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CROP PRODUCTION

Plant growth analysis describing the soybean plants response on dryland field to seed co-inoculation

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ABSTRACT: *Plant growth analysis can be used for soybean plants evaluation to identify morphologic changes caused by soil microbes after seed inoculation. The objective was to measure changes of inoculated soybean plants grown under regular field Brazilian production conditions. The experiment was carried out to compare 5 inoculation treatments: T1 (Bradyrhizobium japonicum and B. diazoefficiens), T2 (T1 and Azospirillum brasilense), T3 (T1 and Trichoderma asperellum), T4 (T1 and T. virens, and Bacillus amyloliquefaciens) and T5 (T1 and Penicillum bilaiae). Leaf area, shoot and root dry matter were measured at vegetative and reproductive growth stages. Results of this study validate the contribution of plant growth analysis of soybeans plants for identifying their responses promoted by the combined inoculation of their seeds with selected microbes. Also, the microbial effects on plant growth vary not only between microbes but also between parts of the plants and through the plant development stages. The introduction of different microbes in soybean rhizosphere combined with Bradyrhizobium sp. strains can contribute to increase crop dry matter productivity during its growing cycle.*

Key words: *Glycine max, plant growth promoter microorganisms, shoot and root dry matter, leaf area index, plant growth rate.*

Análise de crescimento de plantas descrevendo a resposta do cultivo de soja no campo à coinoculação de sementes

RESUMO: *Análise de crescimento de plantas pode ser utilizada para avaliação de plantas e identificação de mudanças causadas por microrganismos de solo, depois de serem inoculados nas sementes. O objetivo deste estudo foi medir as mudanças no crescimento de plantas de soja inoculadas em condição de cultivos extensivos de campo no Brasil. O experimento foi conduzido para comparar cinco tratamentos inoculados: T1 (Bradyrhizobium japonicum e B. diazoefficiens), T2 (T1 e Azospirillum brasilense), T3 (T1 e Trichoderma asperellum), T4 (T1 and T. virens, e Bacillus amyloliquefaciens) e T5 (T1 e Penicillum bilaiae). A área foliar e a massa seca da parte aérea e de raízes foram medidas nos estádios vegetativos e reprodutivos. Os resultados validam a contribuição da análise de crescimento de plantas de soja para identificar as respostas causadas pela inoculação de sementes com combinação de microrganismos selecionados. Os efeitos dos microrganismos no crescimento das plantas não variam apenas entre os microrganismos, mas também entre as partes das plantas e ao longo do seu desenvolvimento. A introdução de diferentes micróbios na rizosfera de soja, combinados com cepas de Bradyrhizobium sp. pode aumentar a produtividade de massa seca das culturas durante o seu ciclo de crescimento.*

Palavras-chave: *Glycine max, microrganismos promotores de crescimento, matéria seca de parte aérea e raízes, índice de área foliar, taxa de crescimento da cultura.*

INTRODUCTION

Plant growth analysis widely used methodology for estimating the productivity of plants, being necessary to evaluate the development and measure the contribution of different physiological processes on plant growing. Hereupon, dry matter is the ideal variable for measuring plant increment because it reflects the ability of plants to capture solar energy and transform such energy through photosynthesis (HASANAH et al., 2014). The base of this analysis is the periodic determination of

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biomass of the plant's parts, to evaluate its evolution (MAGALHÃES, 1985).

Diverse beneficial mechanisms occur in the rhizosphere of plants and are attributed to some desirable microorganisms, such as the production of phytonutrients, antibiotics, siderophores, solubilization of minerals, antagonism and resistance to phytopathogens, and tolerance to abiotic stresses such as drought and salinity (MARTÍNEZ-VIVEROS et al., 2010). The selective isolation and inoculation of plant growth promoting microorganisms is a promising alternative to increase plant production in more sustainable agricultural systems (STEFAN et al., 2013).

Reported responses attributed to *Azospirillum* spp. inoculation, an important plant growth promoter microorganism, are, among others, the improvement in elasticity of the cell wall, higher root growth and plant height and higher biomass production (CORRÊA et al., 2008). Several studies confirm that *Azospirillum* spp. produces phytohormones that stimulate the growth of the roots of diverse species of plants, by the synthesis of indole-acetic acid (IAA), gibberilines and cytokinins (TIEN et al., 1979). Further, development of the roots by inoculation with *Azospirillum* spp. may generate several other benefits, such as increases in the uptake of water and minerals, increased tolerance to stresses, resulting in a more vigorous and productive plant (BASHAN et al., 2014).

Germination rate and seedling growth indices including root length, seedling length, and dry weight of root and leaf area per soybean plants were increased using *Trichoderma* spp. strains (ENTESARI et al., 2013). MILANESI et al. (2013) also find root growth promotion effects and increased of fresh and dry weight of soybean seedlings inoculated by *Trichoderma* spp.. The chemical communications between these fungi induce several systemic effects in plants, so that, besides only roots are typically colonized, the effects also can be perceived in leaves and stems (HARMAN, 2011).

Bacillus genus demonstrated a variety of plant growth promotion effects on many crop species (JOSEPH et al., 2012). Greenhouse studies showed significant growth stimulus, of multiple growth variables on soybean plants (AKINRINLOLA, 2018). These potential plant growth promoters can usually increase the dry weight of the plant, the production of flower and grain; but many of these studies target the physiological interactions between the microorganisms and the plants, without relating the co-inoculation to some morphological response (PÉREZ-MONTAÑO et al., 2014). JAIN et al. (2016) described *Bacillus* spp. as enhancing shoot and root length, and shoot and root biomass on soybean, besides an increase in number of lateral roots, indicating this genus plant growth promoter capacity.

Penicillium species are specially related and described its capability to phosphorous solubilization, but according to some researchers, this effect may explain only part of the growth promotion of plants caused by them (WHITELAW et al., 1997). Investigating other potential growth promotion mechanisms, WAKELIN et al. (2006) described the *in vitro* production of plant hormone precursors that would stimulate root branching and thus contribute to plant growth (PATTEN & GLICK, 2002). The growth promotion of *Penicillium* spp. isolates was also observed by the enhancement of shoot length, root length, and fresh and dry seedling weight (RADHAKRISHNAN et al., 2014).

The simultaneous inoculation of more than one microorganism, that is, co-inoculation also benefits plant crops of interest, but its efficiency is dependent on factors such as soil characteristics and the stage of plant development at the time of co-inoculation (MWANGI et al., 2013). Likewise, inoculant microorganisms can be used in several agricultural crops, but their effects vary in each plant species (GONG et al., 2014).

New alternatives to increase crop yields are being studied, among them, the combination of more than one inoculant (co-inoculation) in soybean [*Glycine max* (L.) Merrill] is a promising technique, generating gains in root system growth and yield when co-inoculated *Bradyrhizobium* spp. with *A. brasilense* (HUNGRIA et al., 2013; HUNGRIA et al., 2015; QUEIROZ REGO, 2018). However, *A. brasilense* Az39, co-inoculated with *Bradyhizobium* spp., promoted nodulation increase, but did not differ for root length, compared to the treatment inoculated only with *B. japonicum* (CASSÁN et al., 2009). According to DARDANELLI et al. (2008), the effect of the co-inoculation of *Azospirillum* spp. and *Rhizobium* spp. was the increase in root development, nitrogen fixation, and signal molecule production, among other evaluated variables in soybeans and in common beans (*Phaseolus vulgaris*).

Other microorganisms were studied on soybean co-inoculation, as well as *Rhizobium*, *Bacillus subtilis*, *Bacillus thurigiensis*, *Serratia proteomaculans*, *Serratia liquefaciens* and *Pseudomonas aureofaciens*, for example; but, many of these studies target physiological interactions between microorganisms and plants, without relating co-inoculation to some morphological response on plants (PÉREZ-MONTAÑO et al., 2014)

It is known that compensatory soybean growth changes are responsible for the maintenance of yields as changes occur in the crop environment (YUSUF et al., 1999). Among these environmental changes, the availability of nitrogen (N) is one of them and, in this sense, the biological nitrogen fixation (BNF), through the symbiotic relationship between bacteria of the genus *Bradyrhizobium* spp. and soybean is widely diffused in the Brazilian fields. However, there are few studies on growth over the whole cycle of inoculated and co-inoculated soybeans crops. In this perspective, the growth analysis can evaluate the development of the soybean plants during the whole cycle, seeking to identify the changes caused by the treatments tested (CRUZ et al., 2010).

The introduction of selected plant growth promoters in the rizosphere of soybean crops would, differently, modify the growth of the plants during the different growth stages, as well as, between its shoots and roots partition. The use of plant growth analysis approach will provide better information for the soybean responses description to co-inoculation treatments, than independent shoot or root biomass measurements.

This study described the growth of soybean plants inoculated with *Bradyrhizobium japonicum* and *Bradyrhizobium diazoefficiens* in combination with *Azopirillum brasilense*, *Trichoderma asperellum*, *Trichoderma virens, Bacillus amyloliquefaciens* or *Penicillium bilaiae* cultivated under subtropical dry land field conditions.

MATERIALS AND METHODS

The study was performed at the Agricultural Experimental Station Campos Gerais (EEACG, 25º 25 ‘44.33 “ S, 50º 03’ 10.80” W, 836 m above sea level) located in Palmeira, Parana State (Brazil) on soil classified as a Cambisol. The climate in the region is mesothermic subtropical with fresh summers and a mean temperature in the hottest month (January) lower than 22 ºC and without a defined dry season (NITSCHE et al., 2019).

On November 9th of 2015, a soybean crop (cultivar BMX Potência, with maturation group 6.5, indeterminate growth habit, highly branched, high stability and yield potential) was sown at a mean rate of 19 seeds m^{-1} and fertilized with 320 kg ha^{-1} of NPK (02-20-20 formulation) (BRASMAX, 2014). The previous crop was wheat (*Tritichum aestivum* L.) harvested approximately 15 days before planting soybean, weeds were chemically controlled during all the study. The selected soybean genotype, as well as the used crop management practices (i.e. planting date, chemical seed treatment, fertilization, weeds, pest and diseases control, etc.) was currently recommendations to reach high productive crops in the region. The table 1 describes monthly meteorological conditions during soybean crop growth, measured at the site, compared to normal regional conditions.

On the planting day, the seeds were chemically treated with carbendazim (150 g l^{-1}) and with tiram (350 g l^{-1}), at a rate of 2 ml kg^{-1}, soon after drying, over treated with five combinations of selected plant growth promoter microbes (Table 2) mixed with a microbial protectant, rich in sugars, added at a rate of 1.4 ml kg^{-1}. The microorganisms and doses were defined based on previous results of tests realized on green house and growth chamber. At each application, the seeds were agitated inside inflated plastic bags, for complete homogenization, and dried after products application.

The experimental plots (5 lines spaced at 0.45 m and with a length of 12 m) were placed in a randomized complete block design with 5 replicates. Five sequentially succeeding plants were randomly collected from the central 10 m of the second and the fourth line of each plot, alternately in each evaluated stage, maintaining a border of 0.5 m from the previous collected plants. The first plant collection was on November 11th 2015, at VC stage, there were four other collections on around ten days period each until R1. The intervals were of around 20 days for next 4 collections, until R8, on March 03rd 2016.

The entire plants were collected, with soil volume sampled of 0.2 m on each side of the plant, by 0.2 m depth. The roots of the freshly collected plants were kept in mineral water until complete analyzes, which occurred before 48 hours after collection.

The fresh leaves of each plant were identified and analyzed with a scanner and the program WinFolia Pro 2013a (REGENT INSTRUMENTS, 2014), to determine the leaf area (AF in cm^2). The plant structures were separated and dried at 65 ± 4 °C until constant weight, to determine the dry matter of shoot, composed of stems, leaves with petioles, flowers, pods, and grains, when available; dry matter of roots composed of roots and nodules, and total dry matter, the sum of dry matter of shoot and dry matter of roots.

The absolute growth rate ($g.day^{-1}$) was determined by the ratio between the accumulated dry matters during a given period, regardless the amount of previous dry matter, that originated this gain. The absolute root growth rate ($g.day^{-1}$) was calculated by

Table 1 - Average maximum temperature (ºC), average minimum temperature (ºC) and precipitation (mm) in the months of November, December, January, February and March, in the 15/16 harvest and historical average (1989-2015) for the Palmeira region, PR, Curitiba, PR, 2017, NITSCHE et al. (2019).

		---------------------------------------Month---------------------------------------				
		Nov	Dec	Jan	Feb	Mar
Average maximum temperature	Historical average*	24.9	26.3	26.8	26.6	25.7
	Season 2015/16	23.9	25.6	26.6	26.7	25.5
Average minimum temperature	Historical average	14.7	16.2	16.9	17.1	16.1
	Season 2015/16	16.2	18.0	17.3	18.6	16.5
Rainfalls	Historical average	121.9	152.8	205.6	172.7	138.3
	Season 2015/16	173.0	457.5	273.5	295.5	224.0

the ratio between the root dry matter accumulated over a given period.

The leaf area index (m^2 m^{-2}) was calculated by the relation between the sum of the leaf area, and the area of soil that it occupies, indicating its capacity of interception and light absorption (HEIFFIG et al., 2006). The net assimilation rate (g.m.$^{-2}$.day^{-1}) was calculated by the ratio between the accumulation of dry matter of the shoot and the leaf area available in a given period. The leaf area ratio (dm^2.g^{-1}) is the quotient between leaf area and dry matter of the whole plant. The root mass ratio (g.g^{-1}) was calculated by the ratio of root dry matter and total dry matter of the plant.

The temporal variation of the dry matter and the leaf area were adjusted via logistic regression using the equation $y = a.[1 + (X.b^{-1})^{c}]^{-1}$. These analyzes were performed using Statistica Statistical Program v.12 (STATSOFT, 2013) to represent the growth progression along the crop season, where (y) is the predicted response variable as a function of time (X), with the statistically estimated empirical coefficients "a" the maximum dry matter accumulation, "b" the integration parameter, or the moment, in time, which plants are half of total dry matter accumulation, and "c" the maturity rate (YUSUF et al., 1999).

A soybean crop was sown at November 26th of 2014, with same cultivar, fertilization, site, seedling density and previous crop. Same procedure was used to collect plants, and evaluate shoot dry matter, root dry matter, leaf area index and yield. Means were separated using Skott-Knott test, with P>=0,10.

RESULTS

Statistically estimated coefficients "a", "b" and "c" were used to create tendency lines of shoot, root and total dry matter (Table 3).

Table 2 - Treatments, microorganisms and doses used in soybean seeds treatment, at field trial, carried out at Agricultural Experimental Station Campos Gerais, city of Palmeira, PR, season 2015/16. Curitiba, PR, 2019. cfu = colony forming units.

	--------------------------------Inoculant----------------------------------		------------------------Co-inoculant------------------------	
Treatment	Microorganism	dose.seed^{-1}	Microorganism	dose.seed^{-1}
1	*Bradyrhizobium japonicum* SEMIA 5079 and *Bradyrhizobium diazoefficiens* SEMIA 5080	1,200,000 cfu		
2	*Bradyrhizobium japonicum* SEMIA 5079 and *Bradyrhizobium diazoefficiens* SEMIA 5080	1,200,000 cfu	*Azospirillum brasilense* AbV5 and AbV6	23,000 cfu
3	*Bradyrhizobium japonicum* SEMIA 5079 and *Bradyrhizobium diazoefficiens* SEMIA 5080	1,200,000 cfu	*Trichoderma asperellum*	1,500,000 viable spores
4	*Bradyrhizobium japonicum* SEMIA 5079 and *Bradyrhizobium diazoefficiens* SEMIA 5080	1,200,000 cfu	*Trichoderma virens* (GI-3) and *Bacillus amyloliquefaciens* (TJ1000)	14,000 and 71,000 cfu
5	*Bradyrhizobium japonicum* SEMIA 5079 and *Bradyrhizobium diazoefficiens* SEMIA 5080	1,200,000 cfu	*Penicilium bilaiae* P201 and P208	5,000 cfu

Table 3 - Summary of fitted parameters *a*, *b*, and *c* from the model logistic describing the growth of soybean plants inoculated.

	----------------------*a*----------------------				----------------------*b*-----------------------				----------------------*c*----------------------			
	Estimate	Standard error	Confidence limit		Estimate	Standard error	Confidence limit		Estimate	Standard error	Confidence limit	
			Lower	Upper			Lower	Upper			Lower	Upper
SDM1*	1238.4	139.0	968.2	1508.6	85.8	6.6	73.0	98.7	-3.6	0.5	-4.6	-2.7
SDM2	1000.6	123.8	760.0	1241.2	76.3	6.2	64.2	88.4	-4.3	1.0	-6.2	-2.5
SDM3	2018.4	432.8	1177.4	2859.3	125.0	19.9	86.3	163.6	-2.5	0.2	-3.0	-2.1
SDM4	894.8	36.8	823.2	966.4	70.0	2.0	66.1	74.0	-4.2	0.4	-4.9	-3.5
SDM5	1835.0	335.3	1183.4	2486.6	110.1	14.4	82.1	138.1	-2.9	0.3	-3.4	-2.3
RDM1	74.1	6.0	62.3	85.8	48.2	3.5	41.4	55.0	-5.0	1.6	-8.2	-1.8
RDM2	69.5	8.2	53.5	85.5	46.3	5.1	36.4	56.2	-4.8	2.3	-9.3	-0.4
RDM3	69.7	3.5	62.8	76.6	44.6	2.1	40.5	48.7	-5.5	1.3	-8.1	-3.0
RDM4	68.8	7.3	54.7	82.9	45.1	4.4	36.5	53.7	-5.4	2.6	-10.5	-0.4
RDM5	73.0	4.8	63.7	82.4	45.6	2.8	40.1	51.1	-4.7	1.2	-7.1	-2.3
TDM1	1281.9	131.9	1025.5	1538.2	81.7	6.0	70.0	93.4	-3.6	0.5	-4.5	-2.7
TDM2	1063.6	132.2	806.6	1320.5	73.6	6.3	61.3	85.9	-4.2	0.9	-6.0	-2.3
TDM3	1845.0	318.3	1226.5	2463.5	110.5	15.2	80.9	140.0	-2.5	0.2	-3.0	-2.1
TDM4	938.2	35.1	870.0	1006.5	66.5	1.8	63.0	70.0	-4.3	0.4	-5.0	-3.6
TDM5	1759.7	239.2	1294.9	2224.6	100.7	10.4	80.5	121.0	-2.8	0.3	-3.3	-2.3

*SDM = shoot dry matter, RDM = root dry matter and TDM = total dry matter. 1 = *Bradyrhizobium japonicum* and *Bradyrhizobium diazoefficiens*; 2 = 1 and *Azospirillum brasilense*; 3 = 1 and *Trichoderma asperellum*; 4 = 1 and *Trichoderma virens* and *Bacillus amyloliquefaciens*; and 5 = 1 and *Penicillum bilaiae*.

Considering VC stage, when there were minimum values for dry matter; for the shoot observed dry matter, the values were between 4.8 g.m^{-2} and 5.2 g.m^{-2}; for root dry matter 1.05 g.m^{-2} to 1.22 g.m^{-2}, total mass from 5.9 g.m^{-2} to 6.4 g.m^{-2}, and leaf area index from 0.12 m^{2}m^{-2} to 0.13 m^{2}m^{-2}.

The maximum values for shoot dry matter were always got at R8, from 821 g.m^{-2} in treatment with *Bradyrhizobium* spp. and *T. virens* and *B. amyloliquefaciens* to 1067 g.m^{-2} in treatment *Bradyrhizobium* spp. and *P. bilaiae*. For root dry matter, the maximum observed value was reached at R6 in treatments 1 and 2, with 79 g.m^{-2}, and at the earlier stage (R5) in treatments co-inoculated with *T. asperellum*, *T. virens* and *B. amyloliquefaciens* and *P. bilaiae*, with 73 g.m^{-2}, 81 g.m^{-2} and 77 g.m^{-2}, respectively. Total mass was observed at R6 for treatment 2 (*Bradyrhizobium* spp. and *A. brasilense*), with maximum of 957 g.m^{-2}, and at R8 for other treatments, varying from 872 g.m^{-2} to 1129 g.m^{-2}, and higher leaf area index found at R3 for *Bradyrhizobium* spp. alone, with 10.28 m^{2}m^{-2}, and R5 for other treatments, from 10.09 m^{2}m^{-2} to 11.82 m^{2}m^{-2}.

The regression equations present high adjustments to the predicted points for the variables of dry matter of shoot (R^2> 0.98), leaves (R^2> 0.89), roots (R^2> 0,90) and total (R^2> 0,98). The dry matter of shoot at R3 was 400 g.m^{-2} for tested treatments, with a tendency to maintain mass accumulation rates up to R6 (Figure 1A). It was observed that treatments with *T. asperellum* and *P. bilaiae* had a higher mass accumulation in the shoot and total mass after R6, promoted by the increase of mass in the stems and mainly in the grains (Figure 1A and C). These treatments (*T. asperellum* and *P. bilaiae*) also allowed higher points of maximum (coefficient a) and integration parameter (b) (Figure 1).

In the present study, considering the most complete variables, shoot dry matter and total dry matter, in which the masses of vegetative structures (sources) and reproductive structures (drains) are added, growth is observed throughout the whole period studied. As a result, these variables present growth until the end of the cycle, which is adequately represented by the logistic model with R^2> 98%.

A similar trend is observed for the total dry matter variable (Figure 1C), for *T. asperellum* and *P. bilaiae* treatments, since they allowed a greater accumulation of total dry matter in the reproductive period, with 1000 gm^{-2} and 1100 gm^{-2}, respectively, presented a marked difference between treatments in the reproductive phase.

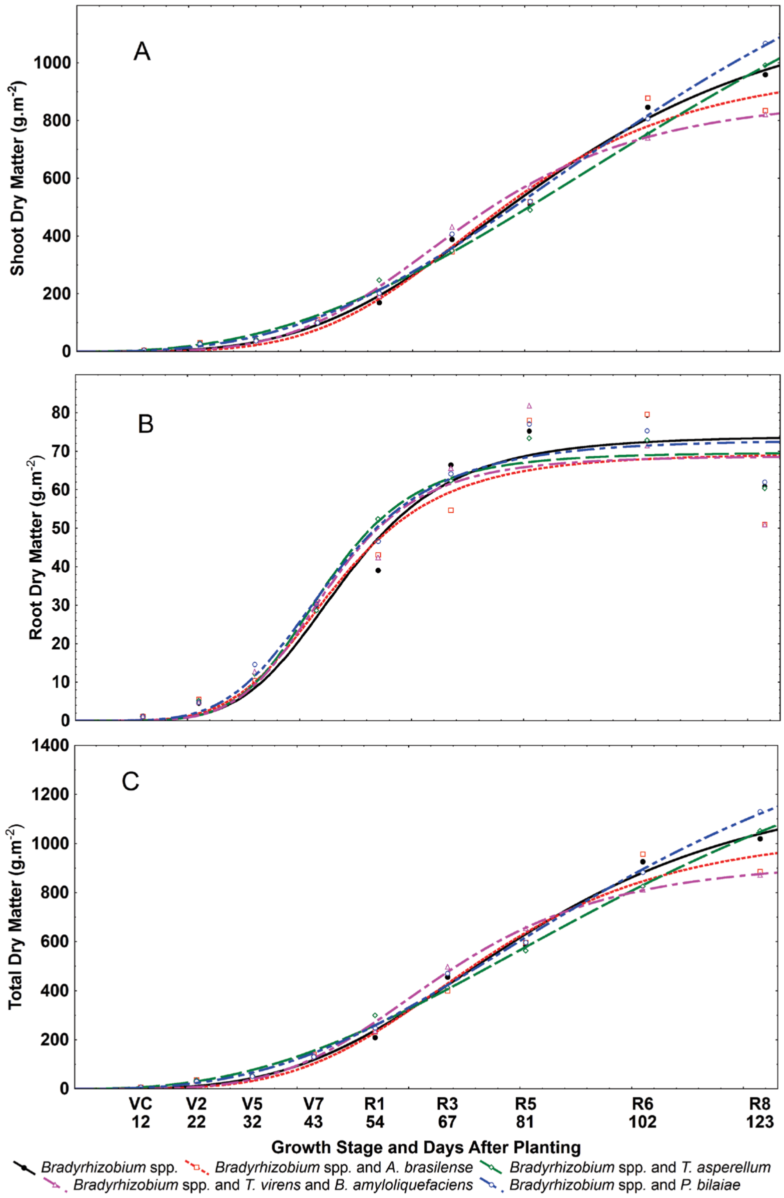

Figure 1 - A) shoot dry matter; B) root dry matter; and C) total dry matter; per square meter, in nine evaluation periods of soybean plants, in Palmeira, PR, season 2015/16. Curitiba, PR, 2019.

The root dry matter of the co-inoculated treatments was higher than the control treatment (*Bradyrhizobium* spp.), up to R1 (Figure 1B).

The absolute growth rate for treatments *T. asperellum* and *P. bilaiae* were higher than other tested treatments until V7 (Figure 2A). From this period, treatments *A. brasilense* and *T. virens* and *B. amyloliquefaciens* were higher, reaching the maximum in R4 and R3, respectively. When comparing root dry matter accumulation (Figure 1 B) at R3, it is observed that these two treatments are below treatment *Bradyrhizobium* spp. alone in main growth stages, with the highest absolute growth rate, indicating reduction of root growth and increase of absolute mass of leaves at this phase.

In R5, treatment *A. brasilense* reached the peak of absolute growth rate (predicted 14.46

g.day^{-1}) and below it is treatment *T. asperellum* in R6 (predicted 11.96 g.day^{-1}) (Figure 2 A), the maximum points and stages were 14.04 g.day^{-1} in R5, 13.98 g.day^{-1} in R3 and 13.24 g.day^{-1} in R6 for treatments *Bradyrhizobium* spp., and co-inoculated with *T. virens* and *B. amyloliquefaciens*, and *P. bilaiae*, respectively. In summary, the leaves of the plants in the treatments *A. brasilense* and *T. virens* and *B. amyloliquefaciens* had a higher growth rate until the first reproductive stages, while the leaves of the plants in treatments *T. asperellum* and *P. bilaiae* presented lower growth rates; however, the growth period was higher, persisting until the grain filling (R5).

The inflection point of the absolute root growth rate curves is between V7 and R1, immediately before the reproductive phase (R1). Co-inoculated treatments anticipate the development stages of the roots in about six days, displacing the maximum points of the absolute root growth rates of these treatments to the left, on V7, when compared to the control (*Bradyrhizobium* spp.), with maximum predicted value at R1.

Roots grown at lower rates, with a maximum of 2 g.day^{-1} between V7 to R1, a low value when compared to absolute growth rate, which presents values above 2 g.day^{-1} from V5 to R8, reaching values more than 7 times greater (Figures 2A and 2B).

The treatments *T. asperellum* and *T. virens* and *B. amyloliquefaciens* had the highest maximum points of absolute root grow rate, indicating that these treatments may have induced an improvement in the physiological processes responsible for this growth (Figure 2 B). These treatments were still accumulating about 10% more dry matter during the end of the vegetative period, compared to the control.

The treatments *T. asperellum* and *P. bilaiae* presented the relative growth rate initially high at VC, predicted respectively 0.137 g.g^{-1}.day^{-1} and 0.099 g.g^{-1}.day^{-1} (Figure 2 C). In V2, treatments *Bradyrhizobium* spp., and co-inoculated treatments *A. brasilense* and *T. virens* and *B. amyloliquefaciens* reach the maximum point, when these treatments presented the relative growth rate predicted values 0.220 g.g^{-1}.day^{-1}, 0.263 g.g^{-1}.day^{-1}, 0.255 g.g^{-1}.day^{-1}. After the period of intense accumulation of dry matter, it reduces in V5, and treatments *T. asperellum* and *P. bilaiae* grow with lower rates. At the reproductive stages, all treatments continue with relative growth rates showing the same trend until the end of the cycle (R8) (Figure 2C).

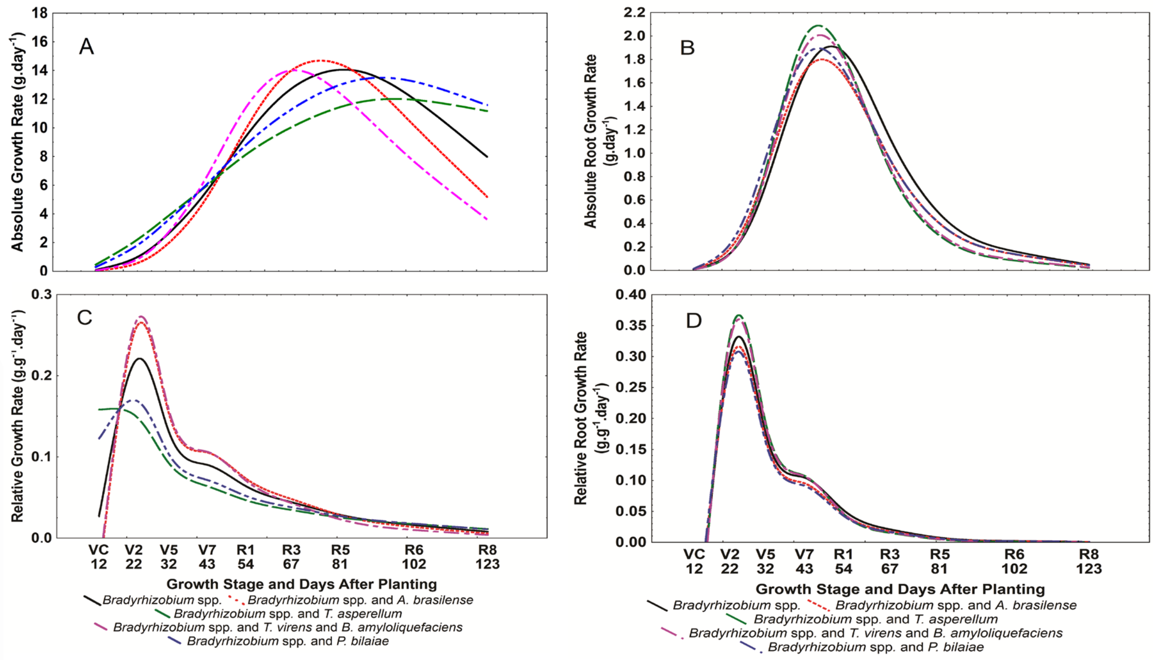

Figure 2 - A) absolute growth rate; B) absolute root growth rate; C) relative growth rate and D) relative root growth rate; in nine evaluation periods of soybean plants, in Palmeira, PR, season 2015/16. Curitiba, PR, 2019.

It was observed that the treatments treatment *T. asperellum* and *P. bilaiae* had a higher absolute growth rate between V2 and V7 (Figure 2A), which is the denominator of the equation for calculating the relative growth rate. Thus, the higher the absolute grow rate, the lower the relative grow rate in that period.

The relative growth rate of roots follows the relative growth rate, in other words, they present a fast growth in the period of crop establishment until reaching the point of maximum that, in the present work, occurred at same grow stage V2, for these two variables. The treatments *T. asperellum* and *T. virens* and *B. amyloliquefaciens* presented a higher relative root growth rate (predicted at V2 with 0.332 $g.g^{-1}.day^{-1}$ and 0.326 $g.g^{-1}.day^{-1}$ respectively).

Leaf area index in of *T. asperellum* is higher than other treatments between V5 and R1. In the following evaluation (R3), *Bradyrhizobium* spp. and *P. bilaiae* treatment appears with higher leaf area index (Figure 3A). The maximum observed points occur in R3 for the control (*Bradyrhizobium* spp.) and in R5, for the treatments co-inoculated. The leaf area index diminished at R5, suggesting physiological maturity and translocation of assimilates to the reproductive parts, later to the grains.

The treatments 2 and 4 presented higher leaf area ratio than other treatments, from the beginning of crop development (VC) to the maximum point at V7 stage for treatments 2 (1.71 $dm^2.g^{-1}$) and 4 (1.59 $dm^2.g^{-1}$) (Figure 3 B).

The treatments *Bradyrhizobium* spp., and co-inoculated *T. asperellum* and *P. bilaiae* had the maximum growth point of leaf area ratio later, at R1, reaching, respectively, 1.39 $dm^2.g^{-1}$, 1.49 $dm^2.g^{-1}$ and 1.42 $dm^2.g^{-1}$, and the reduction of these rates occurred along with the rates of other treatments.

Similar to the leaf area ratio, the root mass ratio of treatment *A. brasilense* was higher than others, reaching maximum point at V5, with a value of 0.31 $g.g^{-1}$, followed by the treatment *T. virens* and *B. amyloliquefaciens*, with maximum point between V5 and V7, both periods with predicted root mass ratio of 0.24 $g.g^{-1}$, followed by treatments *Bradyrhizobium* spp., and co-inoculated with *P. bilaiae* and *T. asperellum*, with their maximum predicted points 0.23$g.g^{-1}$, 0.22$g.g^{-1}$ and 0.20$g.g^{-1}$, respectively at V7 (Figure 3C).

Treatments with *Trichoderma asperellum*; *T. virens* and *Bacillus amyloliquefaciens* and *Penicillum bilaiae* presented higher shoot dry matter and root dry matter for season 14/15, but only *T. virens* and *Bacillus amyloliquefaciens* treatment presented higher leaf area index (Table 4). On following season, 15/16, leaf area index was higher for treatments with *Azospirillum brasilense; Trichoderma asperellum*; *T. virens* and *Bacillus amyloliquefaciens*. *Bradyrhizobium* spp. alone, and together with *Azospirillum brasilense*; *T. virens* and *Bacillus amyloliquefaciens* and *Penicillum bilaiae* presented higher yield.

DISCUSSION

The present study showed that growth analysis enables the perception of differences, on applied treatments, all over plant stages, and in different studied variables. This view of seed treatment products and co-inoculants can help researchers to determine when the best moment is to find the effect, where, and for how long it should be apparent.Knowing the growth changes caused by microorganisms on soybean plants could be an advantage to understand their agronomic effects under extensive field conditions.

The root dry matter of the co-inoculated treatments at the present study was higher than the control *Bradyrhizobium* spp. alone, up to R1 (Figure 1B). Once there is no negative growth, the reduction of observed values of root dry matter, at the final growth stages (R6 and R8), means that the roots were dying, rotting, and lost at sampling moment. Root dry matter is at maximum values at R5, indicating translocation of assimilates to the reproductive structures after that stage. In a situation of water stress, larger roots would be able to better exploit the soil and capture water at greater depths (FARIAS et al., 2007); under these conditions, co-inoculated plants could be benefited by the stimuli of the microorganisms, better supporting possible adverse conditions in the implantation of the crop.

Bacillus genus demonstrated a variety of plant growth promotion effects on many crop species (JOSEPH et al., 2012). Greenhouse studies showed significant growth stimulus, of multiple growth variables on soybean, with differences between *Bacillus* spp. strains on growth promotion, higher for the root growth than for shoot growth. Percent growth increase on root mostly exceeded 90%, whereas it was less than 50 % for shoot growth. None of the strains induced a significant increase in shoot height. (AKINRINLOLA, 2018).

Three isolates were found to increase soybean weight when plants were co-inoculated with one of the isolates and *Bradyrhizobium japonicum* (co-inoculation) under nitrogen-free conditions, compared with plants inoculated with *B. japonicum*

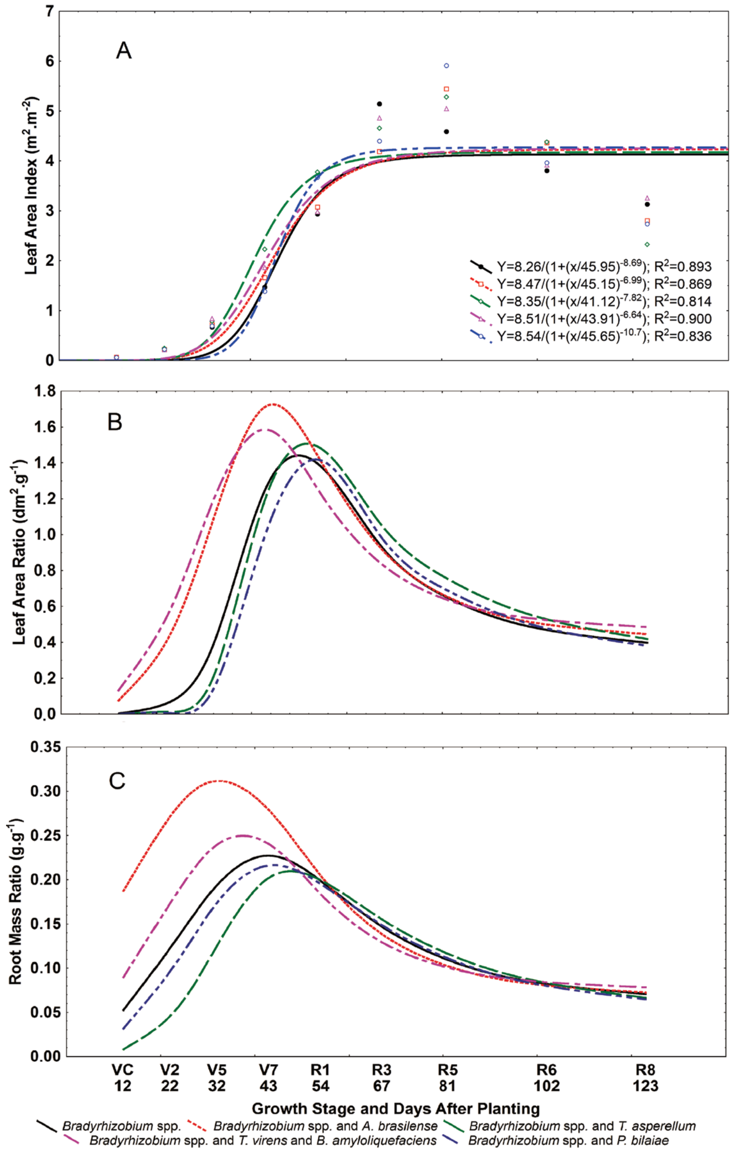

Figure 3 - A) leaf area index; B) leaf area ratio and C) root mass ratio; in nine evaluation periods of soybean plants, in Palmeira, PR, season 2015/16. Curitiba, PR, 2019.

alone. In the absence of *B. japonicum*, these isolates neither nodulated soybean, nor affected soybean growth. All three isolates were Gram-positive spore-forming rods (BAI et al., 2002).

Considering the masses of the roots, stems, leaves and pods, and using data adjusted by an exponential polynomial function, CRUZ et al. (2010) observed the maximum dry matter at 90 days for the first soybean season, evaluated in western Bahia state.

CLAWSON et al. (1986) showed that the aerial plant part dry matter evaluated at 65 days was 400 g.m^{-2} and at 105 days was 800 g.m^{-2}, however, the masses of the reproductive structures (flowers, pods and grains) did not add up to these dry matter values and fall from 115 days after planting. In this sense, the inflection of the regression curve for total dry matter (that includes roots, stem and leaves) occurs at 112 days, regardless of the growth regulator

Table 4 - Shoot dry matter, root dry matter, leaf area index and yield, in crop year 2014/15 and 2105/16 of soybean plants, in Palmeira, PR, Curitiba, PR, 2019.

Crop year	Treatment	Shoot Dry Matter		Root Dry Matter		Leaf Area Index		Yield	
		----------g.plant^{-1}----------		---------g.plant^{-1}----------		---------m^{2}.m^{-2}--------		----------kg.ha^{-1}----------	
2014/15	T1[1]	4.26	b	0.68	b	2.68	b	2330.6	a
	T2	4.10	b	0.70	b	3.16	b	2328.2	a
	T3	4.94	a	0.80	a	2.95	b	2294.0	a
	T4	5.13	a	0.85	a	3.81	a	2433.1	a
	T5	5.00	a	0.80	a	3.02	b	2508.8	a
	CV%	6.38		8.86		9.46		6.2	
2015/16	T1	2.56	a	0.47	a	1.46	b	3170.4	a
	T2	2.58	a	0.50	a	1.65	a	3168.7	a
	T3	2.45	a	0.48	a	1.73	a	2902.5	b
	T4	2.55	a	0.48	a	1.86	a	3063.0	a
	T5	2.29	a	0.50	a	1.38	b	3206.6	a
	CV%	16.17		14.57		16.37		5.9	

[1] T1 = *Bradyrhizobium japonicum* + *B. diazoefficiens*, T2 = T1 + *Azospirillum brasilense*, T3 = T1 + *Trichoderma asperellum*, T4 = T1 + *Trichoderma virens* + *Bacillus amyloliquefaciens* and T5 = T1 + *Penicillum bilaiae*.
Averages followed by same letter do not differ each other (Skott-Knott test, P >= 0.10).

used (CAMPOS et al., 2008). Conversely, when comparing only root dry matter accumulation (Figure 1 B) at R3, it can be observed that all treatments show similar development, indicating the positive effect of the inoculation with *Bradyrhizobium* spp., alone. The application of N and other two treatments with manure were efficient to increase the absolute growth rate, it soybean at 38 days after planting, compared to control without inoculation (HASANAH et al., 2014).

Confirming the results from this study, the values for the absolute growth rate, the maximum point occurs between 12.5 and 19.0 g.day^{-1}, but may vary according to the cultivar (CLAWSON et al., 1986). The growth-promoting microorganisms co-inoculated not only accelerate the development of the roots, strengthening this structure in the vegetative period (BELLONE, 2011), but also make changes in the ratio of plants parts, along plants grow, indicating that plants are always seeking out to restore balance between their parts.

The highest points for absolute root growth rate were reached by treatments *T. asperellum* and *T. virens* and *B. amyloliquefaciens*, probably that the various mechanisms of action attributed to Trichoderma are corroborating that way, such as direct increase of root growth, increase of systemic resistance to diseases, increase in systemic resistance to stresses, better vigor in seeds and increase in N use efficiency (HARMAN, 2011). Also, both *Trichoderma* treatments presented higher root growth rate (0.37 g.g^{-1}.day^{-1}), indicating that these treatments may contribute to root growth (LOBO JUNIOR et al., 2009; HARMAN, 2011).

The treatment inoculated with *B. japonicum* presented relative growth rate superior to the fertilized treatments and control at 25 DAP (HASANAH et al., 2014).

Leaf area index maximum points were between 60 and 90 days after planting and values between 3 and 4 according to MOREIRA et al. (2015). The same trend was observed in no-tillage and conventional tillage soybeans, with a maximum point occurring in R5 (63 DAP), but with a difference between such treatments for leaf area index at R4 phase and maximum values of only 2 m^{2}.m^{-2} (YUSUF et al., 1999).

The leaf area ratio of treatments 2 and 4 may have been favored, between VC and R1, probably, by the mechanisms of action of *A. brasilense* and *B. amyloliquefaciens*, especially by phytormons production (PERRIG et al., 2007). In this sense, the application of plant regulators, with similar functions, can increase the leaf area ratio in soybean between 60 and 86 days after sowing (CAMPOS et al., 2008).

Like the leaf area ratio, the root mass ratio of treatment *A. brasilense*, followed by treatment *T. virens* and *B. amyloliquefaciens*, were highest. This increase of the root mass ratio and increase of the root system in the early stages of development may contribute to the establishment of the crop, fundamental to maintain the stand and the desired productive potential (FARIAS et al., 2007).

At R1 all treatments showed the same trend of leaf area ratio and root mass ratio. At this stage, the root dry matters are at their maximum points. With the conversion of growth to the reproductive portions, the variables shoot dry matter and total dry matter continues to increase, while growth in vegetative portions of the plant drops and tends to cease. In the case of root mass ratio, after R1, all treatments present a decrease in these rates and, between R5 and R8, the maintenance of some roots seems to occur to maintain the minimum functions from the plant until the end of the cycle.

The changes of growth variables on soybean plant and its correlation were described by NAOE et al. (2019) studying coinoculation with *Azospirillum brasilense*, found a positive correlation between root length and yield. In the present study, shoot and root matter gain did not represent a yield gain, probably because there was no drought during this crop growth (Table 4).

Robust trials and data need to be used, over seasons and environments, in order to confirm yield gains of coinoculation on soybean, although the interference of co-inoculated microorganisms on plant growth variables is clear.

CONCLUSION

The microorganisms *Trichoderma asperellum* and *Penicillium bilaiae*, co-inoculated with *Bradyrhizobium* spp. increased the absolute growth rate up to V5 and net assimilation rate throughout the vegetative phase and provided a greater shoot and total dry matter accumulation at the end of the reproductive period.

The microorganisms *Azospirillum brasilense*; *Trichoderma virens* and *Bacillus amyloliquefaciens* co-inoculated with *Bradyrhizobium* spp. in soybean seeds can increase the relative growth rate between V2 and R1, the root mass ratio in the vegetative phase, and the growth rate between V7 and R5.

The co-inoculation of *Trichoderma asperellum* and *Trichoderma virens* and *Bacillus amyloliquefaciens* increased the absolute root growth rate between V7 and R1, and the relative growth rate of roots between V3 and V4.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final.

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