradyrhizobium japonicum* (Cepa Semia 5079).

The experimental plot consisted of seven lines of 15 m in length, and the spacing between lines was 0.45 m. Thus, the experimental design was randomized blocks composed of the seven treatments mentioned above with five replications, in a total of 35 experimental plots..

CONDUCTING THE EXPERIMENT

Before installing the experiment, soil samples were collected from the experimental area for further chemical and granulometric analysis.

Soil samples for chemical (RAIJ et al., 2001) and granulometric (DAY, 1965) characterization were collected in October 2020, in the 0-0.25 m depth layer, and the results obtained were: pH ($CaCl_2$) = 5,60; M.O. = 8,00 g dm^{-3}; CO = 5,8 g dm^{-3}; P = 21,00 mg dm^{-3}; K = 1,7 mmolc dm^{-3}; Ca = 20,80 mmolc dm^{-3}; Mg = 5,80 mmolc dm^{-3}; H + Al = 13,00mmolc dm^{-3}; V = 68,52 %, Total Sand = 892 g kg^{-1} of soil; Clay = 72 g kg-1 of soil and Silt = 36 g kg-of soil, and the test was installed in direct seeding under sugarcane straw. Sowing fertilization was carried out with fertilizer formulated 0-20-20, at the dose of (350 kg. ha^{-1}).

The soybean cultivar used was HO IGUAÇU (64HO133) IPRO which requires medium fertility, maturation group 6.4, indeterminate growth, gray pubescence color, purple flower color, adaptation microregions 101, 102, 103, 201, 202 , 203, 204, 301 and 302. Shows resistance to stem canker and phytophthora rot, moderately resistant to frog eye spot and bacterial pustule and root-knot nematode *(M. javanica*) (LAGOA BONITA SEMENTES, 2021).

20 seeds were sown: m^{-1}, with the use of a seeder-fertilizer composed of seven lines, in order to obtain 14 to 15 plants per linear meter, aiming to obtain the final average population of 320,000 plants: ha^{-1}.

Thus, in the laboratory, before sowing, procedures were carried out to treat the seeds with Basf's technological package: composed of the commercial product Standak® Top at a dose of 2 ml per kg of seed, which was used in all treatments tested. Standak ® Top offers protection of the genetic potential of soybean seeds. The product has multiple and complementary functions in its insecticidal and fungicidal effect, shielding the seeds against the attack of pests and soil diseases that interfere with the germination process and seedlings developing in the soybean crop. The solution has three distinct active principles, which provide high efficiency for the management of pests such as caterpillars, coró and soybean anteaters. Standak® Top also offers greater tolerance to water stress and the occurrence of nematodes (BASF, 2019).

As for the practice of co-inoculation, MASTERFIX L DUAL FORCE® inoculant was used: liquid inoculant for soybean, registered (MAPA Registration - N° SP 003718-4.000002) and produced by Stoller do Brasil Ltda, having as a guarantee the presence of two species of bacteria, which are: *Bradyrhizobium japonicum* (strain Semia 5079), in the concentration of 1 x 10^9 UFC/

ml e *Azospirillum brasilense* (strains AbV5 and AbV6), in the concentration of 1 x 10^7 UFC/ml in the different doses recommended according to the treatments shown in Table 1. The inoculant for inoculation with *Bradyrhizobium* used was MASTERFIX L® SOJA at the 800 ml.ha^{-1}, that is, seven doses more than the seed treatment whose indication is from 100 to 200 ml ha^{-1}. And for joint use in the co-inoculation that corresponded to the T3 treatment, MASTERFIX L® GRAMÍNEAS was used at a dose of 200 ml.ha^{-1}. The application of inoculants for inoculation and co-inoculation was carried out through a sprayer coupled to a seeder-fertilizer. Some precautions were taken to ensure greater efficiency in the treatments, such as preparing the solution in the shade, checking the pH of the solution and properly calibrating the spray nozzles for uniform distribution of the inoculants.

The micronutrients cobalt and molybdenum were also applied via foliar spraying at the V4 phenological stage (FEHR; CAVINESS, 1977), in all treatments including the control, and disease and pest control was carried out using fungicides and insecticides when necessary.

All soybean cultivation techniques, such as cultivar choice, sowing time, plant population, weed control, insects and diseases followed the technical recommendations for soybean cultivation by EMBRAPA (2013).

ASSESSMENTS

Assessments in the reproductive stage R8

After harvesting the experimental plots, the following characters were determined:

-Grain yield (PG) = harvested in the two central rows of 15 m in length and spacing between rows of 0.45 m. From the average values referring to the production of the plots of each treatment, the productivity was calculated, being expressed in kg ha^{-1} (values corrected to 13% humidity).

-number of pods per square meter (NV) – count of the number of full pods obtained in one square meter.

- average number of grains per pod (NMGV) = determined by the sum of the average number of grains obtained in three replications of 10 pods per experimental plot divided by the average number of these ten pods per experimental plot;

-thousand grain mass (MMG) = determined by weighing three subsamples of 100 grains, per repetition, multiplying the results by 10 (BRASIL, 2009);

- nitrogen content accumulated in the grains

(NTG) = in g.kg^{-1} determined by the methodology described by Bataglia et al. (1983).

STATISTICAL ANALYSIS OF RESULTS

For the production components, the estimated and applied Box-Cox transformations were performed as proposed by Hawkins and Weisberg (2017), with the average values maintained at the original scale. The variances, standard deviations, coefficients of variation, DMS, analysis of variance and mean comparisons were calculated with the transformed data. Subsequently, the normality of the residues were verified by the Shapiro-Wilk test at 5% probability (ROYSTON, 1995). And also Homoscedasticity through the homogeneity of variances by Levene's test at 5% probability (GASTWIRTH et al., 2009). When significant differences were detected in the analysis of variance, the means were compared by Tukey's test at 5% probability. The analyzes were performed with the aid of the AgroEstat Software, online version (MALDONADO JUNIOR, 2019).

RESULTS AND DISCUSSION

In Table 2, the average results obtained in the agronomic characters and production components are described. The F test detected highly significant statistical significance ($p \leq 0.01$) for MMG and PG.

Regarding NTG, it is noted that the treatments that stood out were those that used the highest doses of MASTERFIX L DUAL FORCE®, that is, T6 and T7 with respectively 46.80 and 43.58 g.kg-1, despite these did not differ statistically by the test of means applied to the T3 treatment that made use of co-inoculation with MASTERFIX® L SOJA inoculants (800 mL.ha^{-1}) + MASTERFIX® L GRAMÍNEAS (200 mL.ha^{-1}). With an intermediate average value, the T5 treatment had an average of 36,06 g.kg^{-1}, which in turn, did not differ statistically from treatments T4 which used the inoculant MASTERFIX L DUAL FORCE® at the lowest dose applied in the furrow and from T2 which used only inoculation with *Bradyrhizobium*. As expected, the control without the addition of biological inputs (T1) expressed the worst value in terms of nitrogen accumulated in the grains, with an average of 25,60 g.kg^{-1}. As for the increment in terms of NTG, it was verified in the present work that treatments T6 and T7, respectively, produced in the grains: 21,2 and 17,98 g.kg^{-1} more when compared to the non-inoculated control (Table 2). Several contributions have already been mentioned by researchers arising from the interaction between plants x bacteria of the genus: *Azospirillum*, among them, increases in the levels of chlorophyll, nitrogen, proline in shoots and roots, stomatal conductance; greater plant height,

TRAT	NV	NTG	NMGV[1]	MMG	PG
	m^2	g.kg^{-1}	Units	g	kg ha^{-1}
T1	2045,60 b	25,60 d	2,20 d	153,31 e	3098,70 d
T2	2088,80 ab	31,84 cd	2,33 bc	159,16 cd	4482,20 c
T3	2185,60 ab	41,02 ab	2,39 bc	160,38 c	5072,90 b
T4	2131,20 ab	30,78 cd	2,31 c	157,92 d	4246,20 c
T5	2217,20 ab	36,06 bc	2,37 bc	162,91 b	5285,80 ab
T6	2252,40 ab	46,80 a	2,42 ab	166,53 a	5541,30 a
T7	2298,00 a	43,58 a	2,51 a	165,27 a	5488,00 a
F	3,78**	20,33**	17,95**	129,25**	200,00**
CV (%)	1,00	10,74	6,80	0,11	3,91
Average	2174,10	36,53	2,36	160,78	4745,00

Mean of five repetitions followed by the same lowercase letters in the column did not differ from each other by Tukey's test at 5%; T1 = Control (without mineral nitrogen fertilization and without inoculation); T2 = MASTERFIX® L SOYBEAN (800 mL.ha-1) – Planting furrow; T3 = MASTERFIX® L SOYA (800 mL.ha-1) + MASTERFIX® L GRASS (200 mL.ha-1) – Planting furrow ;T4 MASTERFIX L DUAL FORCE®: (800 mL.ha-1) – Planting furrow; T5 = MASTERFIX L DUAL FORCE®: (1000 mL.ha-1) – Planting furrow; T6 = MASTERFIX L DUAL FORCE®: (1200 mL.ha^{-1}) –Planting furrow ;T7 = MASTERFIX L DUAL FORCE®: (1500 mL.ha-1) – Planting furrow; NV = number of pods obtained in one square meter; NTG = Nitrogen content in grains; NMGV = average number of grains per pod; MMG = thousand grain mass; PG = grain yield.[1]average of three replications of 10 pods per experimental plot.

Table 2.Production components evaluated at the R8 stage in a co-inoculation experiment in soybean with the use of a compound inoculant based on *Bradyrhizobium* and *Azospirillum brasilense* installed in an area of sugarcane renovation. Agricultural Year 2020/21. Otter-SP.

biomass production, grain production, root development and tolerance to water stress, among others (HUNGARY, 2011; YADAV; YADAV; SINGH, 2011; LANA et al., 2012).

When analyzing the NV, the T7 treatment that used the inoculant MASTERFIX L DUAL FORCE® at the highest application dose, stood out statistically with 2298.00 pods, only differing statistically only from the non-inoculated control (T1) with an average of 2045 .60 pods per square meter. In terms of increment related to this production component, it can be observed that T7 provided an increase of 252.40 pods per square meter more compared to the T1 control.

Regarding the NMGV, the results showed that the general average of the experiment was 2.36 grains per pod. And for the treatments tested, it is noted that T7 and T6 also provided the highest averages of grains per pod of respectively (2.51 and 2.42). The treatments T2, T3 and T5 had the lowest average value, which did not differ statistically from T4, with an average value of 2.31 grains per pod. And finally, the non-inoculated control (T1) with an average value of 2.20 grains per pod. It is noteworthy that, in the evaluation of this production component, the cultivar used in the present study always presented at most pods with three grains.

As for MMG, the results showed that the overall mean of the experiment was 160.78 g. And for the treatments tested, it is noted that treatments T6 and T7 (application of the inoculant MASTERFIX L DUAL FORCE® at the highest doses – 1200 and 1500 mL. ha^{-1}) produced the highest mean values of mass of a thousand grains respectively (166.53 and 165.27 g), statistically differing from the other analyzed treatments. Then, with an intermediate mean value, the T5 treatment was positioned with a dose of 1000 mL.ha^{-1} presenting an average of 162.91 g. On the other hand, treatments T2 and T3 presented lower mean values in relation to this production component, being statistically lower than those mentioned above and higher than the T6 treatment associated with the use of MASTERFIX L DUAL FORCE® at the lowest dose in the furrow with 157 .92 g, which in turn was statistically superior only to the control treatment (T1) with 153.31 g. In relation to the non-inoculated control, there were increases of respectively 12.59 g more in MMG when treatments T7 and T6 were used, that is, treatments which used the inoculant for co-inoculation of soybean "MASTERFIX L DUAL FORCE ®" in its highest application doses.

As for grain yield (PG), (Table 2) there was a general average of the treatments of 4745,00 kg.ha^{-1}, which demonstrates the excellent performance of the soybean crop, in the 2020/21 Agricultural Year, when considering the area of sugarcane renewal. According to estimates by Conab (2020), in the state of São Paulo, the oilseed was highly benefited by climatic conditions throughout its vegetative development and productivity was estimated at 3.650 kg ha^{-1} appearing as a state record, and an increase of 20.5% in relation to the previous crop.

Treatments T5, T6 and T7 did not differ statistically from each other and stood out providing higher PG values of respectively 5285.80, 5541.30 and 5488 kg.ha-1. With an intermediate position by the average test applied, treatments T3 remained, which were statistically equivalent to each other with T5, with an average of 5072,90 kg ha^{-1}. Then, treatments T2 and T4 were positioned, which were statistically similar with mean values of respectively.e 4482,20 and 4246,20 kg ha$^{-1.}$ With the lowest mean value was the treatment T1 (non-inoculated control) with 3098,70 kg ha^{-1}. In terms of increment, it is noted that considering the average value of the three best treatments (T5, T6 and T7) which was

5438,37 kg ha^{-1}, there was a very high increase in terms of PG, that is, of 2339,70 kg ha^{-1} or, 39 bags ha^{-1} when compared to uninoculated control (T1).

Still in relation to PG, when analyzing the best treatments with MASTERFIX L DUAL FORCE® (T5, T6 and T7) in comparison with T4, which consisted of the use of the same inoculant applied at the lowest dose, it is noted that the average of the first three promoted an increase in terms of PG of 1192.17 kg ha-1 or 19.87 bags per hectare more, thus recommending if you are going to use the MASTERFIX L DUAL FORCE® product, in an area of sugarcane reform, in a sowing furrow that is in the highest doses. Braccini et al. (2016) found that the use of the association of *Bradyrhizobium japonicum* with *Azospirillum brasilense*, via sowing furrow, provided increments in physiological characters, as well as promoted increases in soybean grain yield, when compared to the control. They also corroborate the work of Hungria et al., 2013 and Embrapa, 2014, who confirmed the agronomic efficiency in the field of soy co-inoculation with *Bradyrhizobium* in the seeds and *A. brasilense* in the sowing furrow.

CONCLUSIONS

It is recommended to use the compound inoculant (*Bradyrhizobium* + *Azospirillum brasilense*) (treatments T7, T6 and T5) which consisted of the application of the product via the sowing furrow in higher doses for co-inoculation in soybean, in an area of sugarcane renewal for the conditions of the present work.

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