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Mineral and organic fertilizer combined with doses of *Azospirillum brasilense* in an orchid hybrid

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Abstract

The cultivation of orchids requires many procedures, such as managing cultural treatments and fertilization to produce high quality floral stems. The objective of this study was to evaluate the growth of transplanted seedlings of a *Cattleya virginia* x *Brassocattleya pastoral* orchid hybrid with *Azospirillum brasilense* added and different sources of fertilization. The experiment was conducted in a private orchid nursery in Marechal Cândido Rondon, Paraná State, Brazil. The experimental design was randomized blocks in a 2 x 4 factorial scheme (two sources of fertilization x four concentrations of *A. brasilense* (Masterfix Gramíneas®)), with four repetitions and five plants per repetition, totaling 160 seedlings. The sources of fertilization were Bokashi® as an organic fertilizer and 10-10-10 NPK as a soluble mineral. After ten months, the plants underwent phytotechnical evaluations such as the total number of roots, length of the largest root, number of leaves, length and width of the largest leaf, number of pseudobulbs, number of sprouts, and diameter of the pseudobulb. The use of *A. brasilense* in the growth of the *Cattleya virginia* x *Brassocattleya* pastoral hybrid increased the diameter of the pseudobulb, the number of roots, and the length of the largest root. The inclusion of *A. brasilense* in the mineral fertilization with 10-10-10 NPK resulted in the greatest number of sprouts.

Keywords: *Cattleya* Lindl., Orchidaceae, fertilization, growth promoting bacteria

Introduction

Orchid culture represents one of the most economically significant activities in the global nursery industry (Silva, 2013). Some of the main products are orchid species of the genus *Cattleya* (Schnitzer et al., 2015), which includes 114 species and numerous hybrids (Pivetta et al., 2010). These are all classified as epiphytic with sympodial growth in the form of rhizomes, from which pseudobulbs, leaves, and flowers grow (Assis et al., 2011).

Orchid cultivation and production require several procedures, such as irrigation management, pest and disease control, substrate choice, and fertilization (Faria et al., 2016). Fertilization management in orchid cultivation can decrease the juvenile period of plant species. In the case of cattleyas, which take a mean of four to five years to bloom, fertilization management can increase the number of leaves and flowers per plant compared with plants in nature (Rodrigues et al., 2010).

Orchid growers implement several empirical fertilization practices. There are a variety of fertilizers on the market, with the possibility of numerous combinations and formulations of organic and chemical fertilizers. However, most fertilizers were not developed considering the specificities and different stages of the development of orchids (Santos, 2010).

Naik et al. (2009) state that organic fertilization has the advantages of gradually releasing nutrients, increased biological activity, and nutrient diversity. One of the organic fertilizers that stands out is Bokashi®, a fermented compound with more than 90 species of microorganisms that act to improve natural fertility and nutrient absorption by plants (Lasmini et al., 2018). According to Faria et al. (2016), *Cattlianthe* 'Chocolate drop' orchid plants fertilized with Bokashi® had increased number of sprouts and leaf area.

As for chemical fertilization, nitrogen (N),

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phosphorus (P), and potassium (K) can be cited among the main nutrients provided. The concentration used varies according to the requirements and stage of each crop. Each nutrient plays an essential and specific role in plant metabolism, with N acting directly on all growth, flowering, and fruiting phases. Phosphorus acts in energy formation in all phases of plant development, and K increases root formation (Rodrigues et al., 2010). Chemical fertilization can be applied on leaves or roots, or simultaneously on both (Lone et al., 2010).

In recent years, the use of beneficial microorganisms in agricultural systems has been studied. Brazil has a long tradition of researching biological nitrogen fixation by *Azospirillum* in association with grass species (Ribeiro et al., 2018). These bacteria, when inoculated in several species (especially of the Poaceae family), present mechanisms that influence plant development and biological nitrogen fixation (El-Lattief, 2013).

Ribeiro et al. (2018) report a growing interest in the use of inoculants containing bacteria to promote growth and increase plant productivity due to the high cost of chemical fertilizers and the awareness for sustainable and less polluting agriculture.

Besides its use on grasses, *Azospirillum* can be used in other crops, such as olive trees (Dalla Rosa et al., 2018). A study by Naik et al. (2013) showed that the maximum height and number of sprouts per *Dendrobium* plant were obtained with 10-5-10 NPK fertilization combined with a root treatment of *Azospirillum* and *Phosphobacteria*.

Considering its ability to widely colonize the plant, the use of *Azospirillum* is not restricted to seed inoculation, with its use expanded to application as a foliar spray (Fukami et al., 2016). Some studies with this bacterium used seed and foliar inoculation (Battistus et al., 2015; Spolaor et al., 2016; Silva & Pires, 2017).

Thus, the objective of this study was to evaluate the growth of orchid hybrid transplanted seedlings added with *Azospirillum brasilense* and different sources of fertilization.

Material and Methods

The experiment was conducted in a commercial orchid nursery in Marechal Cândido Rondon, Paraná, Brazil (latitude 24°33'22"S and longitude 54°03'24"W, at about 400 m above sea level). The nursery has a greenhouse with 70% luminosity retention obtained through a black polypropylene shading screen.

The selected seedlings measured about 6 cm height and had four expanded leaves. The hybrid from the crossing of *Cattleya virginia* x *Brassocattleya pastoral* was obtained by artificial pollination in the greenhouse.

The seedlings were removed from size six pots (with an upper diameter of 6 cm, a lower diameter of 5 cm, and 7 cm height) and conditioned in size 10 pots (with an upper diameter of 10 cm, a lower diameter of 7.5 cm, and 9 cm height) containing a substrate composed of 33% charcoal and 67% *Pinus* bark (Table 1).

Table 1. Physicochemical analysis, with average values of hydrogen potential (pH), electrical conductivity (EC), water buffering capacity (WBC), substrate density (D) and nutrients founds in the substrate.

Substrate	pH	EC (µS cm^{-1})	WBC (mL L^{-1})	D (g cm^{-3})
A	4.45	66.90	498.45	0.16

Substrate	N	P	K	Ca	Mg	B	Fe
	g kg^{-1}					mg kg^{-1}	
A	0.3	0.1	0.6	0.2	0.1	5.6	15.6

A = substrate of fine granulometry, composed of 33% of coal and 67% of *Pinus* bark.

Sixty days after transplanting the seedlings, the commercial product Masterfix Gramineae® with Ab-V5 and Ab-V6 strains of *Azospirillum brasilense* was applied, with 2 x 10^8 viable cell colony forming units (CFU) per ml. The product was used at four concentrations (C; ml L^{-1} water), C1 = 0 ml L^{-1}, C2 = 1.0 ml L^{-1}, C3 = 1.5 ml L^{-1}, and C4 = 2.0 ml L^{-1} (adapted from the methodology of Dalla Rosa et al. (2018)). A sprayer was used to apply 6 ml of solution per seedling at the end of the day, between 7:00 and 7:30 pm, with the water supply subsequently suspended for 36 h, according to Galindo et al. (2015).

Bokashi® organic fertilizer and 10-10-10 NPK were used as sources of nutrients and as a mineral fertilizer. The organic fertilizer was applied every 45 days at the edge of the pot using 3 g of the product. The soluble mineral fertilizer was applied for ninety days by adding 50 ml per vessel of the diluted fertilizer at a 3 g L^{-1}concentration.

The treated pots were kept on a wooden bench at 80 cm above the floor. Micro-sprinkler irrigation was performed daily, being manually triggered in the morning period for ten minutes. Manual weed control was conducted weekly. Pest and disease control was not necessary because there was no incidence of either of these.

Phytotechnical characteristics were evaluated ten months after the beginning of the experiment, including: the number of roots (NR), obtained by counting the total number of roots in each plant (with a mean caliber of 4 mm); length of the largest root (LLR), obtained by measuring the longitudinal length of the root system, from the crown to the end of the roots, and expressed in centimeters (cm); number of leaves, obtained by counting the leaves of each plant; and the length and width of the largest leaf (cm), obtained by measuring the latitudinal and longitudinal length of the leaf from the base of the pseudobulb to the end of the leaf. The number of pseudobulbs and sprouts (NS) was determined by counting these, with the pseudobulbs that did not have fully expanded leaves considered as sprouts, while the pseudobulb diameter (DP) was determined by the latitudinal length (mm). The variables involving length were measured using a graduated ruler and a pachymeter.

The experimental design was randomized blocks in a 2 x 4 factorial scheme (two types of fertilization x four *Azospirillum brasilense* concentrations), with four repetitions and five plants per repetition, totaling 160 seedlings. The data were tabulated and subjected to the Shapiro-Wilk normality test, being transformed in to (Y + 1.0) ^ 0.5 when necessary, and subsequently to an analysis of variance (ANOVA). The means of the qualitative variables were compared using the Tukey test at a 5% significance level, and the means of the quantitative variables were analyzed by regression using the SISVAR® statistical software (Ferreira, 2011).

Results and Discussion

The mean number of leaves, width of the largest leaf, and number of pseudobulbs found in the experiment did not significantly differ among treatments and there was no effect of *A. brasilense* concentrations.

The number of sprouts (NS) showed a significant interaction between the concentration of Gramineae Masterfix® with mineral and organic fertilization (Figure 1). Increased *A. brasilense* concentrations in the treatments resulted in distinct responses according to the type of fertilization. Increased *A. brasilense* concentrations in plants under mineral fertilization resulted in increased NS; however, plants under organic fertilization presented with a decreased NS, which is an important characteristic considering orchid production and commercialization, since they will later result in floral buds and seedlings for propagation (Assis et al., 2011).

Naik et al. (2009) studied the orchid *Dendrobium nobile* Lindl. and reported that the maximum height and NS per plant were obtained with 10:5:10 NPK fertilization combined with an *Azospirillum* and *Phosphobacteria* treatment on the roots of the plants, corroborating the results of the present study. The balance between nutrient availability provided by chemical fertilization and the concentration of *A. brasilense* present in the treatments resulted in the greatest benefit for the plants.

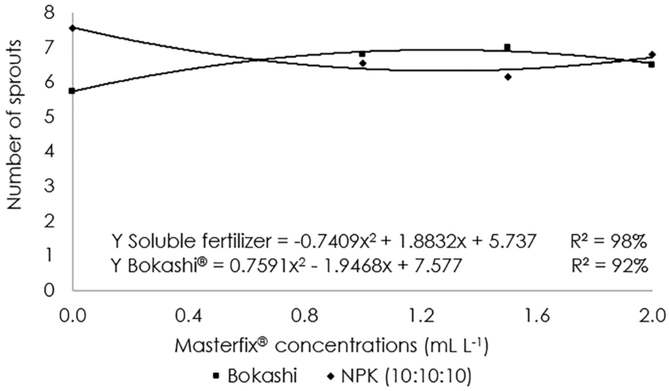

Figure 1. Number of sprounts of *Cattleya virginia* x *Brassocattleya* pastoral hybrids due to the different *Azospirillum brasilense concentrations and* sources of fertilization.

As Bokashi is a fermented compound (Magrini et al., 2011) containing more than 90 species of microorganisms (Faria et al., 2016), the excessive use of it may have an antagonistic effect on nutrient availability, justifying the decreased NS observed in the treatments. According to Puente et al. (2018), a high concentration of *A. brasilense* individuals may cause an inhibitory or deleterious effect to plants due to the excess of auxins.

As for the NR, LLR, and DP, there was a significant difference only for the addition of *A. brasilense* to the plants. Lone et al. (2010) analyzed a fortnightly 10:10:10 NPK fertilization treatment in an intergeneric hybrid of *Cattleya intermedia* Graham ex Hooker x *Laelia purpurata* Lindley, and reported no significant difference in the LLR between treatments.

As for the NR, the treatments with *A. brasilense* added at a concentration of 1.0 ml L^{-1}presented superior results compared with the other treatments, with the development of more roots per plant. At the other concentrations, this variable decreased (Figure 2).

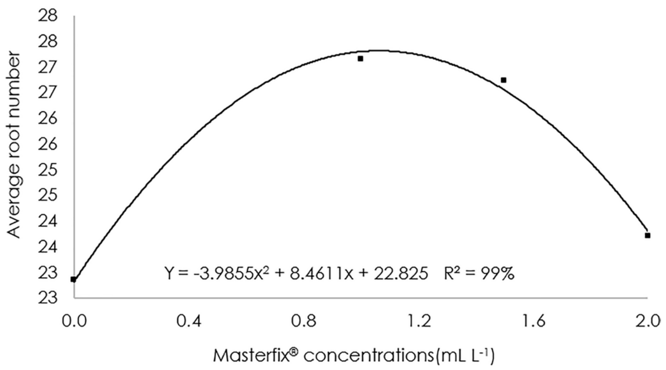

Figure 2. Average root number of *Cattleya virginia* x *Brassocattleya* pastoral hybrids due to the different *Azospirillum brasilense concentrations and* sources of fertilization.

This result may be due to the ability of *Azospirillum* to expressively stimulate plant hormone production, including auxins. Masciarelli et al. (2013) studied *Azospirillum* spp. isolates inside cassava roots *in vitro* and reported that hormones such as indole-3-acetic acid were excreted.

As previously mentioned, the high quantity of bacteria present in the highest dosage analyzed may have produced too many auxins, causing an inhibitory effect on the plant and thus reducing the NR. Corroborating this statement, Fukami et al. (2016) reported that high concentrations of *A. brasilense* inoculated in maize and wheat plants, both monocots, intensified hormonal secretion, thus decreasing root development.

The DP increased with the addition of the highest *A. brasilense* concentration and subsequently stabilized (Figure 3). The estimated point was reached with 2.0 ml L^{-1} of Masterfix Gramineae®, with a maximum DP of 2.56 cm.

Variations in the DP may occur depending on nutrient availability (He et al., 2013) in the substrate and NR. Auxin production by *A. brasilense* inoculation may increase lateral roots and root trichomes, resulting in a higher volume of exploited soil (Fukami et al., 2016; Kazi et al., 2016), consequently increasing the water and nutrient uptake and the size of the pseudobulb.

The increased *A. brasilense* concentration in the treatments resulted in an increased LLR. According to Morais et al. (2015) and Hungria et al. (2010), positive associations between the microorganism and plant species have been demonstrated, with inoculation increasing the root length in most cases. The concentration of 2.0 ml L^{-1} resulted in a mean LLR of 37.82 cm (Figure 4).

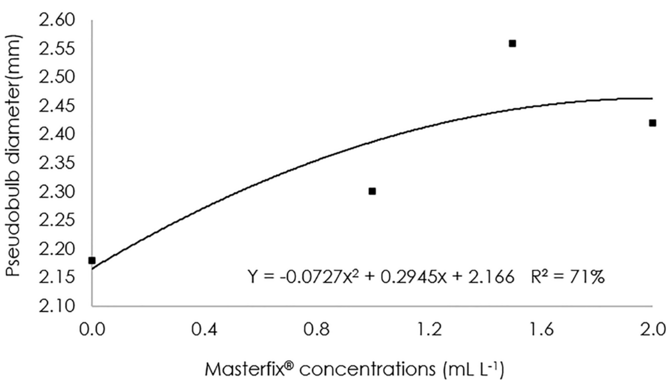

Figure 3. Average pseudobulb diameter (mm) of *Cattleya virginia* x *Brassocattleya* pastoral hybrids due to the different *Azospirillum brasilense concentrations and* sources of fertilization.

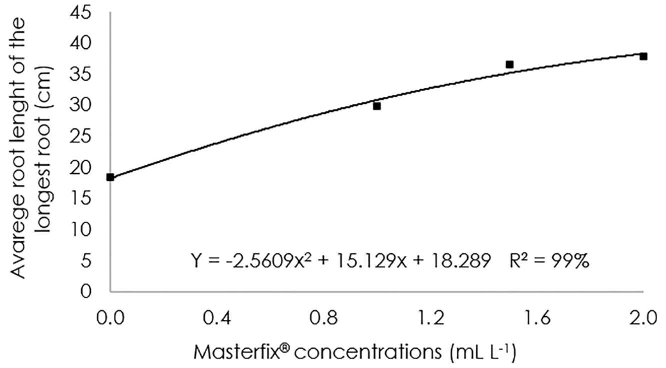

Figura 4. Root lenght of the longest root (cm) of *Cattleya virginia* x *Brassocattleya* pastoral hybrids due to the different *Azospirillum brasilense concentrations and* sources of fertilization.

Rodrigues et al. (2017) studied maize seeds and observed that inoculation with diazotrophic bacteria increased the development of the roots in inoculated plants compared with plants that were not inoculated. This was also described by Larraburu et al. (2015), who stated that the increased NR was possibly due to auxin production and biological nitrogen fixation by this bacterium.

The results obtained in this experiment show the benefit of using *A. brasilense* in the vegetative growth of the *Cattleya* hybrid studied. Further studies on the sources of nutrients to attend to the nutritional needs of orchids are necessary, especially to clarify the interaction between the microorganism and the nutrient sources in *Cattleya virginia* x *Brassocattleya pastoral* hybrids.

Conclusions

The use of *Azospirillum brasilense* in the growth of *Cattleya virginia* x *Brassocattleya pastoral* hybrids increased the DP, NR, and LLR, making it a promising tool in orchid production.

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References

Assis, A.M., Unemoto, L.K., Lone, A.B., Souza, G.R.B., Faria, R.T., Roberto, S.R., Takahashi, L.S.A. 2011. Cultivo de orquídea em substratos à base de casca de café. *Bragantia* 70: 544-549.

Battistus, A.G., Hachmann, T.L., Mioranza, T.M., Muller, M.A., Madaloso, T., Favorito, P.A., Guimarães, V.F., Kein, J., Kestring, D., Inagaki, A.M., Bulegon, L.G. 2015. Synergistic action of *Azospirillum brasilense* combined with thiamethoxam on the physiological quality of maize seedlings. *African Journal of Biotechnology* 13: 4501-4507.

Dalla Rosa, D., Villa, F., Silva, D.F., Corbari, F. 2018. Rooting of semihardwood cuttings of olive: indolbutyric acid, calcium and *Azospirillum brasilense*. *Comunicata Scientiae* 9: 34-40.

El-Lattief, A. 2013. Impact of integrated use of bio and mineral nitrogen fertilizers on productivity and profitability of wheat (*Triticum aestivum* L.) under upper Egypt conditions. *International Journal of Agronomy and Agricultural Research* 3: 67-73.

Faria, R.T., Hoshino, R.T., Alves, G.A.C., Melo, T.R., Barzan, R.R., Fregonezi, G.A.F. 2016. Adubação mineral e orgânica no crescimento da orquídea *Cattleya* 'Chocolate Drop'. *Horticultura Brasileira* 34: 475-482.

Ferreira, D.F. 2011. Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia* 35: 1039-1042.

Fukami, J., Nogueira, M.A., Araujo, R.S., Hungria, M. 2016. Accessing inoculation methods of maize and wheat with *Azospirillum brasilense*. *AMB Express* 6: 1-3.

Galindo, F.S., Ludkiewicz, M.G., Bellote, J.L.M., Santini, J.M.K., Teixeira Filho, M.C.M., Buzetti, S. 2015. Épocas de inoculação com *Azospirillum brasilense* via foliar afentando a produtividade da cultura do trigo irrigado. *Tecnologia & Ciência Agropecuária* 9: 43-48.

He, J., Norhafis, H., Qin, L. 2013. Responses of green leaves and green pseudobulbs of CAM orchid *Cattleya laeliocattleya* Aloha case to drought stress. *Journal of Botany* 2013: 1-9.

Hungria, M., Campo, R.J., Souza, E.M., Pedrosa, F.O. 2010. Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. *Plant and Soil* 331: 413-425.

Kazi, N., Deaker, R., Wilson, N., Muhammad, K., Trethowan, R. 2016. The response of wheat genotypes to inoculation with *Azospirillum brasilense* in the field. *Field Crops Research* 196: 368-378.

Larraburu, E.E., Llorente, B.E. 2015. *Azospirillum brasilense* enhances *in vitro* rhizogenesis of *Handroanthus impetiginosus* (pink lapacho) in different culture media. *Annals of Forest Science* 72: 219-229.

Lasmini, S.A., Nasis, B., Hayati, N., Edy, N. 2018. Improvement of soil quality using bokashi composting and NPK fertilizer to increase shallot yield on dry land. *Australian Journal of Crop Science* 12: 1743-1749.

Lone, A.B., Takahashi, L.S.A., Faria, R.T., Assis, A.D., Unemoto, L.K. 2010. Desenvolvimento vegetativo de orquídeas submetidas a diferentes formulações de macronutrientes e frequências de adubação durante a fase de aclimatização. *Bulletin of the World Health Organization* 83: 92-99.

Magrini, F.E., Camatti-Sartori, V., Finkler, R., Torves, J., Ventirun, L. 2011. Características químicas e avaliação microbiológica de diferentes fases de maturação do biofertilizante Bokashi. *Agrarian* 4: 146-151.

Masciarelli, O., Urbani, L., Reinoso, H., Luna, V. 2013. Alternative mechanism for the evaluation of indole-3-acetic acid (IAA) production by *Azospirillum brasilense* strains and its effects on the germination and growth of maize seedlings. *Journal of Microbiology* 51: 590-597.

Morais, T.P., Brito, H.C., Ferreira, A.S., Luz, J.M.Q. 2015. Aspectos morfofisiológicos de plantas de milho e bioquímico do solo em resposta à adubação nitrogenada e à inoculação com *Azospirillum brasilense*. *Revista Ceres* 62: 589-596.

Naik, S.K., Bharathi, T.U., Barman, D., Devadas, R., Ram, P., Medhi, R.P. 2009. Status of mineral nutrition of orchid: a review. *Journal of Ornamental Horticulture* 12: 1-14.

Naik, S.K., Barman, D., Medhi, R.P. 2013. Evaluation of electrical conductivity of the fertiliser solution on growth and flowering of a *Cymbidium* hybrid. *South African Journal of Plant and Soil* 30: 33-39.

Pivetta, K.F.L., Martins, T.A., Galdino Júnior, R.F., Gimenes, R., Faria, R.T., Takane, R.J. 2010. Crescimento *in vitro* de plântulas de *Caularthron bicornutum* em diferentes concentrações de sacarose. *Ciência Rural* 40: 897-1902.

Puente, M.L., Gualpa, J.L., Lopez, G.A., Molina, R.M., Carletti, S.M., Cassán, F.D. 2018. The benefits of foliar inoculation with *Azospirillum brasilense* in soybean are explained by an auxin-signaling model. *Symbiosis* 76: 41-49.

Ribeiro, R.H., Besen, M.R., Figueroa, L.V., Iwasaki, G.S., Guginski-Piva, C.A., Sartor, L.R., Piva, J.T. 2018. Seed and leaf inoculation with *Azospirillum brasilense* and increasing nitrogen in wheat production. *Revista Brasileira de Ciências Agrárias* 13: e5550.

Rodrigues, D.T., Novais, R.F., Alvarez, V., Dias, V.H., Moreira, J.M., Vilani, E.M.A. 2010. Orchid growth and nutrition in response to mineral and organic fertilizers. *Revista Brasileira de Ciência do Solo* 34: 1609-1616.

Rodrigues, L.A., Alves, C.Z., Rego, C.H.Q., Silva, T.R.B., Silva, J.B. 2017. Humic acid on germination and vigor of corn seeds. *Revista Caatinga* 30: 149-154.

Santos, A.F. 2010. *Nutrição e fertilização de orquídeas - estudo de caso - Cattleya walkeriana*. Núcleo de Pesquisa e Conservação de Orquídeas. Viçosa: UFV. http://files.croorquideas. webnode.com.br/200000032-d7251d81ef/ Nutri%C3%A7%C3%A3o%20e%20 fertiliza%C3%A7%C3%A3o%20de%20 orqu%C3%ADdeas. pdf.<Access on 17 Mar. 2021>

Schnitzer, J.A., Su, M.J., Ventura, M.U., Faria, R.T. 2015. Doses de extrato pirolenhoso no cultivo de orquídea. *Revista Ceres* 62: 1301-106.

Silva, J.T. 2013. Orchids: advances in tissue culture, genetics, phytochemistry and transgenic biotechnology. *Floriculture Ornamental Biotechnology* 7: 1-52.

Silva, S.R., Pires, J.L.F. 2017. Resposta do trigo BRS Guamirim à aplicação de *Azospirillum*, nitrogênio e substâncias promotoras do crescimento. *Revista Ciência Agronômica* 48: 631-638.

Spolaor, L.T., Gonçalves, L.S.A., Santos, O.J.A.P., Oliveira, A.L.M., Scapim, C.A., Bertagna, A.B., Kuki, M.C. 2016.

Bactérias promotoras de crescimento associadas a adubação nitrogenada de cobertura no desempenho agronômico de milho pipoca. *Bragantia* 75: 33-40.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.