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Does soil cultivation practices and inoculation with *Azospirillum brasilense* affect the performance of maize genotypes?

As práticas de cultivo do solo e inoculação com *Azospirillum brasilense* afetam a performance de genótipos de milho?

Marcelo Henrique Santos[1], Wagner Emerson Aguiar[2], Eliane Cristina Gruszka Vendruscolo[2], Robson Fernando Missio[2*]

[1]Universidade de São Paulo/ESALQ/USP, Pós-graduação em Genética e Melhoramento de Plantas, Piracicaba, SP, Brasil
[2]Universidade Federal do Paraná/UFRP, Palotina, PR, Brasil
[*]Corresponding author: rfmissio@ufpr.br
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ABSTRACT

One of the bottlenecks for maize (*Zea mays* L.) production is the need for a large supply of nitrogen fertilizers, which burdens farmers. A solution to this problem is the use of proteobacteria *Azospirillum brasilense* as an inoculant. However, experimental results regarding this inoculant vary depending, for instance, on the soil cultivation practices used. The objective of this study was to evaluate the influence of inoculation with *Azospirillum brasilense* on morphophysiological characters and yield characteristics of three maize genotypes in two off-season cultivated in no-tillage system (NT). Fallow soil (FS) was used as control. The results showed that soil cultivation had a significant effect on morphophysiological and productivity parameters compared to inoculation with *Azospirillum brasilense*. There were no distinct morphophysiological differences among the treatments. FS (inoculated or not) was more closely related to productivity parameters (grain yield-YI, weight of 100 seeds-100SW, plant height-PH and number of leaves-NL) than NT in both off-seasons. Among the hybrids, Dekalb 255 PRO3 (DKB 255) showed the highest YI and 100SW. Thus, this hybrid was considered well-adapted to off-seasons in western Paraná, Brazil. The inoculation effect was practically null and did not influence the estimated productivity of the different chosen hybrids.

Index terms: Maize hybrids; no-till system; fallow soil; productivity; farmers business network.

RESUMO

Um dos gargalos da produção de milho (*Zea mays* L.) é a necessidade de uma grande oferta de fertilizantes nitrogenados, o que onera os agricultores. Uma solução para esse problema é o uso da proteobactéria *Azospirillum brasilense* como inoculante. No entanto, os resultados experimentais com esse inoculante variam dependendo, por exemplo, da prática de cultivo do solo O objetivo deste estudo foi avaliar a influência da inoculação com *Azospirillum brasilense* em caracteres morfofisiológicos e características de produtividade de três genótipos de milho em duas entressafras cultivados em plantio direto. O sistema de pousio (PS) foi usado como controle neste processo. Os resultados mostraram que esses sistemas tiveram um efeito significativo nos parâmetros morfofisiológicos e de produtividade em comparação com a inoculação com *Azospirillum brasilense*. Não houve diferenças morfofisiológicas distintas entre os tratamentos. O PS (inoculado ou não) apresentou maior relação com os parâmetros de produtividade (rendimento de grãos, peso de 100 sementes, altura da planta e número de folhas) do que o PD em ambas as safras. Entre os híbridos, Dekalb 255 PRO3 apresentou maior produtividade e peso de 100 sementes. Assim, este híbrido foi considerado bem adaptado para a entressafra no oeste do Paraná, Brasil. O efeito da inoculação foi praticamente nulo e não influenciou na produtividade estimada dos diferentes híbridos escolhidos.

Termos para indexação: Híbridos de milho; plantio direto; pousio; produtividade; agronegócio.

INTRODUCTION

Maize (*Zea mays* L.) is a widely used cereal grain and the largest crop in the world with an average annual production of 1 billion tons (Contini et al., 2019). Currently, Brazil is the third largest maize producer in the world; the country produced almost 115 million tons of maize in 2021-2022 (Companhia Nacional de Abastecimento - CONAB, 2022).

The scenario of off-season maize crop – which is made right after the early soybean crop – has changed in recent years: it was quite unrepresentative and had low yields in the 1980s (Ferreira et al., 2020); but it has been surpassing in total production the summer season crop since 2012 (Contini et al., 2019). Moreover, it has become one of the most important crops in terms of productivity and growing area due to advances in agricultural technologies. This highlights the need for management techniques that increase productivity without increasing costs and consequently ensure profit (Reunião Técnica Anual da Pesquisa do Milho et al., 2017, Rosa, 2020; Santos; Nogueira; Hungria, 2021).

One of the most representative investments that maize farmers have to make during the off-season crop is nitrogen fertilization. This element is one of the most limiting nutrients to the maize crop and its availability influences not only productivity but also the quality of the final product since it enhances ion absorption and is a component of amino acids and proteins, which directly links nitrogen to the grain protein content (Souza et al., 2019; Galindo et al., 2016; Marini et al., 2015). Nitrogen fertilization in the off-season crop represents a large part of the investment, being the same less profitable than the first crop since the risks with climatic factors are greater. However, when maize is sowed after the soybean crop, costs with nitrogen fertilization can be reduced because the previous culture leaves the necessary amount of the element in the soil. Another alternative is to use microorganisms that perform biological nitrogen fixation, making it available to plants (Galindo et al., 2016; Marini et al., 2015; Zeffa et al., 2019, Pavani et al., 2022; Vendruscolo; Mesa; Souza, 2022).

Azospirillum brasilense is one of the most promising strains: a free-living, motile, variable, and aerobic proteobacteria that stimulates plant growth through beneficial association with plants (Zago et al., 2019; Vendruscolo; Mesa; Souza, 2022). Because of that, it is widely used in the formulation of inoculants. In addition to nitrogen fixation, this strain can produce and supply plants with hormones such as auxins, gibberellins, and cytokinins, which stimulate root system development. Consequently, plants are able to better explore the soil by absorbing more water and nutrients and this contributes to increasing productivity in most cases (Fukami; Cerezini; Hungria, 2018; Vendruscolo; Mesa; Souza, 2022, Vendruscolo; Mesa; Missio, 2023).

Another decisive factor for increasing productivity is soil management. No-till system (NT) is one of the most widespread conservationist techniques among farmers (Copec et al., 2015; Derpsch et al., 2010; Rembon; MacKenzie, 1997). The fallow soil (FS), in turn, consists of the total rest of the soil with no management, which allows natural restoration. This practice is very useful to observe changes that NT can cause on soils because FS involve reduced levels of chemical fertilizers, pesticides, herbicides, limestone, or crop rotation (*e.g.* soybean-maize-soybean).

The objective of this study was to evaluate the effect of inoculation and of both NT and FS systems on morphophysiological traits and productivity characteristics of different commercial maize genotypes. This evaluation was based on the hypothesis that NT compared to FS would present different microbial interaction dynamics that could affect the application of inoculants and alter their interaction with maize in terms of morphophysiology and productivity.

MATERIAL AND METHODS

Soil and climate characterization

The experiments were carried out in the city of Palotina, Paraná, Brazil, where the soil is classified as a typical eutrophic Red Latosol (Santos et al., 2018). The climate in this city is Cfa (humid subtropical) according to the Köppen climate classification (Alvares et al., 2013). Monthly rainfall and air temperature were assessed from February (sowing) to May (harvesting). It is worth mentioning that temperature ranged from 33 ^{o}C to 16 ^{o}C (higher and lower temperature, respectively) during this period, as shown in Figure 1.

The area where we conducted the experimental trials had used NT for about 25 years. In recent years, this soil has been alternating between soybean and maize cultures and intercropped with *Brachiaria ruziziensis*. The fallow soil (FS) first had an orchard for 15 years and more recently (4 years) had only grasses as vegetation. We made furrows in the soil using hoes for planting and then we sowed the area with maize seeds. In order to preserve the quality of the soil, that is, to avoid the introduction of *A. brasilense*, the off-season experiments were conducted in the same area but in different places, as shown in Table 1. The chemical characteristics of both soils are also presented in Table 1.

Experimental design

The experimental trial was conducted twice (2019 and 2020) and followed a completely randomized design in a factorial arrangement (2x3x2), with three replications. The research treatments were: (A) FS and NT; (B) maize hybrids: Dekalb 255 PRO3 (DKB 255), Agroceres 9000 PRO3 (AG 9000), and Dekalb 330 PRO3 (DKB 330) (Table 2); and (C) inoculation with *A. brasilense* or no inoculation (as control).

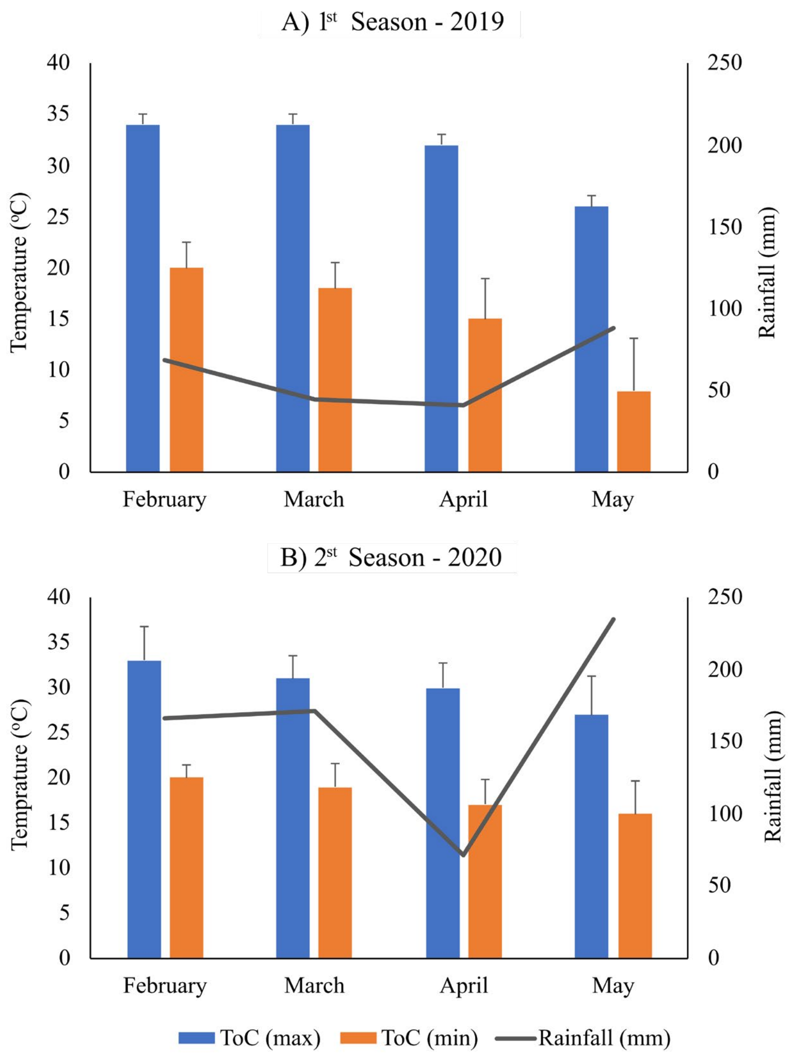


Figure 1: Rainfall (mm), maximum (T max) and minimum (T min) air temperatures (°C) in Palotina, Paraná, related to the experimental period for the first (2019) off-season (A) and second (2020) off-season (B).

Table 1: Location and chemical analysis of the soils used in the experiment of different soil cultivation practices.

Soil cultivation practices	Off-Seasons	Location	P	C	O.M	pH	K^+	Ca^{2+}	Mg^{2+}	H+Al	Al^{3+}	BS[1]	CEC[2]	V[3]
			mg dm^{-3}	g dm^{-3}		$CaCl_2$	cmol$_c$ dm^{-3}							%
NT	1st	24º 16’15.1” S 53º 49’02.9” W	28.80	16.25	27.95	5.20	1.11	5.59	2.26	5.76	0.00	8.96	14.72	60.87
	2nd	24º16’20.4” S 53º49’07.1” W	24.01	8.3	11.43	5.23	0.65	6.41	1.52	4.12	0.00	8.58	12.7	67.56
FS	1st	24º 16’14.7” S 53º 49’03.3” W	32.10	13.50	23.22	4.60	0.29	4.79	2.71	8.36	0.26	7.79	16.15	48.24
	2nd	24º16’19.5” S 53º49’09.8” W	16.78	14.82	25.70	5.37	0.78	7.04	2.83	4.28	0.00	10.64	14.92	71.32

NT-no-till system; FS-fallow soil; O.M: organic matter; [1]sum of bases; [2]cation exchangeable capacity, [3]base saturation = (BS/CEC). 100.

Table 2: Agronomic traits of maize hybrids used in the experiments.

Hybrids	Company	Cycle	Plant height (cm)	Type of hybrid	Number of degree-days
DKB 330 PRO3	Dekalb	Precocius	240	Simple	810
AG 9000 PRO3	Agroceres	Precocius	229	Simple	800
DKB 255 PRO3	Dekalb	Precocius	250	Simple	830

The experimental units were 3 m long and each treatment included four lines. The lines were 0.65m away from each other and the population was of approximately 60 thousand plants per hectare; 0.50 m of soil was discarded from each border to evaluate agronomic characteristics. The hybrids were sown on February 13, 2019 (first off-season) and February 20, 2020 (second off-season).

Fertilization was carried out with 300 kg ha^{-1} of the formulated (NP) 16-20 and about 90 kg ha^{-1} of N (urea-45% N), which were applied to the cover (phase V4); 100 g of the liquid form of Nitro 1000™ (2 x 10^8 Units Forming Colonies mL^{-1}) were applied per 20 kg-bag (60 thousand seeds) in the treatments inoculated with *A. brasilense*; the product was applied directly to the seeds and homogenized until a uniform seed coverage was obtained. Subsequently, the seeds were manually sown. Thirty days later, plant thinning was carried out; a population of about four plants was kept per linear meter. Weeds were controlled manually, and pest and disease control were carried out according to agronomic recommendations.

Morphophysiological and yield evaluations

After weighing all the plants, the following variables were recorded: (a) plant height (PH) was measured from the base of the plant, near the ground, to the insertion of the tassel using a graduated ruler; (b) ear insertion height (EIH) was measured from the base of the plant, near the soil, to the point of its insertion using a graduated ruler; (c) stem diameter (SD) was measured at the first internode above the adventitious roots using a digital caliper; (d) number of leaves (NL) was determined by counting the number of leaves inserted above the ear; and (e) leaf chlorophyll index (Chl a and Chl b) was evaluated in the middle third of the leaf below the index leaf using the portable chlorophyll meter (ClorofiLOG® CFL 1030).

At the end of the vegetative cycle the productivity parameters: weight of 100 seeds (100SW) and grain yield (YI) were calculated. The 100 seeds weight was calculated after threshing the ears of each useful parcel; the batch was homogenized and subsequently 100 grains were separated from each parcel and weighed on a digital scale. This assessment was repeated five times. YI was calculated by weighing the grains harvested in the plot used for the experiment with moisture adjusted to 13% (storage moisture for maize grains) and data adjusted to kg ha^{-1}.

Statistical analysis

All data were initially submitted to normality, homogeneity, and analysis of variance tests (Anova). When significant effects were detected, the averages of the treatments were compared using the Tukey test at 5% probability using the Sisvar® software (Ferreira, 2011). Principal Component Analysis (PCA) was also conducted using the Past software (Hammer et al., 2001). All data were obtained on the Pearson correlation matrix using the broken-stick model which retains the principal components (CPs).

RESULTS AND DISCUSSION

Effect of inoculation on morphophysiological traits

All morphophysiological data are presented in Table 3. The results showed that there was no significant difference in relation to PH between the evaluated genotypes independently from the considered system or period. DKB 255, AG 9000, and DKB 330 presented similar heights: from 1.70 m to 2.17 m. Sangoi et al. (2002) correlated PH and EIH with the balance of hormones (auxin and cytokinins) in plant growth zones. When plants have a greater accumulation of this hormone in the stem growth regions, tissue changes can cause an increase in plant and ear insertion height, which could also be induced by *A. brasilense*. Kappes et al. (2013) observed higher PH in the presence of *A. brasilense* and attributed this fact to growth-promoting substances produced by the bacteria. Our results showed that inoculation with this strain had no effect on PH and EIH. Likewise, Cunha et al. (2014) observed no significant difference in PH of different inoculated maize hybrids. Although there was no significant difference between the means, DKB 330 PH under FS without inoculation was numerically higher when compared to NT (see Table 3).

Although Portugal et al. (2016) and Garcia et al. (2017) observed that seeds inoculated with *Azospirillum brasilense* presented higher EIH when nitrogen was not applied during (topdressing) fertilization, we observed that EIH had no significant differences between treatments. In fact, the main determining factor in our experiments in EIH was the soil cultivation practices; DKB 330, AG 9000, and DKB 255 showed higher averages for EIH (91.6 to 114.6 cm) under non-inoculated FS in both off-seasons. The only exception in these experiments was DKB 255 which was the highest in the first off-season in the NT inoculated treatment (Table 3).

Regarding SD, AG 9000 showed the higher average only in the first off-season, and not in the second one, whereas among other hybrids there were no differences. Ferreira et al. (2020) found no differences in SD between the selected hybrids whether *A. brasilense* was present or not. Moreover, this parameter did not seem to be influenced by soil cultivation practices or inoculation since it was found no pattern to explain the stem diameter of the hybrids. This finding is corroborated by Gavilanes et al. (2020) who also found no differences in phytometric assessments of the interaction between four maize genotypes and inoculations with *A. brasilense*.

Comparing off seasons, DKB 330 showed a higher NL (6.7) in the first year but no differences were found in the second one (6.9 to 7.2 leaves per plant). These data were not affected by presence or absence of inoculation. This characteristic seems to be influenced by the soil cultivation practices because the number of leaves of DKB 330 (in both off-seasons), and of AG 9000 and DKB 255 (both only in the first off-season) increased under FS.

Chlorophyll index is a quick, easy, and nondestructive methodology that can be correlated to the water status and photosynthetic rate of plants (Vieira et al., 2014). Moreira, Valadão and Valadão Júnior (2019) and other authors found chlorophyll index values of 41.93-45.61 for Chl a to Syngenta TL during the bolting stage (VT). As for Chl b Marques et al. (2023), described values from 9.50 to 11.42 in the hybrid BRS1040, also in bolting stage (VT). Argenta et al. (2003) correlated the adequate N content to values of Chl a + Chl b of 55.3 during VT regardless of the hybrid used. In our study, Chl a + Chl b average value was similar between hybrids and seasons, but the indexes varied from the first to the second off-season evaluations (Table 3) indicating the effect of water shortage during the 2019 season. However, comparing both off-seasons, the indexes showed the same tendency: no significant difference was noted. Regarding soil cultivation practices, Chl a from AG 9000 and Chl b from DKB 330 were higher under FS in both off-seasons (Table 3).

Effect of inoculation on corn hybrids yield

The productivity parameters are presented in Table 4. The general average of 100SW presented some differences between the hybrids and soil cultivation practices. In the first off-season, the highest average among the hybrids was found in inoculated seeds under FS (38.3g), but no difference was found in the second off-season. This emphasizes that grain yield depends on multiple abiotic and biotic factors. The hybrid AG 9000 showed the highest 100SW (38.8g) in the first off-season, whereas DKB 255 was the one with the highest 100SW (46.5g) in the second off-season.

Regarding inoculation with *A. brasilense*, FS with or without inoculation promoted a higher 100SW in DKB 255 and DKB 330 hybrids (FS inoculated). NT without inoculation also showed a significantly high average in DKB 330 (41.5g). Although it was not convenient to statistically assess differences between the seasons themselves since they had different climatic conditions, it was observed a lower 100SW in the first off-season, which was affected by water shortage, despite the regular irrigation provided during the course of the experiment.

The general estimated yield (YI) was 7648 kg ha^{-1}. Therefore, it was higher than the average YI in Paraná which was around 5172 kg ha^{-1} for off-season crops at the time of this study (Secretaria de Estado da Agricultura e do Abastecimento do Paraná - SEAB-PR, 2019). Although the genotypes did not differ within off-seasons, it was observed a higher estimated average for the first off-season in all hybrids affected by water deficit in 2019. This result is a consequence of the genetic quality of the hybrids used in the experiment. They presented an excellent performance in the field and an adequate soil fertility based on P content, pH, CTC, and V% (Gitti; Rizzato, 2020).

Climatic conditions during the experimentation period

Although summer is historically the rainiest season, the rainfall values were below the ideal range for the off-season crops (41 mm to 88 mm in 2019 and 72 mm to 235 mm in 2020) (Simepar, 2021) (Figure 1). The water demand for off-season maize crops is around 3 mm to 5 mm day^{-1} and the demand for the whole culture cycle ranges from 400 mm to 600 mm of water (Pegorare et al., 2009; Soler et al., 2007). These data indicate water deficit in both analyzed off-seasons. On the other hand, temperatures were normal and ranged from 8 °C to 34 °C in 2019 and from 16 °C to 33 °C in 2020 during the maize-growing period. Considering that temperature in the maize crop must vary between 10 °C and 35 °C for its optimal development (Edwiges et al., 2017), the plants were expected to have a good development.

Table 3: Morphophysiological traits of the inoculated genotypes under different soil cultivation practice.

Soil cultivation practice	DKB 255		AG 9000		DKB 330		Average	
	1st Off-Season	2nd Off-Season	1st Off-Season	2nd Off-Season	1st Off-Season	2nd Off-Season	1st Off-Season	2nd Off-Season
Plant height (cm) — PH								
NT inoculated	222.3Aa	149.6Ba	211.1Bab	207.5Aa	199.4Bb	175.0ABa	210.9A	177.2A
NT	209.8ABb	171.9Aa	211.3Bb	167.6Aa	227.3BCa	164.6Aa	216.2A	168.1A
FS inoculated	211.3ABb	171.9Aa	230.1Aa	179.5Aa	211.8ABb	182.5Aa	217.7A	178.0A
FS	204.4Bb	187.1Aa	214.9ABb	193.1Aa	232.3Ca	188.7Aa	217.2A	189.6A
Average	212.0a	170.1a	216.9a	186.9a	217.7a	177.5a		
Ear insertion height (cm) — EIH								
NT inoculated	115.5Aa	79.5Ab	107.4Ba	76.5Abc	104.5ABa	80.5Aa	109.1A	78.7AB
NT	98.8Ba	79.1Ab	108.2ABa	74.1Ac	106.6ABa	82.7Aa	104.5A	78.7B
FS inoculated	108.5ABb	87.5Aab	123.1Aa	88.6Aab	98.0Bb	91.3Aa	109.9A	89.1AB
FS	106.5ABa	97.1Aa	111.0ABa	95.1Aa	114.6Aa	91.6Aa	110.7A	94.6A
Average	107.3a	85.8a	112.4a	83.60a	105.9a	86.53a		
Stem diameter (mm) — SD								
NT inoculated	17.5Aa	20.7Aab	18.7Ba	20.2Aa	15.6Ab	21.3Aa	17.2A	20.7A
NT	15.2Bb	21.9Aa	18.1Ba	20.4ABa	15.8Ab	18.7Ba	16.4B	20.3A
FS inoculated	18.1Aa	17.7Ab	18.2Ba	18.9Aa	17.5Aa	19.4Aa	17.9A	18.7A
FS	17.3Ab	20.2Ab	20.4Aa	19.8Aa	17.2Ab	19.3Aa	18.3A	19.8A
Average	17.0b	20.1a	18.8a	19.8a	16.5b	19.7 a		
Number of leaves — NL								
NT inoculated	6.6ABa	6.7Aa	6.5Ba	6.7Aa	6.4Ba	6.9Ab	6.5B	6.8A
NT	6.8Aa	7.1Aa	6.4Bb	7.0Aa	7.0Ca	6.9Ab	6.7A	7.0A
FS inoculated	6.4Bb	7.0Aa	7.0Aa	6.9Aa	6.7BCb	7.5Aab	6.7A	7.1A
FS	6.6ABb	7.4Aa	6.6Bb	7.1Aa	7.1Ca	7.7Aa	6.7A	7.4A
Average	6.6b	7.1a	6.6b	6.9a	6.7a	7.26a		
Chlorophyll A — Chl-a								
NT inoculated	39.5Aa	94.0Aa	37.7Aa	90.1Aab	46.1Aa	91.3Aa	41.1A	91.8A
NT	36.1Aa	87.9Aa	39.1Aa	83.4Ab	38.2Aa	90.1Aa	37.8A	87.1A
FS inoculated	40.9Aa	92.5Aa	42.3Aa	94.5Aa	39.6Aa	96.4Aa	40.9A	94.5A
FS	37.8Aa	93.9Aa	41.4Aa	91.1Aab	40.5Aa	94.9Aa	39.9A	93.3A
Average	38.6a	92.1a	40.1a	89.8a	41.1a	93.2a		
Chlorophyll B — Chl-b								
NT inoculated	15.7ABa	41.3Aa	14.7Bab	40.9Aa	13.5Bb	34.8Aa	14.6B	39.0A
NT	13.8BCa	35.9Aa	14.2Ba	38.7Aa	15.2ABa	34.7Aa	14.4B	36.4A
FS inoculated	16.4Aa	31.2Aa	17.0Aa	37.8Aa	15.8Aa	36.1Aa	16.4A	35.0A
FS	13.1Cb	36.3Aa	16.7ABa	31.5Aa	15.4ABa	35.1Aa	15.1B	34.3A
Average	14.8a	36.2a	15.6a	37.3a	15.0a	35.2a		

Media followed by the same capital letter in the column (Genotypes) and lowercase letter in the line (soil cultivation practice) do not differ significantly by the Tukey test (P>0.05). (n=5). NT-no-till system. FS-fallow soil.

Table 4: Productivity traits of the inoculated genotypes.

Soil cultivation practices	DKB 255		AG 9000		DKB 330		Average	
	1st Off-Season	2nd Off-Season	1st Off-Season	2nd Off-Season	1st Off-Season	2nd Off-Season	1st Off-Season	2nd Off-Season
100 seeds weight (g) — 100SW								
NT inoculated	33.1Bb	43.33ABa	37.5Aa	47.01Aa	32.1BCb	38.96Ba	34.2C	43.1A
NT	35.9ABab	45.97Aa	39.0Aa	42.47Aa	34.9ABb	41.54Aa	36.6AB	43.32A
FS inoculated	37.3Aa	46.88Aa	40.3Aa	40.31Ba	37.4Aa	40.59Aa	38.3A	42.59A
FS	37.5Aa	49.82Aa	38.5Aa	41.19Aa	30.4Cb	41.41Ba	35.5BC	44.14A
Average	35.9b	46.50a	38.8a	42.74ab	33.7c	40.62b		
Estimated productivity (kg ha^{-1}) — YI								
NT inoculated	8712.3Aa	7258.7Aa	6476.9Ba	5851.7ABa	7169.2Aa	5481.0Ba	7452.8B	6197.1B
NT	9095.4Aa	6518.3Aa	8268.1Aba	6740.3Aa	8846.1Aa	5777.3Aa	8736.6AB	6345.3AB
FS inoculated	10188.0Aa	7444.0Aa	8338.5Aba	7888.5Aa	8568.9Aa	6888.5Aa	9031.8AB	7407.0A
FS	9039.2Aa	6222.0Aa	9461.6Aa	7333.0Aa	8969.2Aa	6999.5Aa	9156.7A	6851.5AB
Average	9258.7a	6860.7a	8136.3a	6821.9a	8388.4a	6155.1a		

Average followed by the same capital letter in the column (Genotypes) and lowercase letter in the line (NT and FS) do not differ significantly by the Tukey test (P>0.05). (n=5). NT-no-till system. FS-fallow soil.

Water shortage effect on hybrids yield among off seasons

Maize yield is strongly be affected by water deficit, especially if it occurs during critical stages of culture such as pre-flowering and grain filling (VT) (Li et al., 2021). In an experiment carried out by Marques et al. (2023), the irrigation in the first off-season was sufficient to allow the full development of plants and consequently promote high productivity. In our study, the minimum rainfall (40 mm) was recorded in April 2019 – 60 days after the seed emergence – which is a critical period because it is when female and male reproductive systems are formed (Pegorare et al., 2009). Therefore, our data pointed to lower 100SW but higher estimated productivity (Table 4). The latter is possible due to a greater number of grains per ear. Lopes et al. (2007) argued that higher 100SW and more grains per ear have an effect on the increase of grain yield in single and triple hybrids, whereas in double hybrids only the number of grains per ear has the same effect.

The productivity of the maize crop is highly dependent on an optimum distribution of rain in the critical period of tasseling and grain filling, and not necessarily on the amount of rain during this period. It was observed a water shortage in April 2020 – in this month, it rained half of the total volume of March 2020. Moreover, April corresponds to the period of development of the female and male reproductive systems. Therefore, results were quite similar to those described by Bergamaschi et al. (2004) in 2 crop seasons: a long drought period in the 1st season (46.8 mm of rain in the critical period) allowed a grain yield of about 8 t ha^{-1} without irrigation. In contrast, in the next season, a short drought during the critical period reduced the grain yield to less than 2 t ha^{-1}, affecting the number of ears per plant and the number of kernels per ear. Therefore, it was concluded that, in years of water shortage, crop productivity may be improved if water is supplied in a timely manner during the critical period of plant development.

Under FS, the estimated YI was numerically higher for non-inoculated hybrids in the first off-season and inoculated hybrids in the second off-season. Under NT, inoculation caused a decrease in the generally estimated crop yield. The lowest average YI of AG 9000 was in the first off-season and of DKB 330 in the second off-season; in both cases, the lowest average was observed in inoculated plants under NT.

Effect of *A. brasilense* on corn yield

Regarding yield parameters, Ferreira et al. (2020) observed enhanced productivity of DKB 310 regardless of the absence or presence of *A. brasilense* (231.39 sc ha^{-1}). Schaefer et al. (2019) also found that this strain improves plant growth and yield but does not supplant the effect of N fertilization. Portugal et al. (2016) reported yield increments of up to 14% during two evaluation periods. Lana et al. (2012) also found productivity increases of 15.4% and 7.4% for two subsequent seasons in which *A. brasilense* was used in the absence of nitrogen fertilization.

On the other hand, several authors found that inoculation with *A. brasilense* did not influence maize yield and had little influence on morphophysiological traits (Pandolfo et al., 2015; Zambonin et al., 2019) or productivity (Mumbach et al., 2017; Silva Jr; Freitas; Rezende, 2021) parameters. In accordance with these studies, our results showed that the main factor related to productivity was the soil cultivation practices, not the presence or absence of inoculation.

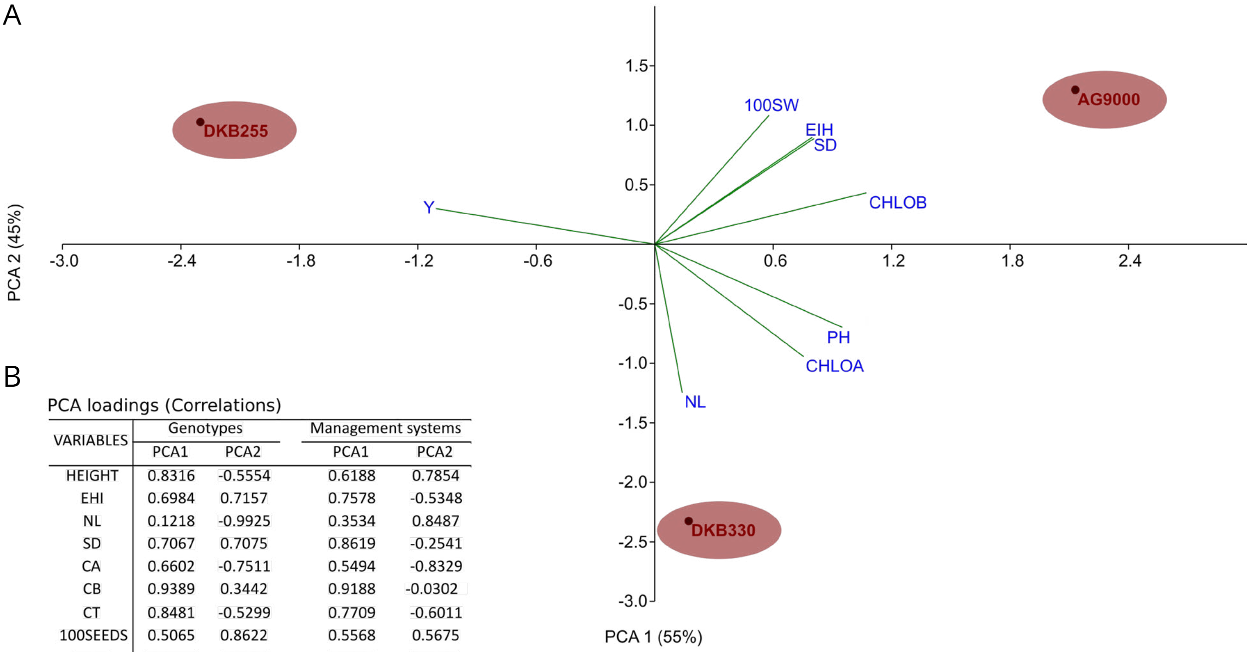
Gavilanes et al. (2020) found incongruities in the responses of maize hybrids to inoculation with *A. brasilense*. This reflects the need for further experimental evaluations to test the possible beneficial effects of inoculation. Another point that must be considered is that plant-bacteria interaction depends on several factors, mainly genotype and strain. These factors can lead to different morphophysiological and productivity results in response to inoculation (Quadros et al., 2014). Zambonin et al. (2019) did not find hybrid-inoculation interaction for any of the morphophysiological and productivity variables in two seasons of experiments involving transgenic maize hybrids. The authors also did not verify any interference of inoculation on grain yield and maize yield.

Principal Component Analysis (PCA) showed that genotypes and soil cultivation practices were separated in both off-seasons (Figures 2 and 3). The association between DKB 255 and productivity is evident from the PCA (Figure 2A), although 100SW and NT were more strongly associated with AG 9000 in the first off-season. Ferreira et al. (2020) found associations between the variables ear diameter with straw (EDS), a thousand-grain mass (TGM), and yield (YI) and the hybrid DKB 310 in the absence of *A. brasilense*. On the other hand, the authors also found that when hybrids 2B610 and 20A78 were inoculated with this proteobacteria, they were highly associated with the strawless ear diameter (SED) and yield (YI) variables according to the analysis of canonical variables.

In our study, the first two components – hybrids and soil cultivation practices – explain a high percentage of variation in the variables (above 88% in all cases) in both PCAs. It was also possible to separate the different treatments (FS and NT) in both off-seasons. The YI was highly associated with FS (Figure 2C and D), thus emphasizing the importance of this system for higher maize productivity.

Although NT is widely recommended as the management system that maintains the best soil conditions in tropical climates, the distinct and positive results of FS regarding the evaluated variables indicate that the continuous use of the soil can decrease plant performance and productivity regardless of inoculation. As a possible perspective for future studies, these results point to a customized use of *A. brasilense* associated with the soil cultivation practices and genotype-hybrid to be used in the off-season crop.

A



B

PCA loadings (Correlations)

VARIABLES	Genotypes		Management systems	
	PCA1	PCA2	PCA1	PCA2
HEIGHT	0.8316	-0.5554	0.6188	0.7854
EHI	0.6984	0.7157	0.7578	-0.5348
NL	0.1218	-0.9925	0.3534	0.8487
SD	0.7067	0.7075	0.8619	-0.2541
CA	0.6602	-0.7511	0.5494	-0.8329
CB	0.9389	0.3442	0.9188	-0.0302
CT	0.8481	-0.5299	0.7709	-0.6011
100SEEDS	0.5065	0.8622	0.5568	0.5675
PROD	-0.9711	0.2386	0.5988	0.7869

C

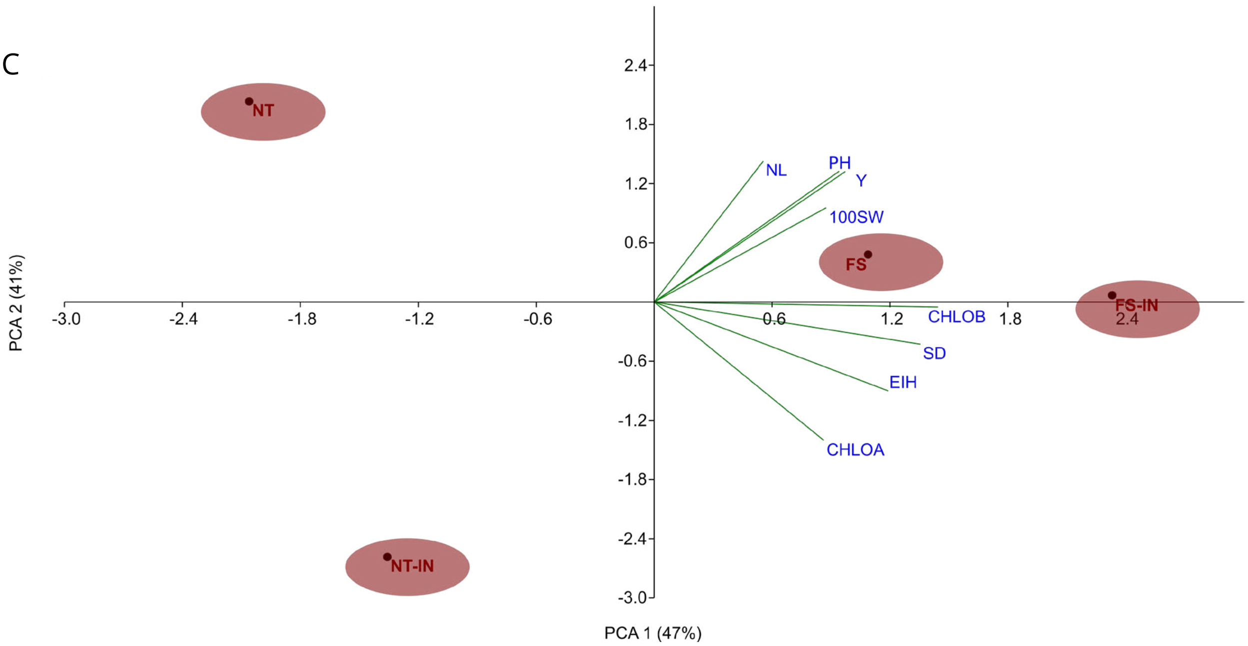
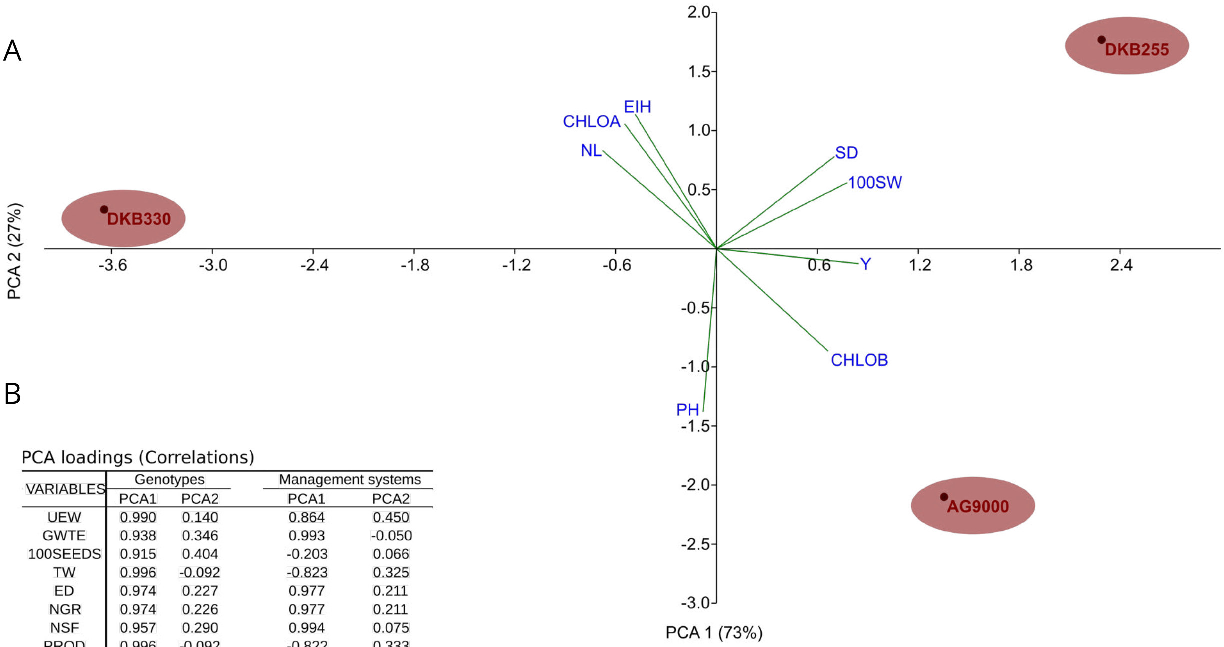


Figure 2: Principal Component Analysis (PCA) for the first (2019) off-season. A) PCA for the genotype factor (PCA1 and PCA2 explain 55% and 45% of the variation, respectively). B) Correlation coefficients between the evaluated variables and the PCA1 and PCA2 for genotypes and soil cultivation practices. C) PCA for the soil cultivation practices factor (PCA1 and PCA2 explain 47% and 41% of the variation, respectively).

A



B

PCA loadings (Correlations)

VARIABLES	Genotypes		Management systems	
	PCA1	PCA2	PCA1	PCA2
UEW	0.990	0.140	0.864	0.450
GWTE	0.938	0.346	0.993	-0.050
100SEEDS	0.915	0.404	-0.203	0.066
TW	0.996	-0.092	-0.823	0.325
ED	0.974	0.227	0.977	0.211
NGR	0.974	0.226	0.977	0.211
NSF	0.957	0.290	0.994	0.075
PROD	0.996	-0.092	-0.822	0.333
HEIGHT	-0.095	-0.996	0.516	0.854
EHI	-0.570	0.822	-0.998	0.060
SD	0.826	0.564	0.310	-0.921
NL	-0.800	0.600	-0.839	0.283
CA	-0.645	0.764	-0.952	0.203
CB	0.780	-0.626	0.972	0.065

C

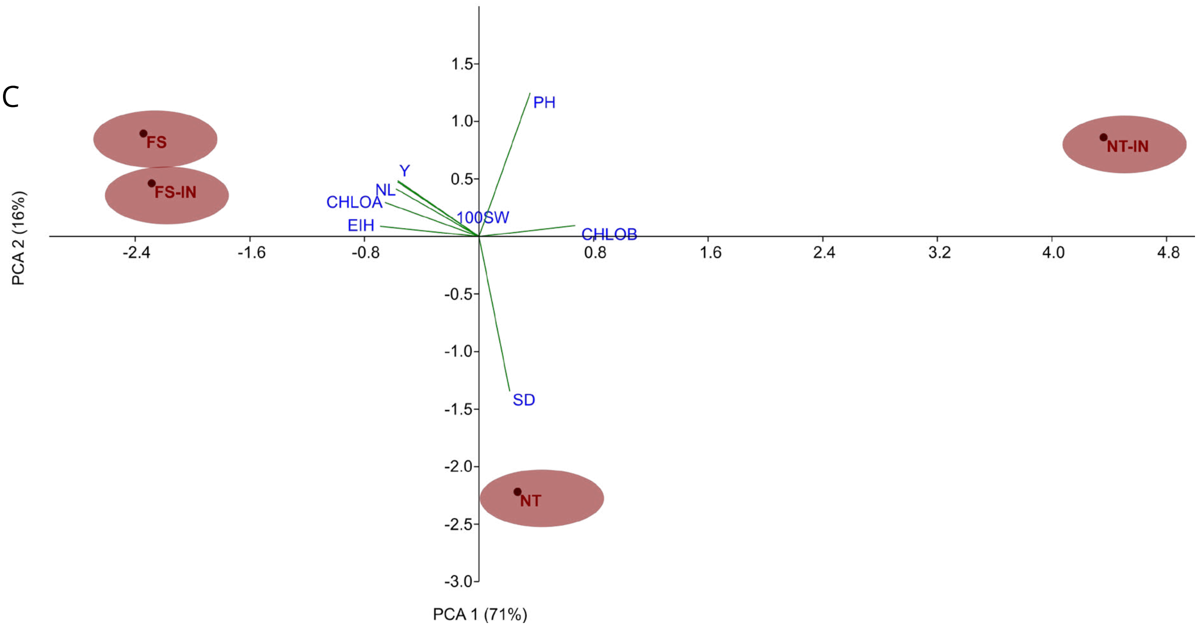


Figure 3: Principal Component Analysis (PCA) for the second (2020) off-season. A) PCA for the genotype factor (PCA1 and PCA2 explain 73% and 27% of the variation, respectively). B) Correlation coefficients between the evaluated variables and the PCA1 and PCA2 for genotypes and soil cultivation practices. C) PCA for the soil cultivation practices factor (PCA1 and PCA2 explain 71% and 16% of the variation, respectively).

CONCLUSIONS

Soil cultivation practices had a stronger effect on morphophysiological and productivity parameters than inoculation with *A. brasilense* in maize hybrids. There were no distinct morphophysiological differences among treatments. FS (inoculated or not) was more closely related to productivity parameters (YI, 100SW, PH, and NL) in both off-seasons when compared to NT, although the cultivation history of the two areas are different. Among the hybrids, DKB 255 showed the highest YI and 100SW and can thus be considered well-adapted to off-seasons in Western Paraná. Inoculation effect was practically null and did not influence the estimated productivity of the analyzed hybrids.

AUTHOR CONTRIBUTION

Conceptual idea: Vendruscolo, E.C.G.; Santos, M.H.; Methodology design: Santos, M.H.; Aguiar, W.E.; Vendruscolo, E.C.G.; Data collection: Santos, M.H.; Aguiar, W.E.; Data analysis and interpretation: Missio, R.F.; Vendruscolo, E.C.G.; Writing and editing: Missio, R.F.; Vendruscolo, E.C.G.

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