Damage caused by Diceraeus (= *Dichelops*) melacanthus in maize plants subjected to combinations: bioinoculation and imidacloprid seed treatment

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ABSTRACT

The aim of this study was to evaluate the damages caused by *Diceraeus* (=*Dichelops*) *melacanthus* in maize plants subjected to bioinoculation with or without imidacloprid seed treatment. In this study, five different combinations of bioinoculants and imidacloprid seed treatment were applied to maize seeds in a completely randomized design. The bioinoculants used were *Azospirillum brasilense* and *Bradyrhizobium japonicum*. From emergence, the plants were subjected to infestation with the stink bug *D. melacanthus* (one stink bug/plant), with permanence up to 21 days after emergence. After this period, the phytotechnical parameters (shoot and root) of the corn plants were evaluated. In general, plant height was higher when imidacloprid was applied, suggesting compatibility with bioinoculants. The chlorophyll *a* content was higher when bioinoculants were applied, regardless of whether imidacloprid was present. Finally, the results indicate that the bacteria *A. brasilense* and *B. japonicum* do not induce resistance to the level of *D. melacanthus* infestation used in the present study. Therefore, these bacteria can be used in combination with imidacloprid, allowing for greater plant height, higher chlorophyll *a* content, and reduced damage caused by *D. melacanthus*.

Keywords: resistance induction; rhizobacteria; corn pests; sucking pests; neonicotinoids.

INTRODUCTION

Maize is the second most important crop in Brazil, second only to soybeans (Sidra, 2021). In the 2019/2020 harvest, Brazilian production of maize amounted to approximately 100 million tons (Sidra, 2021). Within the corn production system, new technologies have been developed to increase production and reduce costs. Recent studies have shown that the use of bioinoculants (rhizobacteria), such as *Azospirillum*, can reduce the demand for nitrogen fertilizers (Picazevic *et al.*, 2017; Caires *et al.*, 2021) and increase maize development (Hafez *et al.*, 2021; Moreno *et al.*, 2021; Skonieski *et al.*, 2019).

Researchers have shown that the use of bioinoculants, in addition to replacing synthetic nitrogen, can contribute to maize defense against herbivory (Amutha *et al.*, 2007; Prischmann-Voldseth *et al.*, 2020**;** Anandh *et al.*, 2010). For example, the inoculation of maize plants with rhizobacteria can harm the development of *Diabrotica speciosa* (Coleoptera: Chrysomelidae) (Santos *et al.*, 2014) and *Mythimna separata* (Lepidoptera: Noctuidae) (Li *et al.*, 2019) on maize plants. This can be attributed to the fact that the bioinoculants and stimulate the production of secondary metabolites associated with defense (Santos *et al.*, 2014).

Currently, one of the main maize crop pests in Brazil is the stink bug *Diceraeus* (= *Dichelops*) *melacanthus* (Hemiptera: Pentatomidae), which causes crop losses of 20% (Cruz *et al.*, 2016) to approximately 100% (Silva *et al.*, 2019; 2021). The use of insecticides for seed treatment

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has been essential to mitigate crop damage. One class of insecticides, neonicotinoids, are effective because of their systemic translocation in plants (Silva *et al.*, 2019), meaning they can protect maize plants shortly after emergence (usually between the first five to 10 days). Common neonicotinoids in Brazil include clothianidin, thiamethoxam, and imidacloprid (Silva *et al.*, 2021).

In this sense, the knowledge of the compatibility between seed treatment with insecticides and bioinoculants is essential for the successful establishment of field crops. For example, it has been demonstrated that the insecticide fipronil is incompatible with *Azospirillum brasilense* because it negatively affects the development of the bacterial population (Santos *et al.*, 2021). In contrast, thiamethoxam has been reported to be associated with *Azospirillum* in a synergic effect, showing better results of growth in roots and shoots (Battistus *et al.*, 2014). These contrasting results highlight the need for further research to allow for a better understanding of treatment compatibility.

Finally, although there are some published studies, research that assesses the development of plants subjected to insect infestation is still scarce. Thus, to better understand the relationships in maize pest management, this study aims to evaluate the damages caused by *D. melacanthus* in maize plants subjected to bioinoculation with or without imidacloprid seed treatment.

MATERIAL AND METHODS

Study location

The study was conducted in a greenhouse in the municipality of Guarapuava, Paraná, Brazil. The research was conducted for a period of 21 days after emergence (DAE), which comprises the period of greatest susceptibility of corn by *D. melacanthus* (Silva e*t al.*, 2021). During the study period, the average, maximum, and minimum temperatures in the study environment were 25.9 °C, 37.4 °C, and 17.5 °C, respectively. The average relative humidity (RH) was 41%.

Treatments used and growing practices

The variety of maize used for the study was hybrid IPS 1706, obtained from the Division of Research in Genetic Improvement of the Instituto de Desenvolvimento Rural do Paraná (IDR-PR), located in Londrina, Paraná. Maize seeds were subjected to five treatments: *A. brasilense* (0.15 mL/100 seeds); coinoculation of *A. brasilense* (0.15 mL/100 seeds) + *Bradyrhizobium japonicum* (0.16 mL/100 seeds); imidacloprid seed treatment (ST; 0.07 mL /100 seeds); *A. brasilense* (0.15 mL/100 seeds) + ST; and coinoculation + ST. The control consisted of seeds with neither bioinoculant nor ST.

The maize was sown on March 8, 2019, in 3 L pots filled with Latosol Bruno dystrophic soil. Five seeds were planted in each pot; however, after emergence, only two plants per pot were kept. To avoid water stress, the soil moisture was checked daily, and the plants were watered when necessary. During the study, no phytosanitary management was performed on the plants.

Stink bug origin and plant infestation details

The insects were obtained from the Entomology Laboratory of the IDR-PR, where they had been raised under controlled temperature (25 ± 2 °C), and moisture (60 ± 20%) conditions. The stink bugs were fed peanuts, snap beans, soybeans, and privet.

The infestation of corn plants occurred at development stage V1 (first expanded leaf) (Magalhães & Durães, 2006), and lasted 15 days (adapted from Bridi *et al.*, 2016). This time interval is when maize is higly susceptive to stink bug damage (Silva *et al.*, 2019). For the plant infestation, two unsexed adult insects were used per sample unit (pot), corresponding to one insect/plant.

To prevent the insects from escaping, the pots were adapted to be in the form of “cages” (40 x 20 cm in height and width, respectively) and covered with voile. The insects were monitored daily, and dead individuals were replaced (Bridi *et al.*, 2016).

Evaluation of leaf damage

The damage to the maize plants was evaluated by adapting the scale described by Roza-Gomes *et al.* (2011): 1) no damage; 2) leaves with small punctuations, plants with no size reduction; 3) injured whorl (partially twisted), plant with size reduction; 4) twisted whorl or “suckering” plants (plants with tillers from the base); and 5) dead whorl.

Evaluation of plant phytotechnical parameters

After subjecting the plants to *D. melacanthus* infestation, the following phytechnical parameters were measured: plant height, chlorophyll *a* and *b* content, fresh weight (shoot and root), and dry mass (shoot and root). All evaluations took place at 21 DAE, except for plant height, which was assessed six days prior (15 DAE).

Plant height was measured with a ruler positioned vertically at the base of the maize stalk. Chlorophyll *a* and *b* contents were measured using an electronic chlorophyll meter (Falker ClorofiLog®, Model CFL1030). The readings were taken on the two plants grown in each pot (n = 20) by placing the device in the middle third of the expanded leaf.

The fresh mass of the aerial parts of all plants (n = 20) were evaluated. Immediately thereafter, the plants were removed from the pot. The aerial parts were sectioned and placed in a paper bag. The material was then sent to the laboratory, where it was weighed using an analytical scale.

All seedlings were harvested at the end of the treatment and were separated into aerial part and roots. In laboratory, the samples were weighed for the determination of fresh weight. Then, the sample was oven-dried to a constant weight at 80 °C, and the dry weight was then measured.

Statistical analysis

The data were initially subjected to normality (Shapiro & Wilk, 1965) and homogeneity of variance (Burr & Foster, 1972) tests to verify whether they met the assumptions of parametric statistics. An analysis of variance (ANOVA) was performed, followed by Tukey's test. The difference was considered significant when $p \leq 0.05$ (SAS Institute, 2001).

RESULTS

Leaf damage

In general, less leaf damage was observed in the maize plants that had been treated with imidacloprid, demonstrating compatibility with bioinoculants (Figure 1). Leaf damage similar to that of the control was observed in maize plants that had been treated with bioinoculants but without imidacloprid (Figure 1).

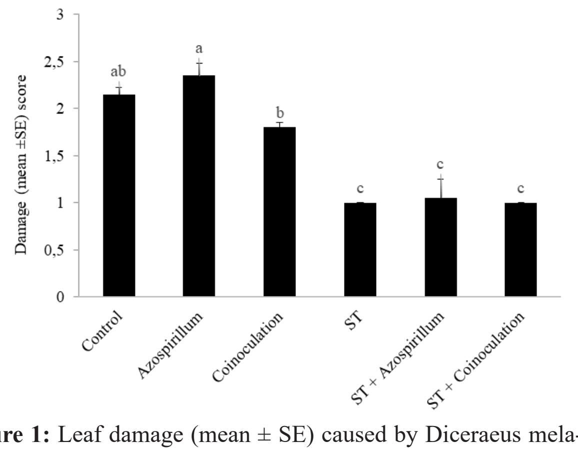

Figure 1: Leaf damage (mean ± SE) caused by Diceraeus melacanthus on maize plants (at 21 DAE) submitted to different treatments. Damage score adapted from Roza-Gomes et al. (2011).

Plant height and chlorophyll content

Imidacloprid seed treatment, both with and without bioinoculants, resulted in higher plant height, especially at 21 DAE (Table 1). In general, the lowest chlorophyll *a* content was observed in the control plants. The plants with imidacloprid seed treatment and the control plants had similar chlorophyll *b* levels, which were lower than those of the plants with bioinoculant treatment (Table 1).

Mass (fresh and dry) of the aerial part and root system

In general, the data did not show a clear relationship between plant mass (both fresh and dry) with seed treatment or bioinoculation (alone or combined) (Table 2). Neither the imidacloprid seed treatment nor the bioinoculation appeared to affect the root system (Table 2). For example, though the combination of seed treatment with coinoculation resulted in a greater fresh mass, the highest dry mass was observed for imidacloprid treatment with or without *A. brasiliense* (Table 2).

DISCUSSION

In general, bioinoculation with *A. brasiliensis* and *B. japonicum* and imidacloprid seed treatment improved the chlorophyll *a* content in maize plants, even under pest infestation conditions. This result suggests that the bioinoculation increased the photosynthetic capacity of plants even under stress conditions, demonstrating the potential importance of bioinoculants in the physiology of corn. Interestingly, the same was observed for bioinoculated seeds treated with imidacloprid, suggesting the compatibility of nitrogen-fixing bacteria with the insecticide.

Leaf damage

It has been previously reported that bioinoculants can promote resistance induction against some pests. For example, incorporation of *Azospirillum* in soil has been shown to repel *Antigastra catalaunalis* (Lepidoptera: Crambidae) in sesame (*Sesamum indicum*) (Anandh *et al.*, 2010). Interestingly, *Azospirillum* can also harm the development of some corn pests, such as the caterpillar *Mythimna sequax* (Li *et al.*, 2019) and the *D. speciosa* (Coleoptera: Chrysomelidae) (Santos *et al.*, 2014). The bioinoculation of the maize in this study does not appear to have had a harmful effect on stink bugs (longevity around 6 d), which explains the greater damage to plants in the absence of seed treatment. However, laboratory studies that assess insect biology may provide a better understanding of the relationship between bioinoculants and pests.

Table 1: Development parameters of corn seedlings (mean ± SE) subjected to infestation (n = 1 stink bug/plant) of *Diceraeus melacanthus* in a greenhouse.

Treatment	Phytotechnical parameters			
	Plant height (15 DAE)	**Plant height (21 DAE)**	**Chlorophyll *a***	**Chlorophyll *b***
Control	12.9 ± 0.32 b	20.66 ± 0.76 b	13.81 ± 1.01 b	2.98 ± 0.14 b
Azospirillum	13.42 ± 0.63 b	19.92 ± 1.03 b	16.63 ± 0.63 a	3.27 ± 0.11 ab
Coinoculation	13.57 ± 0.68 b	23.31 ± 1.18 ab	15.11 ± 0.66 a	3.26 ± 0.06 ab
ST	15.87 ± 0.49 a	26.86 ± 0.41 a	14.74 ± 0.81 ab	2.875 ± 0.16 b
ST + *Azospirillum*	14.85 ± 0.42 ab	25.67 ± 0.44 a	15.96 ± 0.75 a	3.16 ± 0.10 ab
ST + coinoculation	14.32 ± 0.39 ab	26.56 ± 0.69 a	16.66 ± 0.71 a	3.52 ± 0.11 a
CV (%)	11.96	11.29	16.70	12.62
F	4.12	12.60	1.91	3.28
DF error	54	54	54	54
P	< 0.01	< 0.01	n.s	< 0.05

Means ± SEM followed by the same letter in the column do not differ by Tukey test (5% probability). ns = non significant P. ST = seed treatment with imidacloprid; Coinoculation = *Azospirillum* + *Bradyrhizobium*.

Table 2: Mass evaluation (mean ± SE) of shoot and root system of maize plants subjected to infestation (n = 1 bug/plant) of *Diceraeus melacanthus* in a greenhouse.

Treatment	Phytotechnical parameters			
	Fresh aerial mass	**Dry mass of aerial part**	**Fresh root mass**	**Dry root mass**
Control	0.80 ± 0.05 ab	0.12 ± 0.01 ab	1.22 ± 0.1 b	0.30 ± 0.0 c
Azospirillum	0.71 ± 0.03 b	0.11 ± 0.01 b	1.27 ± 0.1 b	0.32 ± 0.0 bc
Coinoculation	0.79 ± 0.05 ab	0.13 ± 0.01 ab	1.25 ± 0.1 b	0.26 ± 0.0 c
ST	0.89 ± 0.04 ab	0.14 ± 0.0 ab	1.17 ± 0.1 b	0.40 ± 0.0 a
ST + *Azospirillum*	0.80 ± 0.04 ab	0.15 ± 0.01 a	1.06 ± 0.0 b	0.38 ± 0.0 ab
ST + coinoculation	0.95 ± 0.05 a	0.13 ± 0.01 ab	1.64 ± 0.1 a	0.35 ± 0.0 b
CV (%)	18.90	18.52	21.17	17.23
F	3.26	3.09	5.46	9.35
DF error	54	54	54	54
P	< 0.05	< 0.05	< 0.01	< 0.01

Means ± SEM followed by the same letter in the column do not differ by Tukey test (5% probability). ST = seed treatment with imidacloprid; Coinoculation = *Azospirillum* + *Bradyrhizobium*.

These results demonstrate that the relationship between bioinoculants and pests is still unknown; however, to date, this is the first study of bioinoculants carried out in association with *D. melacanthus.* Additionally, the pest population density used in the present study (one stink bug/plant) was high (Silva *et al.*, 2021), which probably impaired the development of the plants. Thus, the use of a lower *D. melacanthus* population density could result in different outcomes.

Finally, the importance of studies examining other insecticides should be considered to gain a greater understanding of the relationship among bacteria, plants, and insects. For example, maize seeds inoculated with *Bacillus subtilis* show increased protection against sucking pests because the bacteria increase the absorption of the neonicotinoid thiamethoxam (Myresiotis *et al.*, 2015). Similarly, *Azospirillum* can be recommended in association with a lower dose of thiamethoxam (Battistus *et al.*, 2014). Conversely, *A. brasilense* is incompatible with the insecticide fipronil (Santos *et al.*, 2020), which reduces the bacterial population and impairs biological nitrogen fixation.

Thus, it is important to know the relationship between the bioinoculant and the insecticide to avoid decreased bioinoculant efficiency. In addition, further field studies are needed to explore the impact of bioinoculants and insecticides on plant development and pest damage to ultimately be able to provide recommendations to farmers.

Plant height and chlorophyll content

The seed bioinoculation combined with imidacloprid resulted in greater plant height. This observation suggests the compatibility of rhizobacteria with the insecticide, as both can help support better initial plant development. The relationship between neonicotinoids and increased initial plant growth has previously been reported in the literature (Preetha & Stanley, 2012). Additionally, the low amount of damage caused to the plants that received the imidacloprid seed treatment probably allowed for better initial development of the aerial parts of the plants. The plants that received the imidacloprid seed treatment, whether alone or in combination with bioinoculants, were observed to have 100% stink bug mortality within 48 h after infestation (data not shown), indicating the efficiency of the insecticide. This suggests that these bioinoculants and imidacloprid can be combined for *D. melacanthus* management without loss of insecticide efficiency. An important observation was the higher content of chlorophyll *a*, indicating greater photosynthetic capacity, in the plants that received bioinoculation. Chlorophyll content can be an indication of plant resistance to pest infestation (Melo *et al.*, 2018), as feeding insects generally reduce this component (Golan *et al.*, 2015; Joseph & Jespersen, 2021). Thus, this study demonstrates for the first time that bioinoculation of maize with *A. brasilense* and *B. japonicum*, whether combined with imidacloprid or not, can help to mitigate photosynthetic damage caused by pests. In contrast, those plants treated with imidacloprid alone had a chlorophyll content similar to that of the control plants, indicating two possibilities: 1) neonicotinoids do not directly influence chlorophyll content (Preetha & Stanley, 2012); or 2) there is compatibility between rhizobacteria and imidacloprid (with the dose used in the present study).

Mass (fresh and dry) of the aerial part and root system

The results for fresh and dry masses of the maize plants were variable. This inconsistency has already been observed in other studies on corn (Marques *et al.*, 2020) and may be explained by the genetic material (hybrid) used (Muller *et al.*, 2021). Additionally, Bashan and Dubrovsky (1996) observed that bioinoculants have a greater influence on shoot parameters than on the root system. Thus, two hypotheses can be considered: 1) the hybrid used in this study does not benefit from its root system, and 2) the high stink bug population density did not allow for better root development in plants inoculated without the insecticide. Further research is needed for both hypotheses.

The present study demonstrates that bioinoculants can be used concurrently with imidacloprid maize seed treatment. In Brazil, there is a growing use of rhizobacteria by farmers in maize crops (Santos *et al.*, 2021), highlighting the importance of research on the subject. This study demonstrates that the use of imidacloprid seed treatment does not negatively affect the action of the bioinoculants. Thus, maize farmers will be able to use both treatments in combination to improve plant performance and protect against *D. melacanthus*.

Additional research should be conducted to better understand the compatibility of different doses and strains of bioinoculants and insecticides. This research could include compatibility studies, for example, laboratory observation of the survival rate of the maize rhizobacteria with different insecticides.

CONCLUSIONS

Maize seeds bioinoculated with *A. brasilense* and *B. japonicum*, either with or without imidacloprid, did not allow decrease the chlorophyll *a* content of plants subjected to infestation of *D. melacanthus*. In addition, we conclude that the use of rhizobacteria combined with imidacloprid seed treatment can improve maize plant development and reduce the damage caused by *D. melacanthus*.

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REFERENCES

Amutha M, Chozhan K & Alagar M (2007) Synergistic effect of *Azospirillumm brasilense* and vesicular arbuscular mycorrhiza (VAM *Glomus* spp.) on leaf folder in rainfed rice. Journal of Plant Protection and Environment, 4:15-20.

Anandh GV, Selvanarayanan V & Tholkappian P (2010) Influence of arbuscular mycorrhizal fungi and bio-inoculants on host plant resistance *Antigastra catalaunalis* Duponchel in sesame *Sesamum indicum* Linn. Journal of Biopesticides, 3:152-154.

Bashan Y & Dubrovsky JG (1996) *Azospirillum* spp. participation in dry matter partitioning in grasses at the whole plant level. Biology and Fertility of Soils, 23:435-440.

Battistus A, Hachmann T, Mioranza T, Muller MA, Madalosso T, Favorito PA, Guimarães VF, Klein J, Kestring D, Inagaki AM & Bulegon LG (2014) Synergistic action of *Azospirillum brasilense* combined with thiamethoxam on the physiological quality of maize seedlings. African Journal of Biotechnology, 13:4501-4507.

Bridi M, Kawasaki J & Hirose E (2016) Danos do percevejo *Dichelops melacanthus* (Dallas, 1851) (Heteroptera: Pentatomidae) na cultura do milho. Magistra, 28:301-307.

Burr I W & Foster LA (1972) A test for equality of variances (Mimeo Series No. 282). West Lafayette, University of Purdue. 26p.

Caires EF, Bini AR, Barão LFC, Haliski A, Duart VM & Ricardo KS (2021) Seed inoculation with *Azospirillum brasilense* and nitrogen fertilization for no-till cereal production. Agronomy Journal, 113:560-576.

Cruz I, Bianco R & Redoan ACM (2016) Potential risk of losses in maize caused by *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae) in Brazil. Revista Brasileira de Milho e Sorgo, 15:387-398.

Golan K, Rubinowska K, Kmieć K, Kot I, Górska-Drabik E, Łagowska B & Michałek W (2015) Impact of scale insect infestation on the content of photosynthetic pigments and chlorophyll fluorescence in two host plant species. Arthropod-Plant Interactions, 9:55-65.

Hafez M, Popov AI & Rashad M (2021) Integrated use of bio-organic fertilizers for enhancing soil fertility–plant nutrition, germination status and initial growth of corn (*Zea mays* L.). Environmental Technology & Innovation, 21:101329.

Joseph SV & Jespersen D (2021) Influence of relative humidity on the expression of twolined spittlebug (Hemiptera: Cercopidae) feeding injury in turfgrass genotypes. Arthropod-Plant Interactions, 15:197-207.

Li Z, Parajulee MN & Chen F (2019) Impacts of Bt maize inoculated with rhizobacteria on development and food utilization of *Mythimna separata.* Journal of Applied Entomology, 143:1105-1114.

Magalhães PC & Durães FOM (2006) Fisiologia da produção de milho. Sete Lagoas, Embrapa/Centro Nacional de Pesquisa de Milho e Sorgo. 10p. (Circular, 21).

Marques DM, Magalhães PC, Marriel I, Gomes Junior CC, Silva A, Melo IG & Souza TC (2020) *Azospirillum brasilense* favors morphophysiological characteristics and nutrient accumulation in maize cultivated under two water regimes. Revista Brasileira de Milho e Sorgo, 19:e1152.

Melo CG, Tomaz AC, Soares BO, Kuki KN, Peternelli LA & Pereira Barbosa MH (2018) Anatomical, morphological, and physiological responses of two sugarcane genotypes of contrasting susceptibility to *Mahanarva fimbriolata* (Hemiptera: Cercopidae). Bulletin of Entomological Research, 108:556-564.

Muller TM, Martin TN, Cunha VS, Munareto JD, Conceição GM, Stecca JDL (2021) Genetic bases of corn inoculated with *Azospirillum brasilense* via seed and foliar application. Acta Scientiarum. Agronomy, 43:e48130.

Moreno AL, Krusda JF & Picazevicz AAC (2021) Rhizobacteria inoculation in maize associated with nitrogen and zinc fertilization at sowing, Brazilian Journal of Agricultural and Environmental Engineering, 25:96-100.

Myresiotis CK, Vryzas Z & Papadopoulou-Mourkidou E (2015) Effect of specific plant-growth-promoting rhizobacteria (PGPR) on growth and uptake of neonicotinoid insecticide thiamethoxam in corn (*Zea mays* L.) seedlings. Pest Management Science, 71:1258-1266.

Picazevicz AAC, Kusdra JF & Moreno A de L (2017) Maize growth in response to *Azospirillum brasilense*, *Rhizobium tropici*, molybdenum and nitrogen. Revista Brasileira de Engenharia Agrícola e Ambiental, 21:623-627.

Preetha G & Stanley J (2012) Influence of neonicotinoid insecticides on the plant growth attributes of cotton and okra. Journal of Plant Nutrition, 35:1234-1245.

Prischmann-Voldseth DA, Özsisli T, Aldrich-Wolfe L, Anderson K & Harris MO (2020) Microbial inoculants differentially influence plant growth and biomass allocation in wheat attacked by gall-inducing hessian fly (Diptera: Cecidomyiidae). Environmental Entomology, 49:1214-1225.

Roza-Gomes MF, Salvadori JR, Pereira PRVS & Panizzi AR (2011) Injúrias de quatro espécies de percevejos pentatomídeos em plântulas de milho. Ciência Rural, 41:1115-1119.

Santos MS, Rondina ABL, Nogueira MA & Hungria M (2020) Compatibility of *Azospirillum brasilense* with pesticides used for treatment of maize seeds. International Journal of Microbiology, 2020:01-08.

Santos MS, Nogueira MA & Hungria M (2021) Outstanding impact of *Azospirillum brasilense* strains Ab-V5 and Ab-V6 on the Brazilian agriculture: Lessons that farmers are receptive to adopt new microbial inoculants. Revista Brasileira de Ciências do Solo, 45:e0200128.

SAS Institute Inc. (2001) Statistical Analysis System user's guide. Version 8.2. Cary, Statistical Analysis System Institute. 1028p.

Shapiro SS & Wilk MB (1965) An analysis of variance test for normality. Biometrika, 52:591-611.

SIDRA - Sistema IBGE de Recuperação Automática (2021) Levantamento Sistemático da Produção Agrícola - abril 2022. Available at: https://sidra.ibge.gov.br/home/lspa/brasil. Accessed on: May 20th, 2021.

Silva PR, Istchuk AN, Foresti J, Hun TE, Araújo TA, Fernandes FL, Alencar ER & Bastos CS (2021) Economic injury levels and economic thresholds for *Diceraeus* (*Dichelops*) *melacanthus* (Hemiptera: Pentatomidae) in vegetative maize. Crop Protection, 143:105476.

Silva PR, Istchuk AN, Hunt TE, Bastos CS & Torres JB (2019) Susceptibility of corn to stink bug (*Dichelops melacanthus*) and its management through seed treatment. Australian Journal Crop Science, 13:2015-2021.

Skonieski FR, Viégas J, Martin TN, Mingotti CCA, Naetzold S, Tonin TJ, Dotto LR & Meinerz GR (2019) Effect of nitrogen top dressing fertilization and inoculation of seeds with *Azospirillum brasilenseon* corn yield and agronomic characteristics. Agronomy, 9:01-11.

Santos F, Peñaflor MFGV, Pare PW, Sanches PA, Kamiya AC, Tonelli M, Nardi C & Bento JMS (2014) A novel interaction between plant-beneficial rhizobacteria and roots: colonization induces corn resistance against the root herbivore *Diabrotica speciosa*. PLoS One, 9:e113280.