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Path Analysis in Soybean Cultivars Grown under Foliar Spraying and Furrow Inoculation with *Azospirillum brasilense*

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Abstract

The objective of this study was to evaluate, through path analysis, the influence of agronomic characters as a function of foliar spraying and furrow inoculation by *Azospirillum brasilense* on soybean yield. Two experiments were conducted in the crop years of 2013/14 and 2014/15, grown in Lavras, Minas Gerais. In the first experiment, the experimental design was a randomized block in a factorial 4 × 6, four cultivars (Anta 82 RR®, BRS Favorita RR®, BRS 780 RR®, BRS 820 RR®) and six doses of *A. brasilense* (0, 300, 400, 500, 600, 700 mL ha^{-1}), with three replications. In the second experiment, the experimental design was a randomized block design, arranged in a 4 × 2 factorial scheme, four cultivars (Anta 82 RR®, BRS Favorita RR®, BRS 780 RR®, BRS 820 RR®) and two treatments with *A. brasilense* (inoculated and non-inoculated) with three replications. For both experiments, it was established plant height, phytomass of the aerial part, plant height at harvest, first legume insertion, number of legumes, number of grains per legume, mass of one thousand grains and grain yield. In the study with foliar spraying of soybean with *A. brasilense*, plant height at harvest was the only variable that had a direct effect on soybean grain yield. As such, in the study with furrow inoculation of *A. brasilense* in soybean, plant height at harvest and number of vegetables were the variables with the greatest direct effects on soybean grain yield.

Keywords: *Glycine max*, indirect selection, productivity, multicollinearity, variability

1. Introduction

Soybeans [*Glycine max* (L.) Merrill] are one of the world's largest oilseeds. Its high protein content allows it to have multiple applications in human and animal feeding, being of great socioeconomic importance for Brazilian agribusiness due to its high productive potential (Cruz et al., 2016; Gallon et al., 2016). The productive chain of Brazilian soy has undergone modernization processes, which provide the increase in grain yield (Zuffo et al., 2015a).

The introduction of bacteria of the genus *Bradyrhizobium*, which perform the biological nitrogen fixation (BNF), was one of the main propellants for large-scale soybean cultivation in Brazil (Zuffo et al., 2015b). However, when considering the current and potential limitations of FBN with soybean and the benefits attributed to different cultures by inoculation with *Azospirillum brasilense*, it is concluded that the use of both bacterium can increase the performance of the soybean crop (Hungria et al., 2007). In grasses, *Azospirillum brasilense* bacteria has provided a significant increase in grain yield (Braccini et al., 2012; Cotrim et al., 2016).

On the other hand, the improvement of the agronomic characters and the final grain yield results from the interaction of several genetic, physiological and environmental factors, and cannot be considered in isolation

(Gondim et al., 2008). Generally, the relations between the characters are evaluated by phenotypic, genotypic and environmental correlations (Espósito et al., 2011).

The correlation between agronomic characters and grain yield has been the object of study in some studies (Alcântara Neto et al., 2011; Nogueira et al., 2012). Despite the usefulness of these estimates in the understanding of a complex character such as grain yield, they do not determine the relative importance of the direct and indirect influences of these characters that compose grain yield (Alcântara Neto et al., 2011).

Because the correlations are not measures of cause and effect, it is necessary to consider some effects that can cause misunderstandings in the strategies of selection of the evaluated characteristics when performing the direct interpretation of its magnitudes. The high or low correlation between two characters can be arising from the indirect effect of a third character on them, or a group of characters (Cruz et al., 2004; Nogueira et al., 2012). In this sense, path analysis is essential for a better understanding of the phenomena of associations between variables (Espósito et al., 2011).

Created by Wright (1921), path analysis allows the correlation coefficients to be split in the direct and indirect effects on a basic variable, where the estimates were obtained through regression equations, and in which the variables are previously standardized (Alcântara Neto et al., 2011).

Based on the above considerations, the aim of this study was to evaluate the influence of agronomic characters, as a function of foliar spraying and furrow inoculation of *Azospirillum brasilense*, as well as the contribution of these characteristics to grain yield in soybean crop.

2. Material and Methods

2.1 Location and Characterization of Experimental Area

The experiments were conducted in the crop years of 2013/14 and 2014/15, in Lavras, MG, in Scientific and Technological Development Center of Agriculture, Fazenda Muquém, located at latitude 21°14′ S, longitude 45°00′ W and altitude of 918 m. Its soil is classified as typical Dystroferric Red Latosol-LVdf (Emprapa, 2013), with clay texture, with the following textural values: Clay: 640 g kg^{-1}; Silt: 200 g kg^{-1}; Sand: 160 g kg^{-1}. The soil chemical composition of the experimental area is presented in Table 1.

Table 1. Chemical composition of a typical Red Distroferric Oxisol (0-0.20m) before the experiment, during 2014/14 and 2014/15 crop years

Crop year	pH	Ca^{2+}	Mg^{2+}	Al^{3+}	$H^{+}+Al^{3+}$	SB	CTC	P	K	OM	V
	H_2O	-------------------------- $cmol_c$ dm^{-3} -------------------------						--- mg dm^{-3} ---		dag/kg^{-1}	%
2013/14	6.4	5.0	1.4	0	2.9	6.7	9.6	11.4	118	3.4	69.8
2014/15	6.2	3.8	0.8	0	0.9	4.8	5.7	20.8	92	2.2	83.5

Note. H + Al: potential acidity; SB: sum of basis; CTC: cation Exchange capacity at pH 7.0; OM: organic matter; V: basis saturation index.

The climate is Cwa, according to Köppen classification, with average annual temperature and 19.3 ºC normal annual rainfall of 1,530 mm (Dantas et al., 2007). The climatic data during the condition of the experiments were collected at the meteorological station of the National Institute of Meteorology (INMET) located at the Federal University of Lavras-UFLA and are presented in Figure 1.

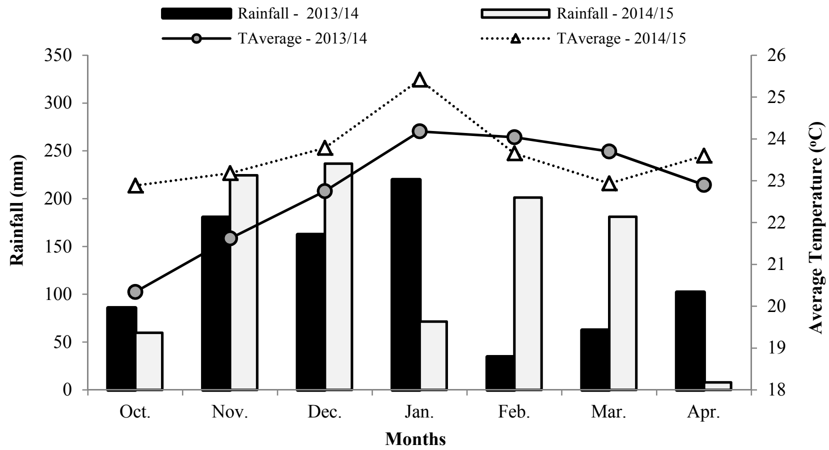

Figure 1. Means for rainfall and air temperature in Lavras, MG, Brazil during 2013/14 and 2014/15 crop years in Lavras, MG, Brazil during the experiments conduction. Source: National Institute of Meteorology (INMET)

2.2 Installation and Conduction of Experiments

In both experiments, the plot consisted of 4 sowing lines of 5 m in length, spaced 0.50 m, the area of each plot being 10 m^2 (5 m × 2 m). As a useful area, we considered the two central rows. The fertilization consisted of 350 kg ha^{-1} of the formulation N-P_2O_5-K_2O (02-30-20), applied through the furrow.

For the experiment with soybeans cultivated under aerial spraying of *A. brasilense* the *Bradyrhizobium japonicum* bacteria were inoculated through the furrow after sowing the soybean. The dose of *B. japonicum* was 18 mL p. c. kg^{-1} of seed-SEMIA 5079 and 5080 strains, containing 10.8 × 10^6 UFC/seeds of Nitragin Cell Tech HC® inoculant (3 × 10^9 UFC/mL). The *A. brasilense* was applied at the V3 stage (second open trefoil); it was used Azo® inoculant (1 × 10^8 UFC/mL) AbV_5 and AbV_6 strains.

In the experiment with soybean cultivated under furrow inoculation with A. *brasilense*, the bacteria *B. japonicum* (Brad) and *A. brasilense* (Azos) were inoculated through the furrow after sowing of the soybean. The doses of *B. japonicum* was 18 mL p. c. kg^{-1} of seed-SEMIA 5079 and 5080 strains, contendo 10.8 × 10^6 UFC/seeds of Nitragin Cell Tech HC® inoculant (3 × 10^9 UFC/mL). For *A. brasilense* was used the dose of 6 mL p. c. kg^{-1} of seed-AbV_5 and AbV_6 strains, contendo 24 × 10^4 UFC/sementes do inoculante Azo® (1 × 10^8 UFC/mL).

The inoculation of the microorganisms was performed using a motorized costal sprayer, attached to the XR 110.02 bar with four spraying tips, applying a volume of syrup equivalent to 150 L ha^{-1}. At first, *B. japonicum*, and later *A. brasilense*, were inoculated.

2.3 Measurement of Characters

For both experiments, at the beginning of flowering (R_1) was determined in five plants per plot: plant height—using a millimeter ruler; aerial phytomass—using a forced air circulation oven, at 60 ºC, for 72 hours, until obtaining a constant mass, with subsequent weighing of the vegetable residues. The leaves were also collected (third trifolium from top to bottom), after which they were washed in deionized water and placed together with dry aerial phytomass for drying. The dried leaves were ground in a Wiley type mill. The chemical analyzes of nitrogen foliar tissues were performed according to the methodology described by Sarruge and Haag (1974). The leaf chlorophyll content—using a portable chlorophyllometer SPAD 502 Plus® model, measuring 3 points on each leaf of the trefoil, in different parts on the same leaf, always in the leaf limb between the ribs in the third trefoil from top to bottom. At the time of harvest the following characters were obtained: height of plants and insertion of the first legume—using a millimeter ruler. Subsequently, five plants per plot were collected to evaluate the number of vegetables per plant and number of grains per legume—through manual counting; mass of a thousand grains—according to the methodology described in Brazil (2009); grain yield—standardized for grain moisture of 13% in Kg/ha^{-1}.

2.4 Experimental Design and Statistical Analyzes

For the experiment with soybean cultivated under aerial spraying of *A. brasilense*, the experimental design was a randomized block design, arranged in a 4 × 6 factorial scheme, four cultivars (Anta 82 RR®, BRS Favorita RR®, BRS 780 RR®, BRS 820 RR®) and six doses of *A. brasilense* (0, 300, 400, 500, 600, 700 mL ha^{-1}), with three replications.

In the experiment with soybean cultivated under furrow inoculation with *A. brasilense*, the experimental design was a randomized block design, arranged in a 4 × 2 factorial scheme, four cultivars (Anta 82 RR®, BRS Favorita RR®, BRS 780 RR®, BRS 820 RR®) and two treatments with *A. brasilense* (inoculated and non-inoculated), with three replications.

The path correlations were calculated, verifying the direct and indirect effects of these on productivity. Path correlations were obtained according to the method proposed by Wright (1921). The statistical analyzes were performed with the help of the Genes computational application (Cruz, 2013). Before the path analysis, a multicollinearity diagnosis was performed, as detailed in Cruz and Regazzi (1997).

The degree of multicollinearity of the correlation matrix between the independent variables of the regression model was established based on its number of conditions, which is the ratio between the highest and the lowest eigenvalue of the phenotypic correlation matrix. Thus, when the number of conditions is less than 100, the multicollinearity is weak and does not cause problem for the analysis; when it is between 100 and 1.000, multicollinearity is moderate to strong; and when it is greater than 1.000, multicollinearity is severe (Montgomery & Peck, 1981).

3. Results and Discussion

3.1 Experiment 1

Path analysis investigates cause and effect relationships and provides the phenotypic coefficients. In the study with foliar spraying of soybean with *A. brasilense*, before conducting the path analysis to evaluate the direct and indirect effects of agronomic characters on soybean yield, the number of conditions of the matrices of phenotypic correlations performed multicollinearity. When using the eigenvalue and eigenvector test of the correlation matrix in the primary independent variables of this model, it was observed that the number of conditions (NC) was equal to 155569.97, which represents a severe multicollinearity. Thus, the crest regression (Hoerl & Kennard, 1970) was used to estimate the coefficients of the model, and thus to circumvent the effects of multicollinearity. Therefore, it adopted the value of $k = 5.84 \times 10^{-4}$ (Table 2) Due to the presence of severe multicolinearity, these proved unstable as K increased, stabilizing $k = 5.84 \times 10^{-4}$, as can be seen from examination of crest trace (Figure 2).

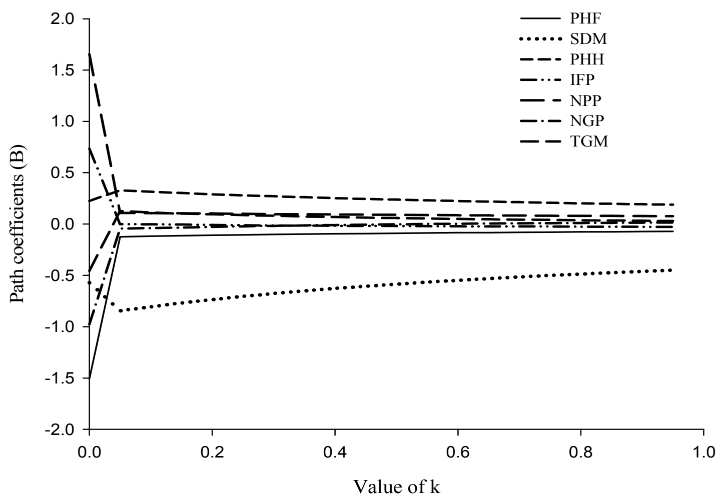

Figure 2. Estimates path coefficients depending on the values of k and obtained from the analysis using as a basic variable the logarithm of productivity, for the experiment of soybeans grown in *A. brasilense* aerial spraying on 2013/14 and 2014/15 crop years

Table 2 shows the unfolding of the phenotypic correlations in components of direct and indirect effect of the explanatory variables on soybean productivity under *A. brasilense* foliar spraying. The value of inflation of variance was below ten, so the path analysis is valid after the value of k is equal to 5.84×10^{-4}. The high value of the coefficient of determination (R^2) in the path analysis model (0.922) and the low effect of the residual variable

(0.279) showed that the explanatory model adopted expressed the relation cause of effect of the primary variables and grain yield of soybean. Thus, through R^2, it can be observed that these variables explained 92.2% of the grain yield variation.

Table 2. Unfolding of phenotypic correlations in direct effect components (diagonal) and indirect of PHF, SDM, PHH, IFP, NPP, NGP and TGM independent variables on the main dependent variable GHI, for the experiment of soybeans grown in aerial spraying *A. brasilense* crop years of 2013/14 and 2014/15

Variable	PHF	SDM	PHH	IFP	NPP	NGP	TGM	Total effect
PHF	<u>-0.118</u>	-0.4	0.216	-0.004	0.08	-0.005	0.077	-0.201
SDM	-0.063	<u>-0.8</u>	-0.033	-0.002	0.011	0.004	0.036	-0.932
PHH	-0.08	0.08	<u>0.318</u>	-0.002	0.104	-0.029	0.015	0.439
IFP	-0.104	-0.4	0.118	<u>-0.005</u>	0.041	0.015	0.108	-0.205
NPP	-0.089	-0.1	0.31	-0.002	<u>0.107</u>	-0.031	0.012	0.228
NGP	-0.014	0.08	0.208	0.002	0.075	<u>-0.044</u>	-0.07	0.228
TGM	-0.079	-0.3	0.042	-0.005	0.011	0.027	<u>0.114</u>	-0.137
VIF	9.62	2.79	8.5	9.24	9.15	7.83	8.23	
Coefficient of determination (R^2) = 0.922								
Effect of the residual variable = 0.279								
Value of k used in the analysis = 5.84×10^{-4}								

Note. PHF: plant height on flowering; SDM: shoot dry mass; PHH: plant height at harvest; IFP: insertion of the first pod; NPP: number of pods per plant; NGP: number of grains per pod; TMG: one thousand grain mass; GHI: grain harvest index.

The variable plant height at harvest (PHH) was the only one that showed a direct effect on the productivity of soybeans (GHI) with a value of 0.318, being higher than the effect of residual variability (0.279). However, this same variable did not present any indirect effect on GHI. The other variables did not present any direct effect on the GHI, always having their values lower than the effect of the residual variable (Table 2). These results differ from those found by Alcântara Neto et al. (2011), because in their studies the variable that had greater influence on soybean yield was the number of pods per plant, due to have greater direct effect on dry matter yield and total grain per hectare, besides suffering indirect effect of number of nodes and plant height. However, according to Carvalho et al. (2002), the plant height at flowering can assist in the selection for productivity.

The variable number of pods per plant (NPP) despite its low estimate of direct effect (0.107) on the GHI was the only exercise indirect effect via PHH (0.31), and its higher coefficient to the residual effect (Table 2). However, in a study by Alcântara Neto et al. (2011), the number of pods per plant had greater direct effect on dry matter yield and total grain per hectare, consequently, higher productivity. When a character positively correlates with ones and negatively with others, extra care must be taken, for selecting one character may cause undesirable changes in others (Coimbra et al., 2005; Hoogerheide et al., 2007).

Therefore, as the variables: plant height on flowering (PHF), shoot dry mass (SDM), insertion of the first pod (IFP), number of grains per pod (NGP) and one thousand grain mass (TGM) had direct effects and indirect effects on grain yield below the residual effect. They are considered insignificant and should not be indicated for direct and indirect selection in studies with aerial spraying of soybean with *A. brasilense* (Table 2). According to Petter et al. (2014), the height of the soybean in the flowering, the dry weight of the aerial part in the flowering and the number of pods per plant do not exert a direct influence on the productivity.

3.2 Experiment 2

For the study with the inoculation of *A. brasilense* in the furrow soybean, previously the path analysis to evaluate the direct and indirect effects of agronomic characters on soybeans was also performed, the multicollinearity was also evaluated using the eigenvalues and eigenvectors test correlation matrix in the primary independent variable, reaching a number of conditions (NC) 159,457.57 equal to, or severe multicolinearity. Thus, the crest regression was used (Hoerl & Kennard, 1970), adopting the value of $k = 6.64 \times 10^{-4}$ for the estimation of the path coefficients (Figure 3).

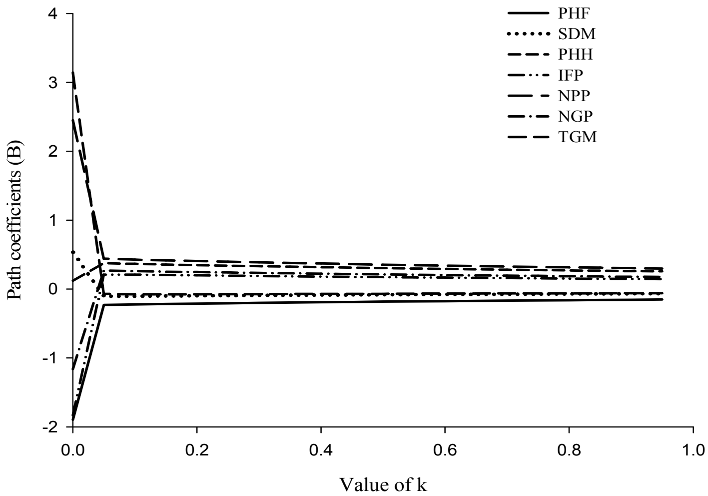

Figure 3. Estimates path coefficients (B) as a function of k values obtained from the analysis using as a basic variable the logarithm of productivity, for the experiment of soybeans grown under planting furrow spraying with *A. brasilense* in crop years of 2013/14 and 2014/15

The unfolding of the phenotypic correlations in components of direct and indirect effects of the explanatory independent variables on the main dependent variable GHI is shown in Table 3. The values of the inflation of variance again were below ten, and the analysis of path is valid when it is used $k = 6.64 \times 10^{-4}$. The coefficient of determination (R^2) in the path analysis model was equal to 0.955 and the effect of the residual variable was 0,084. Thus, the existence of strong cause and effect relationships between the variables under study and the grain yield of the soybean is observed. R^2 shows that 95.5% of productivity is explained by the effect of the analyzed variables.

The variables: plant height at harvest (PHH); insertion of the first pod (IFP); number of pods per plant (NPP); and number of grains per pod (NGP) had a direct effect on GHI, with values of 0.366; 0.212; 0.448; and 0.262, respectively, being higher than the effect of the residual variable (0.084). Thus, the variable that had the greatest effect on soybean grain yield was NPP, presenting the highest direct effect estimate (0.428), followed by PHH, whose direct effect was (0.366) (Table 3). Therefore, PHH, IFP, NPP and NGP variables can be considered as good options for the soybean improvement, in the case of direct selection for productivity. Alcântara Neto et al. (2011) found similar results, where the variable that exerted the greatest influence on soybean yield was the number of pods per plant, since it had a greater direct effect on dry matter and total grain yield per hectare.

Table 3. Unfolding of phenotypic correlations in direct effect components (diagonal) and indirect effect components of the independent explanatory variables PHF, SDM, PHH, IFP, NPP, NGP and TGM on the main dependent variable GHI, for the experiment of soybeans cultivated under spray through sowing furrow with *A. brasilense* in the crop years 2013/14 and 2014/15

Variable	PHF	SDM	PHH	IFP	NPP	NGP	TGM	Total effect
PHF	<u>-0.223</u>	0.02	-0.131	0.112	0.049	-0.192	-0.04	-0.42
SDM	0.046	<u>-0.1</u>	0.197	-0.165	-0.04	-0.134	0.073	-0.135
PHH	0.08	-0.1	<u>0.366</u>	-0.023	0.309	-0.019	0.06	0.748
IFP	-0.118	0.08	-0.041	<u>0.212</u>	0.259	0.017	-0.06	0.371
NPP	-0.025	0.01	0.264	0.128	<u>0.428</u>	-0.013	0.009	0.838
NGP	0.164	0.05	-0.026	0.014	-0.021	<u>0.262</u>	-0.02	0.449
TGM	-0.104	0.1	-0.279	0.15	-0.052	0.062	<u>-0.08</u>	-0.212
VIF	7.46	9.29	8.19	8.71	6.95	6.67	9.67	

Coefficient of determination (R^2) = 0.955
Effect of the residual variable = 0.084
Value of k used in the analysis = 6.64×10^{-4}

Note. PHF: plant height on flowering; SDM: shoot dry mass; PHH: plant height at harvest; IFP: insertion of the first pod; NPP: number of pods per plant; NGP: number of grains per pod; TMG: one thousand grain mass; GHI: grain harvest index.

In addition to the direct effect, the PHH and IFP variables exerted indirect effect on productivity, through NPP, with values of 0.309 and 0.259, respectively, in the same way that the NPP, which in addition to the direct effect, also caused indirect effect on GHI, however, via PHH (0.264) and IFP (0.128). The NGP also had indirect effect on productivity only via plant height on flowering (PHF) (0.164) (Table 3). Therefore, additional care should be taken in the choice of a particular character, as this may cause undesirable changes in others (Coimbra et al., 2005). According to Zuffo et al. (2016) the association between agronomic characteristics is important because it allows to verify the degree of interference of a characteristic over another one of economic interest, as well as to practice the indirect selection.

Despite having a low estimate of direct effect (-0.223) on GHI, PHF exerted an indirect effect via IFP (0.112), its coefficient being greater than the residual effect (0.084). Shoot dry mass (SDM) also had no direct effect on GHI, but presented an indirect effect via PHH (0.197). The same behavior was observed for the one thousand grain mass variable (TGM), which, although it did not have a direct effect on GHI, exerted an indirect effect, however, via SDM (0.1) and IFP (0.15).

Therefore, as all variables (PHF, SDM, PHH, IFP, NPP, NGP, TGM) exercised indirect effect on productivity, all can be considered as a good option in soybean, in the case of breeding purposes, with (PHH), which had the highest indirect effect on productivity (0.309), via NPP.

PHF was the variable that had the least direct and indirect effects, proving to be a variable that shows little cause and effect relation on the studied variables. This result supports those found by Alcântara Neto et al. (2011), where the height of the first pod was the variable that had the least direct and indirect effects.

4. Conclusions

Path analysis indicates that the agronomic characters of the plant with the highest estimates of direct or indirect effects, regardless of the application of *A. brasilense*, air or leaf, will culminate in a higher favorable effect on grain yield in soybean. The plant height at harvest has a direct effect on the productivity of soybean when cultivated under foliar spraying to *A. brasilense*. The plant height at harvest, the insertion of the first pod, the number of vegetables and the number of grains per pod have direct effects on the productivity of soybean when grown under the soil inoculation of *A. brasilense*.

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