

## Efficiency of *Trichoderma asperellum* as a promoter of vegetable growth and soybean productivity

Eficiência de *Trichoderma asperellum* como promotor de crescimento vegetal e produtividade de soja

Eficiencia de *Trichoderma asperellum* como promotor del crecimiento vegetal y la productividad de la soja

Received: 04/14/2022 | Reviewed: 04/29/2022 | Accept: 05/04/2022 | Published: 05/07/2022

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### Abstract

Aiming to achieve greater economic returns by increasing the biomass and productivity of strategic crops, such as soybeans, this article aimed to evaluate the efficiency of TrichoPlus (*Trichoderma asperellum*), as a promoter of plant growth in soybeans. Four independent experiments were carried out in the municipalities of Porto Nacional and Gurupi, Tocantins, Brazil, in the 2017/2018 and 2018/2019 harvests. Four experiments were conducted, each with four treatments with different doses of TrichoPlus (2, 3, 4 and 5 g kg<sup>-1</sup> per seeds), plus two control treatments, one positive control with commercial product based on *Trichoderma asperellum* and one absolute control (without inoculation). For the treatment with the TrichoPlus product, the powder formulation was used, with active ingredient based on *Trichoderma asperellum* 201, formulated with a minimum concentration of 2 x 10<sup>8</sup> CFU g<sup>-1</sup>. The positive results for the biomass characteristics, stand maintenance and productivity were evidenced in the different doses of TrichoPlus, with emphasis on doses close to 5 g kg<sup>-1</sup> of seeds, with gains in productivity above 23% for the 2017/2018 harvest and above 9% for the 2018/2019 harvest in Gurupi. In Porto Nacional the productivity gain for the 2017/2018 harvest was over 21% and for the 2018/2019 harvest it was over 27% in relation to the absolute control. Considering the different doses of TrichoPlus used in the experiments, there were differences between the doses with the best results for treatments with doses of 4 and 5 g kg<sup>-1</sup> of seeds.

**Keywords:** *Glycine max* (L.) Merrill.; Fungus; Inoculation.

## Resumo

Com o objetivo de obter maior retorno econômico por meio do aumento da biomassa e da produtividade de culturas estratégicas, como a soja, este trabalho teve como objetivo avaliar a eficiência do produto TrichoPlus (*Trichoderma asperellum*), como promotor de crescimento vegetal na cultura da soja. Quatro experimentos independentes foram realizados nos municípios de Porto Nacional e Gurupi, Tocantins, Brasil, nas safras 2017/2018 e 2018/2019. Foram conduzidos quatro experimentos, cada um com quatro tratamentos com diferentes doses de TrichoPlus (2, 3, 4 e 5 g kg<sup>-1</sup> de semente), mais dois tratamentos controle, um controle positivo com produto comercial a base de *Trichoderma asperellum* e um controle absoluto (sem inoculação). Para o tratamento com o produto TrichoPlus foi utilizada a formulação em pó, com princípio ativo à base de *Trichoderma asperellum* 201, formulado com concentração mínima de 2 x 10<sup>8</sup> UFC g<sup>-1</sup>. Os resultados positivos para as características de biomassa, manutenção de estande e produtividade foram evidenciados nas diferentes doses de TrichoPlus, com destaque para doses próximas a 5 g kg<sup>-1</sup> de sementes, com ganhos de produtividade acima de 23% para a safra 2017/2018 e acima de 9% para a safra 2018/2019 em Gurupi. Em Porto Nacional o ganho de produtividade na safra 2017/2018 foi superior a 21% e na safra 2018/2019 foi superior a 27% em relação ao controle absoluto. Considerando as diferentes doses de TrichoPlus utilizadas nos experimentos, houve diferenças entre as doses com melhores resultados para os tratamentos com as doses de 4 e 5 g kg<sup>-1</sup> de sementes.

**Palavras-chave:** *Glycine max* (L.) Merrill.; Fungo; Inoculação.

## Resumen

Con el objetivo de obtener mayor retorno económico mediante el incremento de la biomasa y productividad de cultivos estratégicos, como la soja, este estudio tuvo como objetivo evaluar la eficiencia del producto TrichoPlus (*Trichoderma asperellum*), como promotor del crecimiento vegetal en cultivos de soja. Se realizaron cuatro experimentos independientes en los municipios de Porto Nacional y Gurupi, Tocantins, Brasil, en las safras 2017/2018 y 2018/2019. Se realizaron cuatro experimentos, cada uno con cuatro tratamientos con diferentes dosis de TrichoPlus (2, 3, 4 y 5 g kg<sup>-1</sup> de semilla), más dos tratamientos testigo, un testigo positivo con un producto comercial a base de *Trichoderma asperellum* y un testigo absoluto (sin inoculación). Para el tratamiento con el producto TrichoPlus se utilizó una formulación en polvo, con un ingrediente activo a base de *Trichoderma asperellum* 201, formulado con una concentración mínima de 2 x 10<sup>8</sup> UFC g<sup>-1</sup>. Los resultados positivos para las características de biomasa, mantenimiento de rodales y productividad se evidenciaron en las diferentes dosis de TrichoPlus, con énfasis en dosis cercanas a 5 g kg<sup>-1</sup> de semilla, con ganancias de productividad superiores al 23% para la zafra 2017/2018 en adelante. 9% para la cosecha 2018/2019 en Gurupi. En Porto Nacional, la ganancia de productividad en la zafra 2017/2018 fue superior al 21% y en la zafra 2018/2019 fue superior al 27% con relación al control absoluto. Considerando las diferentes dosis de TrichoPlus utilizadas en los experimentos, hubo diferencias entre las dosis con mejores resultados para los tratamientos con dosis de 4 y 5 g kg<sup>-1</sup> de semillas.

**Palabras clave:** *Glycine max* (L.) Merrill.; Hongo; Inoculación.

## 1. Introduction

*Trichoderma* is a naturally occurring soil fungus that has been widely studied and frequently used in agricultural production. Some species of *Trichoderma* can promote plant growth, increase germination and seed emergence. This occurs in an apparently symbiotic and non-parasitic relationship between the fungus and the plant, where the fungus occupies the nutrient niche and the plant can have an influence on growth or development, or even supply its nutritional needs by solubilizing phosphates (Contreras-Cornejo et al., 2016; Bononi et al., 2020), and consequently on crop productivity. The ability of the fungus to colonize the roots is a fundamental factor for its interference in the growth and productivity of the plant.

Laboratory and field studies with *Trichoderma* sp. performed with different crops, have shown a reduction in symptoms caused by abiotic stresses, such as water deficiency, nutrients and salinity (Sofa et al., 2014; Fiorentino et al., 2018). There is an improvement in the development of the plant, an increase in the seedling emergence rate, root system, shoot, chlorophyll content, productivity, size and/or number of flowers and/or fruits (Mendoza-Mendoza et al., 2018; Chagas Junior et al., 2019a, b). Changes in the root system increase the absorption area, favoring the assimilation and translocation of nutrients, which consequently intensifies plant biomass. The plant growth promoting effect is also attributed to the role of *Trichoderma* sp. in the solubilization of phosphate and micronutrients mediated by the release of siderophores and secondary metabolites, or by changes in the content of ethylene and auxin (Contreras-Cornejo et al., 2015; Bononi et al., 2020).

Significant results in increasing plant biomass have been reported in crops such as soybean (Chagas Junior et al., 2019a, b), beans (González-Marquetti et al., 2019), rice (Chagas et al., 2017; Silva et al., 2019), cowpea (Chagas et al., 2015, 2016;

Chagas Junior et al., 2015), with the use of *Trichoderma* spp. found in several formulated products based on this fungus in the world market (Bettiol et al., 2019).

Numerous hypotheses have been proposed to explain this observation including the improvement of chemical solubilization, availability and the absorption of nutrients by the plant (Contreras-Cornejo et al., 2016), as well as the synthesis of growth phytohormones (Zhang et al., 2013). Not only do these processes increase plant growth, but they also stimulate breathing, thus improving photosynthesis or photosynthetic efficiency (Harman et al., 2019), as well as increasing the plant's capacity to withstand abiotic stresses, such as drought, salinity and high temperature (Sofa et al., 2014; Fiorentino et al., 2018).

In order to achieve greater economic returns by increasing the biomass and productivity of strategic crops, such as soybeans, it is necessary to continue the process of generating information, derived from directed research, which seeks and evaluates innovative management practices, such as the use of TrichoPlus product, in the inoculation of soybean seeds, aiming at efficiency as promoters of plant growth and soybean productivity.

This work aimed to evaluate the efficiency of TrichoPlus (*Trichoderma asperellum* 201), as a promoter of plant growth in soybeans and the productive performance in the field in different cultivars and regions.

## 2. Methodology

### 2.1 Location and area preparation

Four independent experiments were carried out in the municipalities of Porto Nacional and Gurupi, Both in the state of Tocantins, Brazil, in the 2017/2018 and 2018/2019 harvests, from December to April of each harvest, with some distinct edaphoclimatic characteristics, in the Macroregions 4: 404 edaphoclimatic region (Gurupi) and 5: Edaphoclimatic Region 501 (Municipality of Porto Nacional), according to Normative Instruction N<sup>o</sup> 1 of 02/02/2012 of MAPA (Mapa, 2020).

In Gurupi, the experiments were carried out at the Experimental Station of the Universidade Federal do Tocantins, Gurupi University Campus. The geographical coordinates of the experimental station correspond to 11°43'45 " S and 49°04'07 " W, with an average altitude of 280 meters. The local climatic characterization is of humid tropical climate with classification of the type small water deficiency (B1wA'a ') / savanna vegetation or Tropical Savanna according to Köppen-Geiger (Peel et al., 2007). The average temperature in the two harvests was 27.1 and 26.8 °C, and 1617 and 1659 mm the average rainfall in both harvests, respectively.

In Porto Nacional, the experiments were carried out at the Research Station ALX Farias Agro Pesquisa Agropecuária do Cerrado LTDA (23°36'45.1 "S - 51°11'01.4" O). The local climatic characterization is of humid tropical climate with classification of type Aw according to Köppen and Geiger (PEEL et al., 2007). The average temperature in the two harvests was 26.1 and 26.8 °C, and 1622 and 1689 mm the average rainfall in both harvests, respectively.

Soil samples were collected from the areas of the experiments, before the implementation of the experiments, in the two harvests, and chemical analyzes were carried out according to Table 1. For the areas in Gurupi, the granulometric characteristics found were: 70.5, 5.0 and 24.5% of sand, silt and clay, respectively, for the area of the experiment in the 2017/2018 harvest; 68.5, 5.5 and 26.0% of sand, silt and clay, respectively, for the experiment area in the 2018/2019 harvest. For the areas in Porto Nacional, the following granulometric characteristics were found: 74.2, 7.1 and 18.7% of sand, silt and clay, respectively, for the 2017/2018 harvest area and 67.0, 14.8 and 18.2% of sand, silt and clay, respectively, for the 2018/2019 harvest area (Embrapa, 2011).

**Table 1.** Chemical analysis of the soil used for cultivation in Gurupi and Porto Nacional, 2017/2018 and 2018/2019 harvests.<sup>1</sup>

	pH	P	K	Al <sup>3+</sup>	H+Al	Ca <sup>2+</sup>	Mg <sup>2+</sup>	SB	T	V	MO
	CaCl <sub>2</sub>	Mg dm <sup>-3</sup>		-----cmol <sub>c</sub> dm <sup>-3</sup> -----						%	%
<b>Gurupi</b>											
2017/2018	5,8	5,7	19,0	0,0	1,5	2,2	1,1	3,35	4,9	69	1,5
2018/2019	5,4	18,8	30,0	0,0	2,5	2,2	0,6	2,88	5,4	54	1,8
<b>Porto Nacional</b>											
2017/2018	5,6	9,0	66,3	0,0	1,5	1,75	1,15	1,49	2,9	62	20
2018/2019	5,6	4,7	27,6	0,0	3,1	1,6	1,2	2,8	0,8	19,6	28,1

Source: Authors.

The soils were classified as medium-textured dystrophic Latossolo Vermelho Amarelo Distrófico (Embrapa, 2011). The areas were prepared by the conventional method, with plowing and harrowing.

In Gurupi, dolomitic filler limestone with 100% RPTN was applied to the areas of the experiments in the two crops, 90 days before planting, for soil correction, in the amount of 1.2 Ton ha<sup>-1</sup>. Seeding fertilization (P-K + S + Ca + Micro) was carried out with the application of 350 kg ha<sup>-1</sup> of formulation 5-25-15. Fertilization with phosphorus (simple superphosphate) was done manually on the planting lines the day before planting. Potassium (KCl) was applied in coverage 30 days after germination, at a dose of 60 kg ha<sup>-1</sup>. In Porto Nacional, mineral fertilization was carried out before sowing in both crops, in which the application of 400 kg ha<sup>-1</sup> of fertilizer containing sulfur, phosphorus and micronutrients, and another 100 kg ha<sup>-1</sup> of KCl in V3 stage.

In the four experiments, the plots consisted of eight lines six meters long and 0.5 m (24.0 m<sup>2</sup>) apart. The three central lines of the 3 m long plot, totaling 4.5 m<sup>2</sup>, were used as a useful plot. In the experiments in Gurupi, 15 seeds were used per linear meter, aiming at a final stand of 12 plants per linear meter. In Porto Nacional, 12 seeds were used per linear meter, aiming at a final stand of 9 plants per linear meter. The experimental design in all experiments was in randomized blocks with four replications.

In all experiments, the seeds were treated one day before sowing with products based on Pyraclostrobin + Methyl Thiophanate + Fipronil, using 100 g for each 50 kg of seeds, according to the manufacturer's recommendations.

## 2.2 Cultivars and treatments

The soybean cultivar used in Gurupi, for both experiments, was M 9144 RR. In Porto Nacional, for both experiments was used the cultivar M 8644 IPRO.

The treatments used in the four experiments were: four treatments with different doses of TrichoPlus (2, 3, 4 and 5 g per kg of seeds), plus two control treatments, one positive control with a commercial product based on *Trichoderma asperellum* (1 x10<sup>10</sup> CFU g<sup>-1</sup>, dispersible granular formulation, dosage of 100 g for 100 kg of seeds, according to the manufacturer's recommendations), and an absolute control (without inoculation). For the treatment with the product TrichoPlus, the powder formulation was used, with active ingredient based on *Trichoderma asperellum* (Table 2), formulated with a minimum concentration of 2 x 10<sup>8</sup> CFU g<sup>-1</sup>, having graphite as an inert in its composition, being applied directly on the seeds before planting.

**Table 2.** GenBank access codes for the *Trichoderma asperellum* 201 strain (TEF Region – translation lengthening factor) used in this study.

Isolated	Species identification	Access GenBank	Similarity	Reference
201	<i>T. asperellum</i> GJS 04-217	DQ381958	99%	Samuels et al. (2010)

Source: Authors.

At sowing seeds were inoculated with rhizobia (*Bradyrhizobium japonicum*, strain SEMIA 5079 and SEMIA 5080), with liquid commercial inoculant recommended for soybeans with  $10^9$  cel·g<sup>-1</sup> (500 mL of the inoculant for each 50 kg of seed), followed by inoculation with TrichoPlus.

During the development of the experiments, phytotechnical and phytosanitary management were carried out according to the recommendations of Henning (2009).

### 2.3 Evaluations

In Gurupi, in both experiments, two evaluations were carried out to determine the height and dry mass of the shoot, at 30 and 60 days after plant emergence (DAPE). In Porto Nacional, in both experiments, two evaluations were carried out at 27 and 52 days after plant emergence (DAPE). The initial stand at 20 DAPE was also determined, and the final stand, at harvest, in the useful area (4.5 m<sup>2</sup>). The biomass evaluation was done through the shoot dry mass (SDM). In all four experiments for biomass assessment, six plants were collected from each plot. The shoot was separated from the roots with a cut made at the base of the stem. Then, the shoot was placed in a paper bag and taken to dry at 65 °C until it reached a constant weight.

At harvest, in V8 stage, plant height, number of internodes, number of pods, and number of grains per pod were determined in the rows next to the experimental plots, using 15 plants per experimental plot, totaling 60 plants per treatment. Grain production was obtained in the central rows of each plot with a useful area of 4.5 m<sup>2</sup>, after the physiological maturation of the plants, when approximately 90% of the pods were dry. Then, the pods were threshed manually, correcting the grain moisture to 13%, being quantified to the productivity in the useful area and estimated in kg ha<sup>-1</sup> and bags ha<sup>-1</sup> (bags = 60 kg).

### 2.4 Statistical Analysis

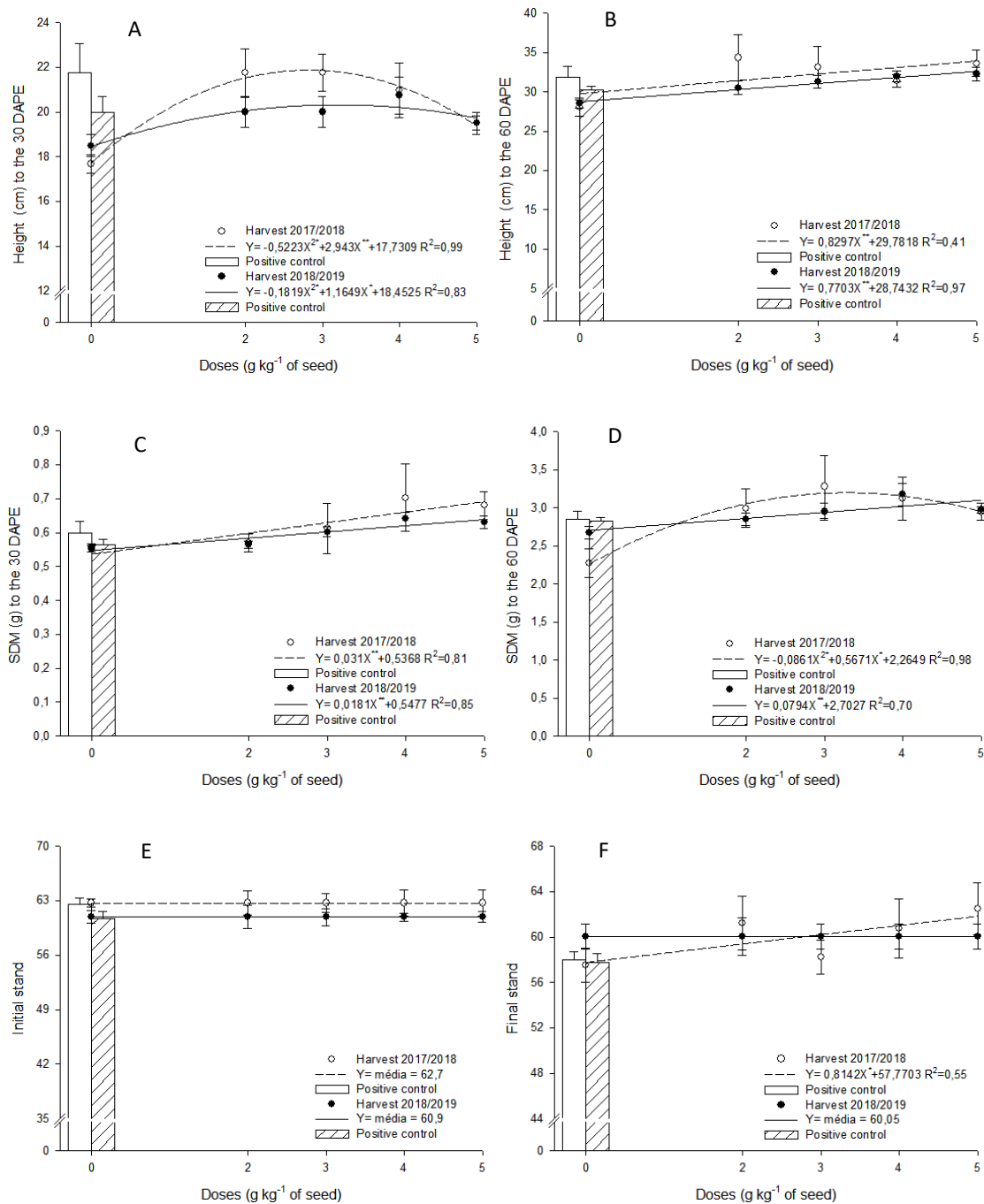
The data were subjected to analysis of variance by the F test. For the purposes of efficiency, the data were subjected to the Tukey test ( $p \leq 0.05$ ), for the effect of doses the regression test was applied and the selection of the models were based on the significance or the betas and the highest coefficient of determination (R<sup>2</sup>), using the SISVAR statistical program. The graphs were plotted using the SigmaPlot version 14.0® program.

## 3. Results and Discussion

### 3.1 Experiments in Gurupi

In the first evaluation, the polynomial model that best adjusted to the behavior of the height variable at 30 DAPE in both harvests as a function of the doses of the TrichoPlus product, was the quadratic model (Figure 1A). The plants height would reach its maximum point with 21.87 and 20.31 cm in the 2017/2018 and 2018/2019 harvest, respectively. These heights would be achieved in doses of 2.81 g kg<sup>-1</sup> of seed in the 2017/2018 harvest and 3.20 g kg<sup>-1</sup> of seed in the 2018/2019 harvest (Figure 1A). In the evaluation of height at 60 DAPE, the polynomial model that best fitted was the linear model (Figure 1B). The plants would reach a height of 33.93 cm in the 2017/2018 harvest and 32.59 cm in 2018/2019 (Figure 1B). For height, they presented coefficients of R<sup>2</sup>=99 and 83%, 2017/2018 harvest and for 2018/2019 harvest, respectively.

**Figure 1.** Plant height at 30 (A) and 60 (B) days after plant emergence, shoot dry mass (SDM) at 30 (C) and 60 (D) days after plant emergence, initial stand (IS) (E) and final stand (FS) soybean cv. M 9144 RR, inoculated with different TrichoPlus doses, Gurupi, TO. 2017/2018 and 2018/2019 harvest \*\*\*. \* Regression equation ( $p \leq 0.05$ ); \*\* Regression equation ( $p \leq 0.01$ ); \*\*\* The error bars express the average standard deviation.



Source: Authors.

The polynomial model that best fitted the characteristic of SDM at 30 DAPE in both crops was the linear one (Figure 1C). The doses used with the TrichoPlus product resulted in a constant increase in the biomass accumulation of soybean plants. The plants had a biomass increase of 12.37% in the 2017/2018 harvest and 11.46% in the 2018/2019 harvest, when compared to the positive control (Figure 1C). In the SDM evaluation at 60 DAPE in the 2017/2018 harvest, the polynomial model that

best fitted was the quadratic one (Figure 1D). The maximum technical efficiency of the product was achieved in the dose of  $3.29 \text{ g kg}^{-1}$  of seed, where the SDM of the seedlings would reach 3.19 g, representing 10% more increase, when compared to the positive control. In the 2018/2019 harvest, this increase was approximately 8.8% more when compared to the positive control, where the soybean plants would reach 3.10 g of dry biomass (Figure 1D).

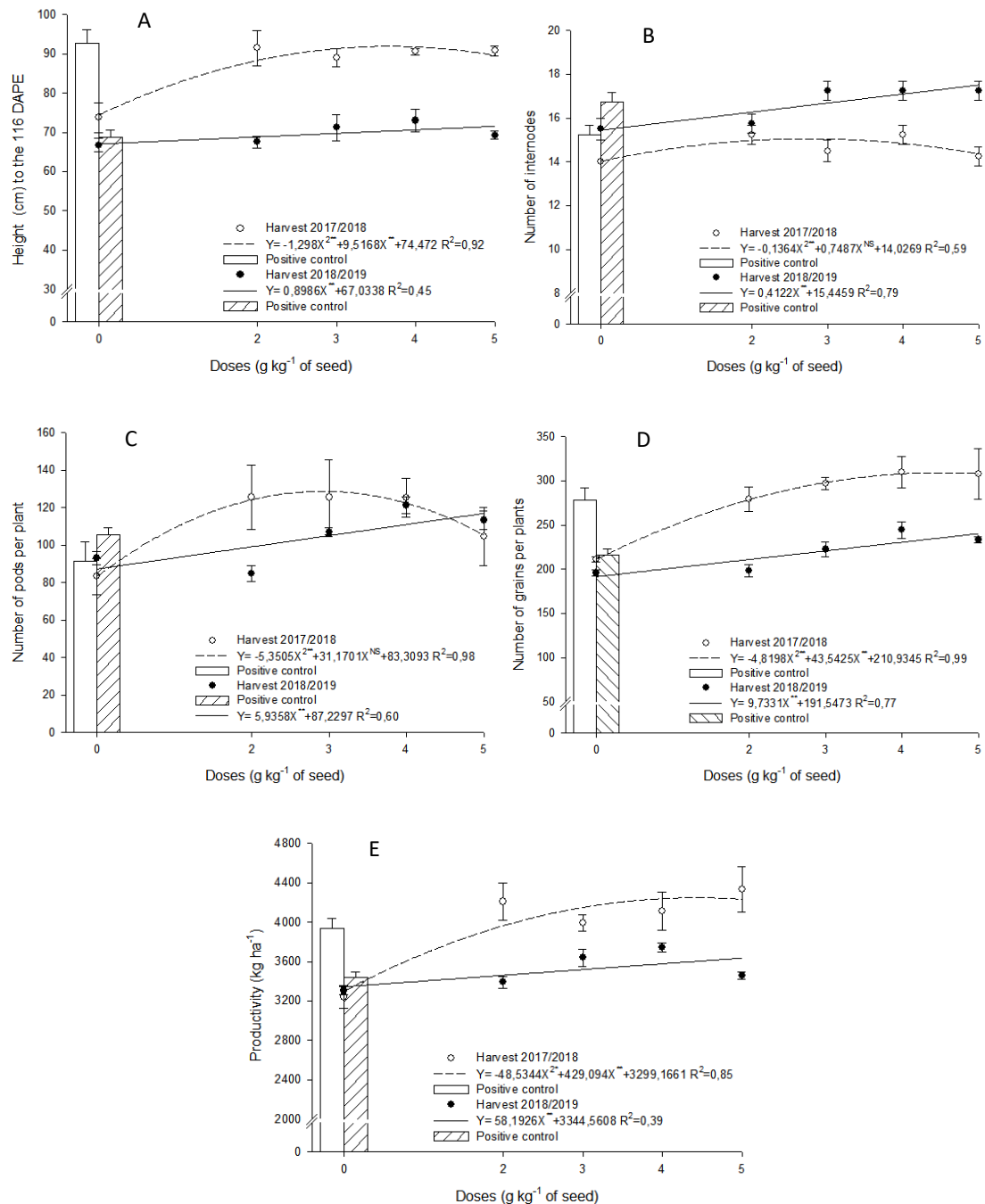
In the SDM evaluation at 60 DAPE in the 2017/2018 harvest, the polynomial model that best fitted was the quadratic one (Figure 1D). The maximum technical efficiency of the product was achieved in the dose of  $3.29 \text{ g kg}^{-1}$  of seed, where the SDM of the seedlings would reach 3.19 g, representing 10% more increase, when compared to the positive control. In the 2018/2019 harvest, this increase was approximately 8.8% more when compared to the positive control, where the soybean plants would reach 3.10 g of dry biomass (Figure 1D). In the evaluation of the initial stand (IS), for the evaluated averages there was no statistical difference in the significance of the betas ( $p \leq 0.05$ ) (Figure 1E). As for the final stand (FS) in the 2017/2018 harvest, a linear effect was observed under the doses of the product used, where it was 6.2% more effective than the positive control and 7% more effective when compared to absolute control (Figure 1F), representing an increase of 9,300 and 10,500 more plants per hectare, in relation to the positive and absolute control (dose 0, without inoculation), respectively. However, in the 2018/2019, there was no significant difference in the significance of the betas ( $p \leq 0.05$ ) for any polynomial model (Figure 1F).

The polynomial model that best adjusted to the behavior of the height variable at the time of harvest in the 2017/2018 due to the Trichoplus doses, was the quadratic model (Figure 2A). For height characteristic (Figure 2A) in the 2017/2018 harvest the maximum point obtained by the doses of the TrichoPlus product was  $3.67 \text{ g kg}^{-1}$  of seed, at which the soybean plants would reach a height of approximately 91.44 cm. For the 2018/2019 harvest, the linear polynomial model that best adjusted for plant height was the linear model. The plants would reach 71.53 cm in height in the respective crop, which represents an increase of 3.61% more when compared to the absolute control (Figure 2A). As for the number of internodes, the best-fitting polynomial model was the quadratic and linear model, 2017/2018 and 2018/2019 harvest, respectively. The dose that obtained the maximum technical efficiency of 15 and 17 internodes in the doses of 2.74 and  $5.00 \text{ g kg}^{-1}$  of seed, in the 2017/2018 and 2018/2019 harvest, respectively (Figure 2B). This represents an increase of 4.32% more internodes in the 2018/2019 harvest.

The number of pods per plant (NPP) in the 2017/2018 harvest showed a quadratic response as a function of TrichoPlus product doses applied from the inoculant by the regression test (Figure 2C). The maximum technical efficiency for NPP was obtained in the dosage of  $2.91 \text{ g kg}^{-1}$  of seed, with an average of 128 pods per plant, which represents an increase of 28.9% more than the positive control. For the 2018/2019 harvest, the NPP presented a linear response, where the best dose obtained an increase of 9.76% more than the positive control (Figure 2C).

The NGP achieved at the maximum point of the TrichoPlus product was approximately 240 grains per plant. This represents an increase in the number of grains between the maximum points evaluated, which correspond to 10.11 and 9.98% when compared to the negative control, 2017/2018 harvest and for the 2018/2019 harvest, simultaneously. For NGP they presented coefficients of  $R^2=99$  and 77%, 2017/2018 harvest and for 2018/2019 harvest, concomitantly.

**Figure 2.** Plant height (A), number of internodes (B), number of pods per plant (NPP) (C), number of grains per plant (NGP) (D) and productivity (E) of soybean cv. M 9144 RR, inoculated with different Trichoplus doses, Gurupi, TO. 2017/2018 and 2018/2019 harvest \*\*\*. \* Regression equation ( $p \leq 0.05$ ); \*\* Regression equation ( $p \leq 0.01$ ); \*\*\* The error bars express the average standard deviation.



Source: Authors.

The productivity of soybean showed a quadratic response for the 2017/2018 harvest, and a linear response for the 2018/2019 harvest, significant in function of the TrichoPlus product doses (Figure 2E). In the 2017/2018 harvest, grain yield would reach approximately 4247 kg ha<sup>-1</sup> (71 bags ha<sup>-1</sup>), where maximum technical efficiency was achieved at the dose of 4.42 g kg<sup>-1</sup> of seed, providing an increase of 7.3% (310 kg ha<sup>-1</sup>) more grains, when compared to the positive control, and an increase



of 23.68% when compared to the absolute control. In the 2018/2019 harvest, productivity would reach 3,635 kg ha<sup>-1</sup> (60 bags ha<sup>-1</sup>) of grains. The increase in relation to the treatment of the absolute control represents about 9.09% (5.5 bags ha<sup>-1</sup>). When the comparison is between the positive control and the product used in the experiment, there was an increase in grains productivity of 198 kg ha<sup>-1</sup> (3.3 bags ha<sup>-1</sup>), which represents about 5.45% increase.

As for the number of grains per plant (NGP), the polynomial model that best fitted the evaluated averages was the quadratic in the 2017/2018 harvest (Figure 2D). The best dose achieved was 4.51 g kg<sup>-1</sup> of seed, 2017/2018 harvest, where the plants obtained averages of 309 grains per plant. For the 2018/2019 harvest, the linear polynomial model that best adjusted to the behavior of the observed averages was the linear model.

### 3.2 Experiments in Porto Nacional

Most of the evaluated characteristics showed that the doses of the TrichoPlus product significantly influenced the phytotechnical evaluations in both crops for the evaluated characteristics. However, for the plant height characteristics (Figures 3A and 3B) in the 2017/2018 harvest and the initial stand (IS) (Figure 3E) in both crops there were no significant differences by the F test ( $p \leq 0, 05$ ).

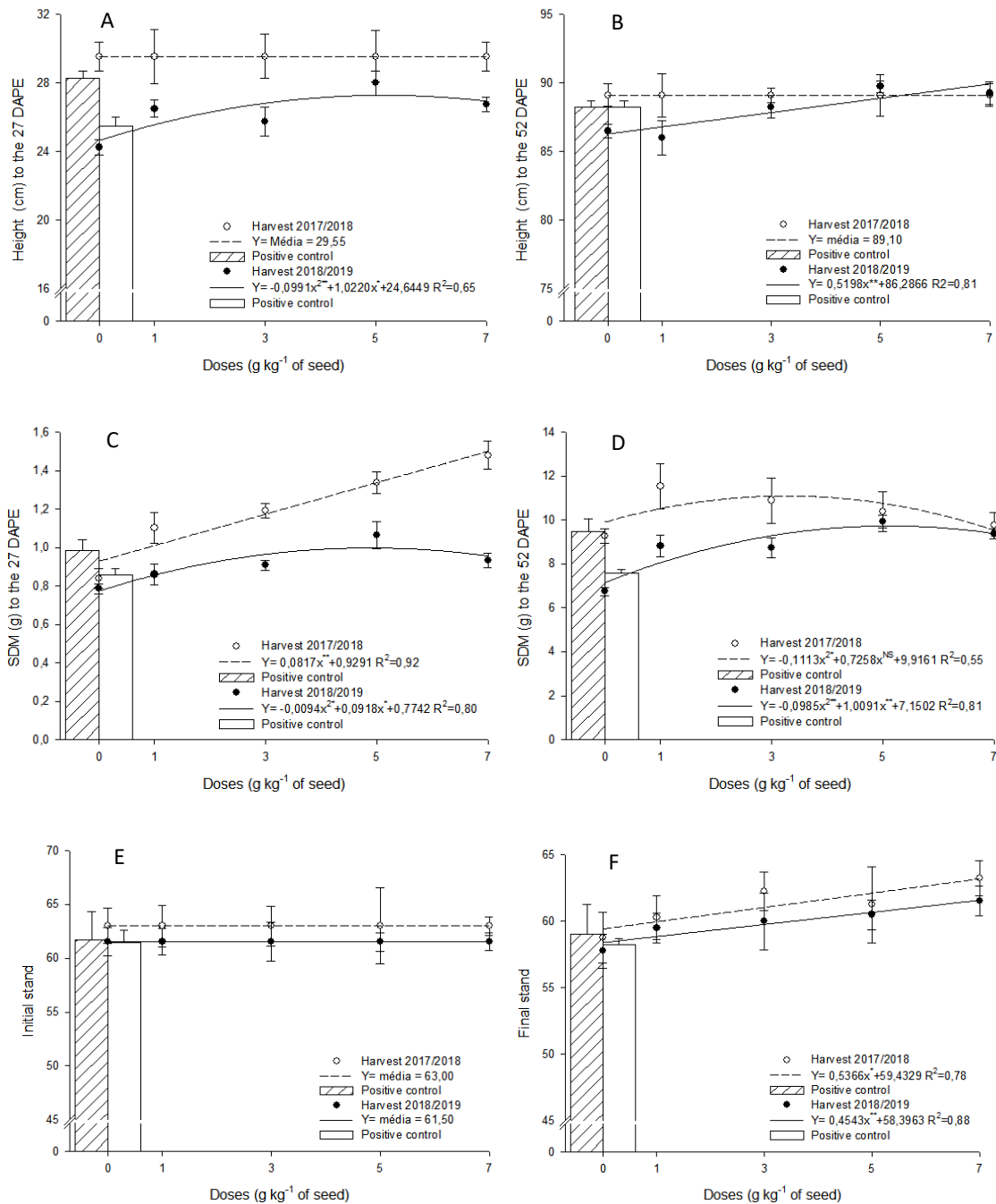
For the height parameter at 27 and 52 DAPE in the 2017/2018 harvest, there were no adjustments in regression curves between treatments in both evaluations ( $Y = \text{average}$ ) (Figure 3A). For the 2018/2019 harvest, at 27 DAPE evaluation, the polynomial model that best adjusted to the behavior of the height variables as a function of the TrichoPlus doses, was the quadratic and at 52 DAPE evaluation it was the linear one (Figure 3B). In the 2017/2018 harvest, the best dose for height in the evaluation at 27 DAPE was 5.16 g kg<sup>-1</sup> of seed, where the plants would reach approximately 27 cm. For the 2018/2019 harvest, in the height evaluation at 52 DAPE the plants would reach approximately 89 cm.

The polynomial model that best adjusted to the behavior of the SDM variable at 27 DAPE in the 2018/2019 harvest and SDM at 52 DAPE in 2017/2018 and 2018/2019, as a function of the doses of the TrichoPlus product, was the quadratic (Figure 3C, Figure 3D). As for SDM at 27 DAPE in the 2017/2018 harvest, the best-fitting polynomial model was the linear one (Figure 3C). In the evaluation of SDM at 27 DAPE, in the 2017/2018 harvest, all doses used with the TrichoPlus product were superior to the positive control, where the plants had an increase above 33% when compared to the positive control. In the 2018/2019 harvest, the maximum technical efficiency of the product was achieved at the dose of 4.88 g kg<sup>-1</sup> of seed, where the SDM of the seedlings would reach 1 g, representing an increase of 14%, when compared to the positive control (Figure 3C).

For the SDM at 52 DAPE, the maximum response of the TrichoPlus inoculant in the 2017/2018 and 2018/2019 harvest was 3.26 and 5.12 g kg<sup>-1</sup> of seed, a dose at which the plants would reach 11.09 and 9,73 g of dry mass (Figure 3D). This represents an increase of 14.7 and 21.9% in relation to the positive control, 2017/2018 and 2018/2019 harvest, respectively.

In the evaluation of the initial stand (IS), for the evaluated averages there was no statistical difference in the significance of the betas ( $p \leq 0.05$ ) (Figure 1E). As for the final stand (FS), a linear effect was observed under the doses of the product used (Figure 3F), showing an average population increase of 6.6 and 5.4% more plants per ha<sup>-1</sup> in the 2017/2018 harvest and 2018/2019, respectively.

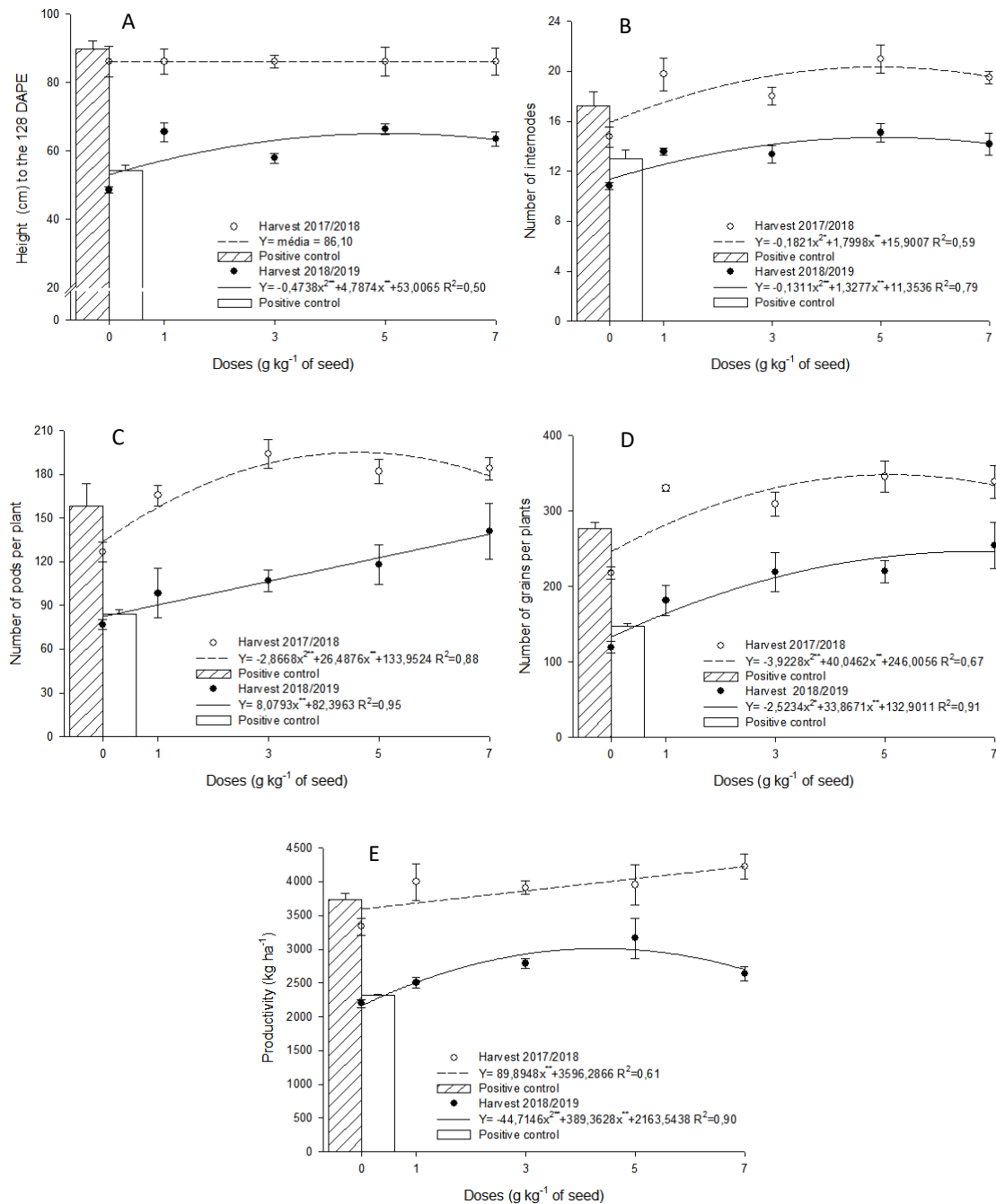
**Figure 3.** Height of plants (cm) at 27 (A) and 52 DAPE (B), shoot dry mass (SMD) at 27 (C) and 52 (D) days after plant emergence, initial stand (IS) (E) and final stand (FS) (E), of soybean cv. M 8644 ipro inoculated with different TrichoPlus doses, in Porto Nacional - TO. 2017/2018 and 2018/2019 harvest \*\*\*, \* Regression equation ( $p \leq 0.05$ ). \*\* Regression equation ( $p \leq 0.01$ ). \*\*\* The error bars express the average standard deviation.



Source: Autors.

For height characteristics (Figure 4A) in the 2017/2018 harvest, there were no adjustments in regression curves between treatments ( $Y = \text{average}$ ). However, the behavior in the 2018/2019 harvest, the soybean plants would reach a height of 65 cm at the dosage of 5.05 g kg<sup>-1</sup> of seed, which had a better performance when compared to the positive control. There was an increase of 16.4% more in the best tested dose, when compared with the positive control, and obtained an increase of 25.5% more, when compared to the absolute control.

**Figure 4.** Plant height (A), number of internodes (B), number of pods per plant (NPP) (C), number of grains per plant (NGP) (E) and productivity (F) of soybean cv. M 8644 ipro inoculated with different TrichoPlus doses, in Porto Nacional - TO. 2017/2018 and 2018/2019 harvest \*\*\*. \* Regression equation ( $p \leq 0.05$ ). \*\* Regression equation ( $p \leq 0.01$ ). \*\*\* The error bars express the average standard deviation.



Source: Authors.

As for the number of internodes, the maximum technical efficiency of 20 and 14 internodes was obtained in the doses of 4.95 and 5.06 g kg<sup>-1</sup> of seed of the TrichoPlus product, in the 2017/2018 and 2018/2019 harvest, respectively (Figure 4C). This represents an increase of 15.2% in the 2017/2018 harvest and 11.6% in the 2018/2019 harvest, in relation to the absolute control. The number of pods per plant (NPP) in the 2017/2018 harvest showed a quadratic response as a function of the applied TrichoPlus doses (Figure 4C). The maximum technical efficiency for NPP was obtained at a dosage of 4.62 g kg<sup>-1</sup> of seed to

which the plants would reach an average of 195 pods per plant, which represents an increase of 19.02% more than the positive control. For the 2018/2019 harvest, the NPP presented a linear response with the doses application of the TrichoPlus product, where the best dose obtained an increase of 39% more than the positive control (Figure 4C).

As for the number of grains per plant (NGP), the polynomial model that best fitted the evaluated averages was the quadratic one (Figure 4D). The best dose achieved was 5.1 and 6.71 g kg<sup>-1</sup> of seed, 2017/2018 and 2018/2019 harvest, respectively, which represents an increase in the number of grains between the evaluated dosages, which corresponded to 20.52 and 40.29% when compared to the absolute control, 2017/2018 harvest and for 2018/2019 harvest, simultaneously.

The productivity (P) of soybeans showed a linear response for the 2017/2018 harvest, and quadratic for the 2018/2019 harvest, significant in function of the TrichoPlus product doses (Figure 4E). In the 2017/2018 harvest, grain yield would reach 4225 kg ha<sup>-1</sup> (70 bags ha<sup>-1</sup>), showing an increase of 11.54% (487 kg ha<sup>-1</sup>) more grains, when compared to the positive control, and an increase of 21.1% when compared to the absolute control. The maximum technical efficiency obtained in the 2018/2019 harvest was achieved in 4.35 g kg<sup>-1</sup> of seed, where productivity would reach 3011 kg ha<sup>-1</sup> of grains (50.2 bags ha<sup>-1</sup>). The increase in relation to plants that did not receive a dose of any TrichoPlus based product represents about 27.04% increase (13.5 bags ha<sup>-1</sup>). When the comparison is between the positive control and the product used in the experiment, there was an increase in seed productivity of 11.5 bags ha<sup>-1</sup>, which represents an increase of 22.92%.

The positive results for the biomass characteristics, stand maintenance and productivity were evidenced in the different TrichoPlus doses, with emphasis on doses close to 5 g kg<sup>-1</sup> of seeds, observed in the Gurupi experiments 2017/2018 and 2018/2019 harvests, with gains in productivity above 23% for the 2017/2018 harvest and above 9% for the 2018/2019 harvest, in relation to the absolute control treatment (without inoculation).

For results in Porto Nacional, 2017/2018 and 2018/2019 harvests, positive results were also observed for the characteristics of biomass and productivity with the inoculation of TrichoPlus in different doses. The productivity gain for the 2017/2018 harvest was over 21% and for the 2018/2019 harvest it was over 27% in relation to the absolute control, also for doses close to the treatment with 5 g kg<sup>-1</sup> of seeds. These results were confirmed with the use of a dose of 5 g kg<sup>-1</sup> of seeds in the 2019/2020 harvest, also in Porto Nacional, with gains in plant biomass above 19% and an increase in productivity estimated at 8.1%.

The positive results observed for the different soybean experiments can be explained in terms of the action of the inoculant used, considering that fungi of the genus *Trichoderma* are used not only in the biological control of phytopathogens, but as promoters of plant growth, due to their versatility of action, such as parasitism, antibiosis and competition, in addition to acting as inducers of resistance to plants against diseases and producing growth hormones, phosphate solubilization, siderophores and secondary metabolites (Chagas Junior et al., 2015; Chagas et al., 2015; Contreras-Cornejo et al., 2016; Bononi et al., 2020). These fungi are found in the rhizosphere, are growth promoters in plant species, and produce a rich source of secondary metabolites, presenting a vast repertoire of genes supposedly involved in the biosynthesis of non-ribosomal peptides, polyketides, terpenoids and pyrones (Mukherjee et al., 2012), and inoculation with a high concentration of these microorganisms can provide positive results regarding the biocontrol of phytopathogens and, consequently, the promotion of plant growth.

These results may be related to the ability that the fungus *Trichoderma* has in promoting the growth of the plants shoots and the production of auxins or analogues to auxins and metabolites such as 6PP (6n- that favors the development of roots, promoting deeper and more vigorous roots, increases the absorption and solubilization of nutrients (Contreras-Cornejo et al., 2016; Bononi et al., 2020;) and favors hydrophobic adherence and the development of absorbent hairs on the lateral roots, with increased absorption surface (Samolski et al., 2012). Also, they increase the dry mass and the content of starch and soluble

sugars of the plants and the photosynthetic efficiency (Harman et al., 2019), the latter being directly related to the assimilation of nitrogen (Domínguez et al., 2016; Monte et al., 2019).

Studies show lines with efficiency for the biocontrol of several pathogens and others efficient in promoting plant growth, via colonization of the rhizosphere, under natural and axenic conditions or making nutrients available to the plant (Martínez et al., 2013). In addition to the ability of *Trichoderma* to control phytopathogens by producing antibiotics, some compounds produced by *Trichoderma* are capable of altering the metabolism of host plants (Patil et al., 2016). The increase in plant productivity is evident when seeds exposed to conidia, but separated by cellophane, without contact, show an increase in their growth, suggesting that metabolites of *Trichoderma* spp. They act not only as growth promoters, but also as signaling molecules (Ramada et al., 2019).

Thus, fungi belonging to the genus *Trichoderma*, in addition to being recognized fungicides, can also be classified as biofertilizers, biostimulants and enhancers of resistance against biotic and abiotic stresses. The result of all interactions is usually the promotion of plant growth (Woo and Pepe, 2018), that is, even when there are no diseases, when the product is used, the producer will have the benefit of increasing the efficiency of nutrient use and productivity.

According to Harman et al. (2019), the net effect of these fungi's residence in plants, such as *Trichoderma*, is to induce greater shoot and root growth, increasing crop yields, which will raise future food production.

These discoveries help to understand the role of *Trichoderma* in natural or cultivated ecosystems and promote its use in agriculture. The work carried out with the TrichoPlus product with potential for plant growth has demonstrated its ability, reflecting the gains in productivity observed in the regions of Gurupi and Porto Nacional.

Some factors are important for obtaining effective results with inoculants that promote plant growth: effective lines in the field against various phytopathogens and as promoters of plant growth, low cost of production involving efficient formulations and form, dose and time of application. This was developed for the product TrichoPlus (*Trichoderma asperellum*) aiming at seed inoculation, providing *Trichoderma* adherence and protection in direct contact with the seed and protection immediately after planting. This adequate formulation aims at maintaining the viability of the active principle aiming at the protection of structures, the persistence and effectiveness in the field, presenting an adequate concentration of structures (conidia) of the fungus and the ease of application (inoculation) of the product.

#### 4. Conclusion

The inoculation of the TrichoPlus product positively influenced biomass, stand maintenance and soybean crop productivity under field conditions in Gurupi and Porto Nacional, in the 2017/2108 and 2018/2019 harvests.

Considering the different doses of TrichoPlus used in the experiments, the recommended dose for soybeans growth and productivity was 4 and 5 g kg<sup>-1</sup> of seeds.

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