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SEED INOCULATION WITH *Azospirillum brasilense*, ASSOCIATED WITH THE USE OF BIOREGULATORS IN MAIZE[1]

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ABSTRACT - The inoculation of seeds with the bacterium *Azospirillum* has been carried out in maize culture and other grasses. The application of growth bio-regulators is another technology whose results in maize culture have yet to become more widespread. Current study evaluates the agronomic effectiveness of seed inoculation with *Azospirillum brasilense* in maize, associated with the use of the growth regulator Stimulate ®. Triple hybrid maize CD 304 underwent the following treatments: 1 - control without nitrogen and without *Azospirillum brasilense*; 2 - Treatment without nitrogen but with *Azospirillum brasilense*; 3 - Treatment without nitrogen but with *Azospirillum brasilense* + Stimulate ®; 4 - Treatment with 50% of nitrogen dose recommended for maize culture; 5 - Treatment with 50% of nitrogen dose and inoculation with *Azospirillum brasilense*; 6 - Same as 5 but with Stimulate ®; 7 – Total N recommended; 8 – Total N recommended + *Azospirillum brasilense*; 9 – Total N recommended + *Azospirillum brasilense* + Stimulate ®. The inoculation of maize seeds with *Azospirillum brasilense* increases plant height and grain yield when compared with rates in control. The use of 50% of N dose in sowing, associated with the inoculation of maize seeds with *Azospirillum brasilense* at 200 mL ha^{-1} (mixed to the seeds) and associated with Stimulate ® (in foliar application), is viable.

Keywords: Maize. Microaerobic diazotrophic bacterium. Bio-regulator.

INOCULAÇÃO DAS SEMENTES COM *Azospirillum brasilense*, ASSOCIADO AO USO DE BIORREGULADOR, NA CULTURA DO MILHO

RESUMO - A inoculação das sementes com bactérias do gênero *Azospirillum* tem sido realizada na cultura do milho e em outras gramíneas, assim como a aplicação de biorreguladores tem sido outra tecnologia disponível, mas com resultados ainda pouco difundidos na cultura do milho. O objetivo deste trabalho foi avaliar a eficiência agronômica da inoculação das sementes com *Azospirillum brasilense* na cultura do milho, associada com o uso do biorregulador Stimulate®. Para tanto, o híbrido triplo de milho CD 304 foi submetido aos seguintes tratamentos: 1 - Testemunha sem nitrogênio e sem *Azospirillum brasilense*; 2 - Tratamento sem nitrogênio, mas com *Azospirillum brasilense*; 3 - Tratamento sem nitrogênio, mas com *Azospirillum brasilense* + Stimulate®; 4 - Tratamento com metade da dose de nitrogênio recomendada para a cultura; 5 - Tratamento com metade da dose de nitrogênio recomendada e inoculada com *Azospirillum brasilense*; 6 - Idem ao anterior, porém com acréscimo do Stimulate®; 7 - N total recomendado; 8 - N total recomendado + *Azospirillum brasilense*; 9 - N total recomendado + *Azospirillum brasilense* + Stimulate®. A inoculação das sementes de milho com *Azospirillum brasilense* promovem aumentos na altura de plantas e no rendimento de grãos do milho, em comparação com a testemunha. A utilização de metade dose de nitrogênio em semeadura, associada à inoculação das sementes de milho com *Azospirillum brasilense*, na dose de 200 mL ha^{-1} (misturado às sementes) e, associado ao Stimulate® (em aplicação foliar), mostra-se viável agronomicamente.

Palavras-chave: Milho. Bactérias diazotróficas microaeróbias. Biorregulador.

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INTRODUCTION

There is currently a growing concern with regard to pollution of the atmosphere and water resources by nitrogenated fertilizers. Since the nutrient may be lost through erosion, leaching, volatilization and de-nitrification (TAIZ; ZEIGER, 2009) and since the product is expensive, other alternatives are sought that would economically maximize the best dose, associated to the nutrient's best application period in maize culture (LOPES et al., 2004; SOUSA; LOBATO, 2004).

Certain microaerobic and diazotrophic bacteria of the genus *Azospirillum*, with their high potential in fixing free atmospheric nitrogen, may be used to achieve the results mentioned above (MIYAUCHI et al., 2008). When these bacteria are associated to the plants' rhizosphere, they contribute towards their nitrogenated nutrition. Biology and soil fertility researchers have shown great interest in the process. The optimization of a possible association of *Azospirillum* spp and maize may increase productivity and decrease production costs, especially in purchasing nitrogenated fertilizers. According to Silva et al. (2005), nitrogen is a mineral nutrient extracted in great amounts by maize, with great influence in grain productivity but with high production costs.

Inoculation by the bacterium *Azospirillum* spp has already been conducted in maize and in other grasses, while several researches have shown an increase of productivity of these cultures due to this technology. Salomone and Döbereiner (1996) analyzed the response of several maize genotypes to the inoculation of four strains of *Azospirillum* spp isolated in Argentina and of three strains of sorghum and maize isolated in Brazil. An increase in grain yield occurred, ranging between 1,700 and 7,300 kg ha^{-1}, with variations according to the different genotypes. Results however are highly affected by soil, environment and genotype conditions.

The use of bio-regulators is another technologically adaptable and compatible practice with high productivity systems for maize culture. Taking into account a determined commercial product, such as Stimulate®, with concentrations of 0.005% indole-butyric acid (IBA) (auxin), 0.009% kinetin (cytokinin) and 0.005% gibberellic acid (gibberellin), synthetic analogues to vegetal hormones which act as mediators for morphological and physiological processes, it is surmised that this bio-regulator may increase growth and vegetal development, stimulate cell division and increase absorption of water and nutrients by plants, due to its composition, concentration and percentage of compounds (VIEIRA; CASTRO, 2001).

Dourado Neto et al. (2006) and Ferreira et al. (2007) achieved positive agronomic results in maize culture and confirmed the efficiency of Stimulate® and other bio-regulators in the treatment of seeds. Several results on seed treatments are extant, especially for soybean (KLAHOLD et al., 2006; ÁVILA et al., 2008; MOTERLE et al., 2008), cotton (SANTOS; VIEIRA, 2005; ALBRECHT et al., 2009) and forest essence (PRADO NETO et al., 2007) cultures.

Research that contributes towards the formation of a solid positioning with regard to the use of bio-regulators, such as Stimulate®, and the association of bio-regulators with the nutritional management of nitrogen comprising alternatives such as the use of *Azospirillum* spp in seed inoculation is highly valid. Current research evaluates the agronomic efficiency of seed inoculation by *Azospirillum brasilense* associated with the bio-regulator Stimulate® in maize culture.

MATERIALS AND METHODS

Experiment was carried out during the agricultural year 2008-2009 on the Iguatemi Experimental Farm of the Universidade Estadual de Maringá (UEM), Maringá, northwestern region of the state of Paraná, Brazil, at 23º25' S and 51º57' W, and average 540 m above sea level.

Soil of the experimental area has been classified as Red Dystrophic Argisol (EMBRAPA, 2006) of medium texture. Table 1 shows the chemical analysis of soil previous to the experiment.

According to Köppen's classification, prevalent region climate type is Cfa, humid mesothermal climate, with abundant rainfall during the hot summers and with dry winters (IAPAR, 1994). Table 2 provides local data for rainfall, daily maximum and

Table 1. Results of soil fertility at the 0 - 20 cm layer for Red Dystrophic Argisol before culture.

Depth	P[1]	pH[2]		H^++Al^{3+}	Al^{3+}	K[1]	Ca[3]	Mg[3]	SB	CTC	V	C[4]
cm	mg dm^{-3}	$CaCl_2$	H_2O	---------------------- $cmol_c$ dm^{-3} ----------------------							%	g dm^{-3}
0 – 20	4.7	5.2	6.0	3.42	0.0	0.27	2.74	1.23	4.24	7.66	55.35	8.52

[1] - Extractor Mehlich 1; [2] - $CaCl_2$ 0.01 mol L^{-1}; [3] - KCl 1 mol L^{-1}; [4] - Walkley-Black method.

Table 2. Mean monthly data of maximum and minimum temperature, rainfall and relative air humidity in the morning and evening, during the experimental period. (Maringá, PR – 2008/2009)[1].

Month	Temperature		Relative humidity		Rainfall
	Minimum	Maximum	Morning	Evening	
	(°C)	(°C)	(%)	(%)	(mm)
November	29.4	18.4	80.3	54.8	137.4
December	31.9	19.3	75.4	50.8	53.4
January	28.3	19.5	84.1	68.4	248.9
February	29.9	20.6	89.0	63.6	227.3
March	28.5	20.5	80.6	57.1	56.9

[1] Source: Meteorological Station of the Iguatemi Experimental Farm (UEM)/Seed Laboratory.

Table 3. Inoculation treatments of maize seeds with *Azospirillum brasiliense* with Stimulate® associations.

Nº	Treatments
1	Control without N and *Azospirillum*
2	Without N but with *Azospirillum*
3	Without N but with *Azospirillum* + Stimulate®
4	With 50% N dose recommended for culture
5	With 50% N dose and inoculate with *Azospirillum*
6	50% N dose + *Azospirillum* + Stimulate®
7	With total N recommended for culture
8	Total N recommended + *Azospirillum*
9	Total N recommended + *Azospirillum* + Stimulate®

minimum temperature and relative air humidity, during the period of the experiment.

Treatments, arranged according to scheme given in Table 3, consisted of nitrogen fertilization in sowing, topdressing and seed inoculation by *Azospirillum brasilense* bacteria in liquid formulation (Table 4), combined or not with the bioregulator Stimulate®.

Stimulate® is a liquid bio-regulator manufactured by Stoller do Brasil Ltda and is composed by three vegetal regulators at concentrations: 0.005% indole-butyric acid (auxin), 0.009% kinetin (cytokinin) and 0.005% gibberellic acid (gibberellin), (STOLLER DO BRASIL, 1998).

Experimental design consisted of complete randomized blocks and six repetitions. Each plot comprised eight 6 m rows, with 0.9 m spacing, totaling 43.2 m^2 per plot. Seeding density was approximately five plants per linear meter, with a population of 55,555 plants / ha^{-1}.

Table 4. Characterístics of product based on *Azospirillum-brasilense*.

Tested inoculants	Strains	Support
Liquid formulation[(1)] *(Azospirillum brasilense)*	AbV5 and AbV6	Water

Manufacturer: Stoller do Brasil Ltda.
Guarantee of products: concentration of 10^8 viable bacteria per mL;
Dose: 200 mL of the commercial product, per hectare, mixed to seeds.

Fertilization was carried out mechanically according to mean extraction of nutrients by the maize culture for the production of approximately 10.000 kg ha^{-1} of maize associated with information related to soil analysis. Further, 180 kg of nitrogen per ha, 90 kg of phosphorus and 120 kg of potassium per ha were applied (FANCELLI; DOURADO NETO, 2004). Half of the potassium was applied at sowing and the other half as topdressing at the V_4 stage. Total nitrogen recommended for fertilization was parceled as follows: 1/3 at sowing and 2/3 at topdressing. Half the dose was applied in treatments 4, 5 and 6 at sowing and at topdressing. Treatments 7, 8 and 9 received the full recommended nitrogen dose.

No-tillage sowing system was carried out on October 13, 2008 with triple cross hybrid and short season maize seeds CD 304; soil had the required humidity for sowing.

Seed inoculation with *Azospirillum brasilense* formulations was undertaken after their treatment with fungicide Derosal Plus at 2.5 mL kg^{-1} of seeds and with insecticide Cropstar at 300 mL ha^{-1}. The inoculant *Azospirillum* was applied to maize seeds as follows: sugared spray with 10% concentration was used for a better adherence of the inoculant to the seeds. After the seeds and the spray were mixed, the inoculant in liquid formulation was applied until all the seeds were uniformly covered with the mixture. In the case of treatments with *Azospirillum brasilense*, 100 mL of the sugared solution were used for the amount (kg) of seeds per hectare + 200 mL of liquid inoculant.

Weed control was carried out by post-emergence application of Atrazine – Atrazina Atanor® (5 L ha^{-1}) and complemented by hoe weeding. Insecticides were applied when required for pest control (FANCELLI; DOURADO NETO, 2004).

Bio-regulator Stimulate® (treatments 3, 6 and 9) were applied at development stage V_4 with 250 mL ha^{-1} dose. A CO_2-propelled backpack sprayer at a constant pressure of 2 BAR (or 29 PSI), with a discharge of 0.65 L min.$^{-1}$ and equipped with a fan nozzle tube (Teejet XR 110 02), was used for leaf applications. At a 50 cm height from the target and at a velocity of 1 m second^{-1}, the sprayer reached a 50 cm-wide band and provided a spray volume of 200 L ha^{-1}. Climatic conditions were adequate (UR = 84%; temperature = 25 ºC; time schedule = 8:50; partially clouded, with a slight breeze).

During harvest time, on the 19th February 2009, the four external rows (1, 3, 6 and 8) were harvested and the area for the experimental units was reduced to 7.2 m^2 (rows 4 and 5). The analyzed variables were height of plant, weight of one thousand grains and productivity. The other rows (2 and 7) were reserved for dry matter sampling.

Plant height was determined at harvest time by randomly measuring 10 plants within the area of each plot, in meters, from the base to the tassel insertion height. Mean plant height for each plot was then calculated.

The dry matter of plants was limited to the aerial part exclusively during tasseling when samplings of 10 plants per plot were harvested randomly within the experimental area. Samples were labeled in the field and then transported to the laboratory where they were weighed (fresh weight), placed in multilayer Kraft paper bags and dried in a forced air circulation buffer at a 65 ºC till constant weight. They were then weighed by a precision scale and the dry matter of 10 plants was obtained from the dry weight.

Grain ears at the physiological maturation point were collected manually, or rather, when 95% of seeds already exhibited the black layer. After the ear harvest, the ears were threshed in a stationary threshing machine. The grains were then cleaned with sieves, dried naturally and stored in multilayer Kraft paper bags. Grain yield for each plot was weighed by an analytic scale and the humidity rate of seeds was calculated by the buffer method at 105ºC for 24 hours (BRASIL, 2009). Productivity in kg ha^{-1} was provided and yield data were corrected for 14% humidity. The weight of one thousand seeds was determined by weighing 8 sub-samples of 100 seeds for each repetition with a milligram precision analytic scale and multiplying the results by 10 (BRASIL, 2009). Results were given in grams of seeds (g).

Data were submitted to variance analysis at 1% probability ($P < 0.01$). Significant means were compared by Scott-Knott grouping method ($P < 0.01$).

RESULTS AND DISCUSSION

Results of variance analysis (Table 5) showed that there was a significant difference at 1% probability ($p < 0.01$) for the variables plant height, dry matter in tasseling and grain productivity (kg ha^{-1}).

Table 6 shows results of the variables under analysis. With regard to plant height, all treatments differed significantly from control (Treatment 1). Without N, seed inoculation and Stimulate®, control had the lowest height and differed significantly from that of the other treatments ($P < 0.01$). Results characterize the role of the other treatments in nitrogen input for the vegetative performance of maize plants.

Results of the weight of one thousand grains, with a mean rate of 296.26 g, did not show any significant differences ($P < 0.01$) among the treatments. Although there was no difference among treatments for this parameter, in many cases no direct relationship with productivity (kg ha^{-1}) was extant. The interaction of other co-relational parameters should be taken into account since they are also related in a direct form with the final productivity, such as dry mass of the plants. Although weight of grains does not explain productivity rise, increases in the accumulation of dry matter since the start of culture development have also favored an increase of ear size and of the number of grains per ear. This fact establishes favorable factors for productivity increases.

The plants' dry matter distinguished treatments with the best results, namely 3, 5, 6, 7, 8 and 9. The importance of inoculation with *Azospirillum* was highly relevant, since treatments 5 (1/2 dose N + *Azospirillum*) and 3 (*Azospirillum* + Stimulate®, without N) were similar to treatments with total nitrogen dose (7, 8 and 9). It should be emphasized that, even without nitrogenated fertilization, treatment 3 with *Azospirillum* and Stimulate® was higher than treatments 4 (with ½ N dose) and 1 and 2 (without N). Perhaps due to the activity of its analogues of promoting hormones, the addition of Stimulate® provides the differential, supposingly due to the mitigating effect of the environmental stress impacts, especially water restriction at stage V_6 up to the start of the reproduction phase.

Zhang and Schmidt (2000) state that bio-

Table 5. Variance analysis, with mean squares, for the variables: plant height, dry matter of plants at tasseling, weight of 1000 grains, productivity of hybrid maize CD 304, as a response to different treatments with *Azospirillum brasilense* associated with bio-regulator Stimulate®.

V.F.	D.F.	Plant height	Dry matter	Weight of one thousand grains	Productivity
Blocks	5	834.2407	206432.5629	566.4296	4236666.8741
Treatments	8	197.5879**	93363.8935**	162.7962^{ns}	1029184.2962**
Waste	40	43.1157	43.1157	1029184.2962	93477.8241

** significant at 1% probability, by test F.
^{ns} not significant by test F.

Table 6. Means of plant height, dry matter at tasseling, weight of 1000 grains, productivity of hybrid maize culture CD 304, as a response to different treatments with *Azospirillum brasilense* associated with the bio-regulator Stimulate®.

Treatments	Evaluated characteristics[1]			
	Plant height	Dry matter	Weight of one thousand grains	Productivity
	------- cm --------	-------- g --------	------- g --------	----- kg ha^{-1} ----
1	157.33 B	730.83 B	284.83 A	3120.33 C
2	171.66 A	877.67 B	294.00 A	3917.00 B
3	173.50 A	941.83 A	298.34 A	4076.33 B
4	167.50 A	850.17 B	294.83 A	3761.83 B
5	172.00 A	975.83 A	302.67 A	3988.17 B
6	174.50 A	959.50 A	296.83 A	4271.67 A
7	175.67 A	1112.83 A	300.33 A	4394.83 A
8	172.00 A	1045.67 A	294.50 A	4428.67 A
9	175.67 A	1108.33 A	300.00 A	4355.50 A
Means	171.09	955.85	296.26	4034.93
C.V. (%)	3.84	17.19	3.90	7.58

[1] Means followed by the same capital letter in the column do not significantly differ by Scott-Knott grouping method, at 1% probability.

regulators are efficient when applied at small concentrations since they favor the good performance of the plants' vital processes and cause greater productivity and high quality products. Russo and Berlyn (1990) suggest that these compounds may increase productivity and resistance of plants during environmental stress. Bio-regulators with bio-stimulating or with bio-promoting features are components that respond to plant growth by an improvement of tolerance to abiotic stress (LONG, 2006), such as droughts.

In the case of high yields maize plants have to establish not only photosynthesis capacity but they have to continue photosynthesis during the formation of seeds and grain formation. This is a very important role since the accumulation of dry matter in maize grains depends on photosynthesis. Most N in the maize leaves is associated to chloroplast (approximately 60% of the leaf's total N) and the proteins are subject to development and remobilization of the resulting amino-acids. Photosynthesis capacity, supply of assimilated things and grain yield decrease as the leaves age. Decline occurs fast for N-lacking leaves, causing smaller ears with a smaller number of grains (TAIZ; ZEIGER, 2004).

In the case of productivity (Table 6), it should be emphasized that low productivity occurred because of water deficit coupled to high temperatures at stage V_6 till the start of the production phase. Since stress caused severe consequences in yield per area, mean productivity of merely 4,355.93 kg ha^{-1} is the response of culture to adverse conditions.

Significant differences among treatments for the variable productivity were relevant. Since rates of control were lowest for all treatments, the importance of N in vegetal nutrition is highly important, especially in maize culture (FANCELLI; DOURADO NETO, 2004). Two other groups were formed in which treatments 6, 7, 8 and 9 were statistically higher than treatments 2, 3, 4 and 5.

Above-mentioned productivity results confirmed the importance of inoculation with *Azospirillum,* since treatments, such as treatment 2 with *Azospirillum* only, had the same performance as treatments 4 and 5, with ½ N dose. This fact suggests that inoculation may replace up to 90 kg ha^{-1} of N to achieve the same productivity of maize grains.

Rates of treatment 2 were higher than those of control (treatment 1) by 20.3%. Results corroborate those by Riggs et al. (2001) with increases between 13 and 25% in grain yield, depending on genotype and with the inoculation of maize seeds. According to Dobbelaere et al. (2003), this occurs because of the biological fixation of nitrogen by the association of *Azospirillum* bacteria with maize.

When inoculated *Azospirillum* is associated with the bio-regulator Stimulate® in leaf applications during the vegetal stage, it may have a very important role in productivity increase or conservation. In fact, treatment 6, *Azospirillum* + Stimulate® with only ½ dose of N, was similar to treatments 7, 8 and 9 with total N dose. A compensatory role of the bio-regulator is presumed and favors the growth and the development of maize culture and, consequently, productivity.

When increase in grain productivity, attributed to treatments 2 and 3, is compared to control, it may be inferred that the fixation of atmospheric nitrogen triggered by *Azopirillum* bacteria, even without the application of nitrogenated fertilizers to the soil, caused the increase of dry matter (observed especially in treatment 3, with the addition of bioregulator) and of productivity of maize culture.

According to Coelho (2006), nitrogen occurs in protein molecules, enzymes, co-enzymes, nucleic acids and phytochromes and also integrates the chlorophyll molecule. On the other hand, its deficiency decreases the plants' vegetal development and the culture's productivity (AMARAL FILHO et al., 2005). With regard to the relationship between photosynthesis capacity and nitrogen contents, N has an important role since photosynthesis requires a substantial number of pigments and proteins for photochemical and carboxilative processes (TAIZ; ZEIGER, 2004). The above confirms the legitimacy of results with regard to increase in plants' dry matter and grains' productivity (Table 6).

Current research, conducted during the harvest year 2008-2009, must also take into account the climatic factors, such as, in current case, the water restrictions related to N dynamic complex in the soil (leaching, volatilization, immobilization, nitrification, de-nitrification and mineralization) and with regard to the plant. This fact may cause great modifications in the availability and the need of the nutrient during the ontogeny of the culture. The above considerations agree with the suppositions by Taiz and Zeiger (2004) and may have affected the associative process of the bacteria with the plant.

During the experiment, the control plants in the plots (treatment 1) manifested symptoms in N deficiency in leaves (yellowing of the older leaves) and ears (grain abortion at the ear tip). It has also been verified that plants of treatment 1 aged early when compared to those of other treatments. It seems that the soil where the experiment was carried out was insufficient to supply the demands of the culture, or rather, the required N amounts. On the other hand, symptoms of nutrition deficiency or phytotoxicity in the plants caused by treatments with *Azospirillum brasilense* were not diagnosed.

Based on productivity results, it may be stated that *Azospirillum* may partially replace nitrogenated fertilization. Further, *Azospirillum* + Stimulate® cause an increase in productivity even when nitrogenated fertilization is reduced. The use of these management alternatives may economically make viable the activity with higher profits as a consequence of a decrease in nitrogenated fertilization and its costs.

CONCLUSIONS

Inoculation treatments of maize seeds with liquid *Azospirillum brasilense* cause an increase in the height of plants and in the yield of maize grains, when compared to control;

The employment of ½ dose of N in sowing, associated with the inoculation of maize seeds with *Azospirillum brasilense* at 200 mL ha^{-1} (on the seeds) and the use of Stimulate® (in leaf application) was agronomically viable;

The inoculation of maize seeds with *Azospirillum brasilense* also causes a significant increase in the plants' dry matter.

REFERENCES

ALBRECHT, L. P. et al. Componentes da produção do algodoeiro e qualidade de fibra em resposta à aplicação de biorregulador. **Scientia Agraria**, Curitiba, v. 10, n. 3, p. 191-198, 2009.

AMARAL FILHO, J. P. R. et al. Espaçamento, densidade populacional e adubação nitrogenada na cultura do milho. **Revista Brasileira de Ciência do Solo**, Viçosa, MG, v. 29, n. 3, p. 467-473, 2005.

ÁVILA, M. R. et al. Bioregulator application, agronomic efficiency, and quality of soybean seeds. **Scientia Agricola**, Piracicaba, v. 65, n. 6, p. 567-691, 2008.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Regras para análise de sementes**. Secretaria de Defesa Agropecuária. Brasília: Mapa/ ACS, 2009. 399 p.

COELHO, A. M. **Nutrição e adubação do milho**. Sete Lagoas: EMBRAPA Milho e Sorgo. 2006. (Circular Técnica, 78)

DOBBELAERE, S.; LEYDEN, J.; OKON, Y. Plant growth-promoting effects of diazotrophs in the rhizosphere. **Critical Reviews Plant Science**, v. 22, n. 2, p. 107-149, 2003.

DOURADO NETO, D. et al. Aplicação e influência do fitorregulador no crescimento das plantas de milho. **Revista da FZVA**, Uruguaiana, v. 11, n. 1, p. 1-9, 2006.

EMBRAPA - EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA. Centro Nacional de Pesquisa de Solos. **Sistema brasileiro de classificação de solos**. 2. ed. Rio de Janeiro, 2006. 306p.

FANCELLI, A. L.; DOURADO NETO, D. **Produ-**

ção de milho. 2. ed. Piracicaba: Livroceres, 2004. 360 p.

FERREIRA, L. A. et al. Bioestimulante e fertilizante associados ao tratamento de sementes de milho. **Revista Brasileira de Sementes**, Lavras, v. 29, n. 2, p. 80-89, 2007.

IAPAR. **Cartas climáticas básicas do estado do Paraná**. Londrina: Instituto Agronômico do Paraná, 1994. 44 p.

KLAHOLD, C. A. et al. Resposta da soja (*Glycine max* (L.) Merrill) à ação de bioestimulante. **Acta Scientiarum Agronomy**, Maringá, v. 28, n. 2, p. 179-185, 2006.

LONG, E. **The importance of biostimulants in turfgrass management**. Disponível em: <http:// www.golfenviro.comlArtic1e% 20ArchivelBiostimulants-Roots.htm>. Acesso em: 10 set. 2006.

LOPES, S. A. et al. **Sistema plantio direto**: bases para o manejo da fertilidade do solo. São Paulo: ANDA, 2004. 110 p.

MIYAUCHI, M. Y. H. et al. Interactions between diazotrophic bacteria and mycorrhizal fungus in maize genotypes. **Scientia Agricola**, Piracicaba, v. 65, n. 5, p. 525-531, 2008.

MOTERLE, L. M. et al. Efeito da aplicação de biorregulador no desempenho agronômico e produtividade da soja. **Acta Scientiarum Agronomy**, Maringá, v. 30, n. 5 (suplemento especial), p. 701-709, 2008.

PRADO NETO, M. et al.. Germinação de sementes de jenipapeiro submetidas à pré-embebição em regulador e estimulante vegetal. **Ciência e Agrotecnologia**, Lavras, v. 31, n. 3, p. 693-698, 2007.

RIGGS, P. J. et al. Enhanced maize productivity by inoculation with diazotrophic bacteria. **Australian Journal of Plant Physiology**, v. 28, n. 5, p. 829-836, 2001.

RUSSO, R. O.; BERLYN, G. P. The use of organic biostimulants to help low input sustainable agriculture. **Journal of Sustainable Agriculture**, v. 1, n. 2, p. 19-42, 1990.

SALOMONE, G.; DÖBEREINER, J. Maize genotypes effects on the response to *Azospirillum* inoculation. **Biology Fertilizer Soils**, v. 21, n. 2, p. 193-196, 1996.

SANTOS, C. M. G.; VIEIRA, E. L. Efeito de bioestimulante na germinação de sementes, vigor de plântulas e crescimento inicial do algodoeiro. **Magistra**, João Pessoa, v. 17, n. 3, p. 124-130, 2005.

SILVA, E. D.; BUZETTI, S.; LAZARINI, E. Aspectos econômicos da adubação nitrogenada na cultura do milho em sistema de plantio direto. **Revista Brasileira de Milho e Sorgo**, Sete Lagoas, v. 4, n. 3, p. 286-297, 2005.

SOUSA, D. M. G.; LOBATO, E. **Cerrado**: correção do solo e adubação. Planaltina: Embrapa Cerrados, 2004. 416 p.

STOLLER DO BRASIL. **Stimulate Mo em hortaliças**: informativo técnico. Cosmópolis: Stoller do Brasil; Divisão Arbore, 1998. v. 1. 1 p.

TAIZ, L.; ZEIGER, E. **Fisiologia vegetal**. 3. ed. Porto Alegre: Artmed, 2004. 613 p.

VIEIRA, E. L.; CASTRO, P. R. C. Ação de bioestimulante na germinação de sementes, vigor de plântulas, crescimento radicular e produtividade de soja. **Revista Brasileira de Sementes**, Brasília, v. 23, n. 2, p. 222-228, 2001.

ZHANG, X.; SCHMIDT, R. E. Hormone-containing products' impact on antioxidant status oftall fescue and creeping bentgrass subjected to drought. **Crop Science**, v. 40, n. 5, p. 1344-1349, 2000.