



Semina: Ciências Agrárias

ISSN: 1676-546X

ISSN: 1679-0359

Universidade Estadual de Londrina

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Semina: Ciências Agrárias, vol. 39, no. 2, 2018, March-April, pp. 855-864

Universidade Estadual de Londrina

DOI: <https://doi.org/10.5433/1679-0359.2018v39n2p855>

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## Compatibility of *Azospirillum brasilense* with fungicide and insecticide and its effects on the physiological quality of wheat seeds

### Compatibilidade de *Azospirillum brasilense* com fungicida e inseticida na qualidade fisiológica de sementes de trigo

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#### Abstract

Seed treatment is a practice that helps the initial establishment of the crop without the effects caused by pests and diseases. The association of diazotrophic bacteria with grasses has been used in the supply of nitrogen to plants; however, these microorganisms produce growth-promoting substances, which promote benefits in the growth and development of the crops. Thus, the objective of this study was to evaluate the compatibility of *Azospirillum brasilense* associated with the fungicide difenoconazole and the insecticide thiamethoxam by observing the effects on the quality of seed emergence of three wheat cultivars (*Triticum aestivum* L.). Three wheat cultivars, arranged in a 4 x 2 factorial system with four replicates, were tested. The treatments were the control; difenoconazole (Spectro® at a dose of 150 mL per 100 kg of seed); thiamethoxam (Cruiser® FS 350 at a dose of 200 mL per 100 kg seed) and difenoconazole + thiamethoxam, applied on wheat cultivars TBIO Mestre, TBIO Itaipu and TBIO Sinuelo. Bacteria from the *Azospirillum* genus were used in the inoculation. The wheat seed retains its quality when it checks the germination, vigor and independent accelerated aging, whether or not fungicide, insecticide and *A. brasilense* were used. The insecticide thiamethoxam increased the length of shoots and roots and provided compatibility with *A. brasilense*, and fungicide inhibited the length of shoots and roots and was antagonistic to the bacterium *A. brasilense*.

**Key words:** Diazotrophic bacteria. Seed treatment. *Triticum aestivum*.

#### Resumo

O tratamento de sementes é a prática que auxilia o estabelecimento inicial da lavoura sem os efeitos causados por pragas e doenças. A associação de bactérias diazotróficas com gramíneas tem sido empregada no aporte de nitrogênio para as plantas, entretanto esses microrganismos produzem substâncias promotoras de crescimento, que promovem benefícios no crescimento e desenvolvimento das culturas. Dessa forma, objetivou-se com esse estudo avaliar a compatibilidade do *Azospirillum brasilense* associado ao fungicida difenoconazole e inseticida thiamethoxam, observando o efeito sobre

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a qualidade de emergência de sementes de três cultivares de trigo (*Triticum aestivum* L.). Foram testadas três cultivares de trigo, arranjadas em fatorial 4 x 2 com quatro repetições. Os tratamentos utilizados foram testemunha; difenoconazole (Spectro® na dose de 150 mL por 100 kg de semente); thiamethoxam (Cruiser® 350 FS na dose de 200 mL por 100 kg de semente) e difenoconazole + thiamethoxam aplicados sobre as cultivares de trigo TBIO Mestre, TBIO Itaipu e TBIO Sinuelo. Na inoculação foram utilizadas as bactérias do gênero *Azospirillum*. A semente de trigo mantém a sua qualidade fisiológica quando se verifica a germinação, vigor e o envelhecimento acelerado independente da cultivar, utilização ou não de fungicida, inseticida e *A. brasilense*. O inseticida thiamethoxam aumenta o comprimento da parte aérea e raiz e apresenta compatibilidade com o *A. brasilense* e o fungicida inibe o comprimento da parte aérea e raiz e é incompatível com a bactéria *A. brasilense*.

**Palavras-chave:** Bactéria diazotrófica. Tratamento de sementes. *Triticum aestivum*.

Wheat (*Triticum aestivum*) is a highly relevant crop due to its various uses, including in human and animal diets and crop rotation, among others. Due to its high genetic diversity and the exploitation of this variability through breeding, it presents wide edaphoclimatic adaptations, being produced from regions with a desert climate to regions with high rainfall (RIBEIRO JÚNIOR et al., 2007).

Fungi can cause leaf spot, which is often transmitted to seeds (DANELLI et al., 2012). The higher the incidence of the pathogen in the seeds is, the higher the percentage of infested wheat plants in the crop. Thus, seed treatments aim to protect the seeds during the early stages of the crop, avoiding pest and pathogen attacks, which may compromise the productive potential of the crop (MENTEN, 1995).

Difenoconazole is a broad-spectrum systemic fungicide used to fight diseases in fruits, vegetables, cereals and other field crops. It has preventive and curative actions, acting to inhibit enzymes that participate in the final stages of ergosterol biosynthesis, generating modifications in the permeability of the fungal membrane and thus inhibiting its growth (PICININI; FERNANDES, 2003).

Thiamethoxam is a synthetic insecticide with a bioactivation effect, triggering several physiological reactions in the plant that promote the activation of membrane transport proteins and enzymes, increasing plant metabolism through the synthesis

of amino acids and protein precursors and the endogenous synthesis of hormones (CARVALHO et al., 2011). In addition, it has indirect effects, such as increased biomass, photosynthetic rate and root growth (ALMEIDA et al., 2011).

In the absence of resistant cultivars that may break the cycle of disease or of other more efficient control methods, chemical control is an alternative to ensure adequate germination and establishment of seedlings in the field (PICININI; FERNANDES, 2003; REIS et al., 2016). In phytosanitary systems, seeds are used in addition to stimulants, such as micronutrients, growth regulators and promoters, systemic resistance inducers and, more recently, inoculants with diazotrophic bacteria (VIEIRA JÚNIOR et al., 2013).

Diazotrophic bacteria of the genus *Azospirillum* can produce substances that promote plant growth. These microorganisms stimulate plant development, especially through biological nitrogen fixation (BNF) (HUNGRIA, 2011), phosphate solubilization (RODRIGUEZ et al., 2004) and the production of hormones (PERRIG et al., 2007) that promote the development of the seed embryo.

Fungicides and insecticides may adversely affect the proliferation of soil microorganisms, impairing their survival or causing the death of the seed-inoculated bacterial cells (ALCÂNTARA NETO et al., 2014; CAMPO et al., 2009) due to the potential toxic effects of their active ingredients. Some pesticides disrupt the interactions between plants

and N-fixing bacteria and consequently inhibit the vital process of biological nitrogen fixation (BNF) (ARAÚJO; ARAÚJO, 2006).

In crop management, the use of practices such as fertilization and pest and disease control, among others, contribute to the establishment and development of the crop. Among the crop management practices that affect the germination rate and vigor of seedlings, inoculation with beneficial microorganisms such as *Azospirillum brasilense* has been shown to be a viable alternative due to its low cost and environmental sustainability (SCHLINDWEIN et al., 2008). Several field and laboratory studies have been developed to assess the effect of pesticides on soil N-fixing microorganisms, indicating that most have a small or transient effect (GALLORI et al., 1991). These studies have focused mainly on the toxic effect of fungicides used in the treatment of legume seeds on BNF (CAMPO; HUNGRIA, 2000). However, studies on the effect of pesticides on the growth of diazotrophic bacteria inoculated into grass seeds should be carried out in order to assess whether toxic effects exist.

Some studies report that seed treatment with fungicide, insecticide and *A. brasilense* bacteria are compatible with the promotion of germination percentage and root dry matter accumulation in maize (DARTORA et al., 2013) and with increased shoot length in wheat seedlings (PEREYRA et al., 2009). In this context, considering the great variety of chemical products indicated for seed treatment and the scarcity of information when these are associated with *A. brasilense*, this study aimed to evaluate the compatibility of *A. brasilense* associated with the fungicide difenoconazole and insecticide thiamethoxam, observing its effect on the quality of seed emergence of three wheat cultivars.

The experiments were conducted at the Didactic and Seed Research Laboratory of the Department of Plant Science of the Federal University of Santa Maria (UFSM), Santa Maria, Rio Grande do Sul-RS, Brazil. The design was completely randomized,

with treatments distributed in a 4 x 2 factorial arrangement (seed treatment x inoculation), with four replications. Seeds of three wheat cultivars (TBIO Mestre, TBIO Itaipu and TBIO Sinuelo) were used.

The treatments applied to the three wheat cultivars were control (without seed treatment); difenoconazole (Spectro®); thiamethoxam (Cruiser® 350 FS); inoculation with *A. brasilense*; difenoconazole + thiamethoxam; difenoconazole + thiamethoxam + *A. brasilense*; thiamethoxam + *A. brasilense*; and difenoconazole + *A. brasilense*. The doses of difenoconazole and thiamethoxam used were 150 and 200 mL of active ingredient (a.i.) per 100 kg of wheat seeds, respectively. In the inoculation, *A. brasilense* strains AbV5 and AbV6 were used at a concentration of  $2.0 \times 10^8$  colony forming units (CFU) mL<sup>-1</sup>, at a dose of 7.5 mL kg<sup>-1</sup> of seed.

The application of the treatments was carried out following the sequence of insecticide, fungicide and inoculant. The treatments were applied directly to the seeds and mixed in polyethylene bags to ensure the homogeneity of the seed treatment; this procedure occurred moments before assembling the tests. The physiological characteristics of the seeds were determined by the following tests: germination (BRASIL, 2009), vigor, shoot and root lengths, dry mass (NAKAGAWA, 1999), accelerated aging (MARCOS FILHO, 1999; MODARRESI et al., 2002) and cold (FANAN et al., 2006).

The data obtained were analyzed individually for each cultivar and subjected to an analysis of variance, and the means were compared by the Scott-Knott test at a 5% probability level using the Sisvar software (FERREIRA, 2011).

The results show that there was no significant interaction between the tested factors on the variables vigor, germination and accelerated aging for wheat cultivars (Table 1). In general, the cultivars presented a minimum germination rate of 80%, which is required for seed commercialization

(MAPA, 2011), confirming that none of the products had an effect on the tests; however, most of the time, the treated seeds presented better performance

than the untreated seeds (KROHN; MALAVASI, 2004) due to the better conditions for initial seed development.

**Table 1.** Percentage of vigor, germination and accelerated aging in wheat seeds of cultivars TBIO Mestre, TBIO Sinuelo and TBIO Itaipu according to the application or not of *A. brasilense*, insecticide and fungicide.

	Mestre				Sinuelo				Itaipu			
	----- Vigor % -----											
	W/ Fung		Wo/ Fung		W/Fung		W/o/Fung		W/Fung		W/o/Fung	
	----- Insecticide -----				----- Insecticide ----				----- Insecticide -----			
	With	W/o	With	W/o	With	W/o	With	W/o	With	W/o	With	W/o
W/Azo	75*	75	76	85	80	77	76	80	72	71	70	72
W/o/inoc	80	80	80	81	73	80	77	75	80	70	77	77
Mean	78	78	78	83	76	78	77	77	76	70	73	74
CV %	7				7				9			
	----- Germination % -----											
W/Azo	81	77	80	81	80	80	80	80	80	80	81	81
W/o/inoc	80	81	81	87	74	80	81	76	83	77	80	81
Mean	80	80	80	84	77	80	80	78	81	78	80	81
CV %	7				7				5			
	----- Accelerated aging % -----											
W/Azo	63	71	67	64	63	71	67	64	64	70	67	71
W/o/inoc	68	76	70	71	68	76	70	71	68	73	65	72
Mean	66	73	68	67	66	73	68	67	66	71	66	71
CV %	10				10				12			

\* not significant by the Scott-Knott test ( $\alpha \leq 0.05$ ). W/fung = with fungicide, W/o/fung = without fungicide, W/inoc = with inoculation, W/o/inoc = without inoculation.

A reduction in shoot length (SL) was observed when cultivar TBIO Mestre was inoculated with *A. brasilense* associated with fungicide and insecticide (Table 2). This reduction may be related to the depletion of seed reserves or the alteration of chemical composition, such as lipid oxidation and partial breakdown of proteins (BORGHETTI; FERREIRA, 2004), negatively affecting amino acids, nucleotides, lipids, carbohydrates and

chlorophyll, which are the primary metabolic processes responsible for sustaining initial shoot growth. Studies have indicated that the bacterial concentration that promotes plant growth is 10 million viable cells mL<sup>-1</sup>; higher concentrations have an inhibitory effect on the growth of inoculated plants, whereas lower concentrations simply have no effect (ARSAC et al., 1990), which can explain the reduction in SL.

**Table 2.** Shoot length in wheat seedlings of cultivar TBIO Mestre according to the application or not of *Azospirillum brasilense*, insecticide and fungicide.

<i>A. brasilense</i>	----- W/Fungicide -----		----- W/o/Fungicide -----	
	W/insecticide	W/o/insecticide	W/insecticide	W/o/insecticide
	(cm)			
With inoculation	4.7 bB*	5.4 aB	6.3 aA	5.7 aB
Without inoculation	5.1 aA	5.1 aA	5.5 bA	5.5 aA
Mean	5.4			
CV %	4.1			

\* Significant by the Scott-Knott test ( $\alpha \leq 0.05$ ); lowercase letters differ in the column, uppercase in the row. W/insecticide = with insecticide, W/o/insecticide = without insecticide; W/fungicide = with fungicide, W/o/fungicide = without fungicide.

However, the treatment with fungicide alone showed lower SL for the cultivar TBIO Mestre, and the highest means were obtained with inoculation of *A. brasilense* and insecticide, alone or in association (Table 2). The increase in SL occurred because the insecticide acts on the expression of the genes responsible for the synthesis and activation of metabolic processes related to plant growth (GRISI et al., 2009), altering the production of the amino acid precursors of hormones. The inoculation with *A. brasilense* acts on embryo germination induced by growth regulators (indole acetic acid, cytokinins, gibberellins and ethylene) (CASSÁN et al., 2009), which are produced by the bacterium and affect the development of plants and increase their green mass (GRAY; SMITH, 2005).

When comparing the means between treatments, with and without fungicide and with and without insecticide, the untreated seeds had higher root lengths (RL) for the cultivar TBIO Mestre (Table 3). The insecticide and fungicide probably caused lesions in the root cells and caused changes in the meristematic activity (CASTRO, 2008) or formed free radicals through the production and oxidative modification of proteins, causing DNA damage and lipid peroxidation of membranes (SOARES; MACHADO, 2007), which can lead to damage to membrane permeability and consequently reduced root development in this cultivar. However, the total dry mass (TDM) did not change when comparing chemically treated and untreated seeds, which may be justified by the SL.

**Table 3.** Root length (CR, cm) and total dry mass (TDM, g) of cultivar TBIO Mestre according to seed treatment with fungicide and insecticide.

----- Root length (cm) -----				----- Total Dry Mass (g) -----		
Insecticide	Mean	Insecticide	Mean	Insecticide	Mean	Insecticide
With insect	5.55 b*	With insect	5.55 b*	With insect	5.55 b*	With insect
W/o insect	7.53 a	W/o insect	7.53 a	W/o insect	7.53 a	W/o insect
Mean	6.5	Mean	6.5	Mean	6.5	Mean
CV%	7.7	CV%	7.7	CV%	7.7	CV%

\* significant by the Scott-Knott test ( $\alpha \leq 0.05$ ); lowercase letters differ in the column, uppercase in the row; with insect. = with insecticide, w/o insect. = without insecticide.



The SL, in the seed treatment with insecticide and fungicide alone or in association, did not show significant differences among them for cultivar TBIO Itaipu (Table 4). Thus, seed treatment with fungicide and insecticide alone or in association did not affect the shoot growth of the cultivar TBIO Itaipu. Root length (RL) decreased when the seeds were treated with fungicide for the cultivar TBIO Itaipu (Table 4). This result indicates that the phytotoxic effects of this product may have altered the hormonal balance of the plant, reducing the number of dividing cells in the apical meristem of the root (CASTRO, 2008), regulating the

elongation of the root cells and thereby reducing the root length. Although the fungicide reduced the root size, the prevention and control of pathogens with fungicides is fundamental for crop establishment (REIS; CASA, 2012).

For SL, in the cultivar TBIO Sinuelo, no positive effect was observed with the inoculation of *A. brasilense*. This can be related to the genotype of the host plant (FIBACH-PALDI et al., 2012), considering that the interaction with the bacterium may vary depending on environmental factors, the genotype (DOBBELAERE et al., 2001) or even the strain selected (HUNGRIA, 2011).

**Table 4.** Shoot length (SL, cm) and root length (RL, cm) of the TBIO Itaipu cultivar, according to seed treatment with fungicide and insecticide.

----- Shoot length (cm) -----			---- Root length (cm) ----	
Insect/Fung	W/fung	Insect/Fung	W/o/fung	Insect/Fung
W/insect	4.8 aA*	5.0 aA	W/fung	5.0 b
W/o/insect	4.9 aA	4.7 bA	W/o/fung	6.7 a

\* significant by the Scott-Knott test ( $\alpha \leq 0.05$ ); lowercase letters differ in the column, uppercase in the row; with insect. = with insecticide, w/o insect. = without insecticide; with fung. = with fungicide, w/o/fung. = without fungicide

The use of the fungicide difenoconazole promoted higher SL when compared to the control without fungicide (Table 5). This result suggests that the fungicide formed a protective coating around the seed, protecting it and providing adequate

conditions for shoot emission and development. Similar results were found by Pereyra et al. (2009), who treated wheat seeds with tebuconazole and observed an increase in coleoptile length 24 h after application of the fungicide.

**Table 5.** Shoot length (cm), root length and cold test of cultivar TBIO Sinuelo according to the application or not of *Azospirillum brasilense*, insecticide and fungicide.

----- Shoot length (cm) -----			
<i>A. brasilense</i>	Mean	Fungicide	Mean
With inoculation	4.5 b*	With fungicide	4.7 a
Without inoculation	4.7 a	Without fungicide	4.5 b
Mean	4.6		
CV%	5.8		
----- Root length (cm) -----			
Insecticide/fungicide	With fungicide	Without fungicide	
Com insecticide	5.5 aB*	7.0 bA	
Without insecticide	5.0 aB	7.8 aA	
Inoculation/fungicide	With fungicide	Without fungicide	
With inoculation	5.3 aB*	7.1 bA	
Without inoculation	5.0 aB	7.6 aA	
Mean	6.3		
CV%	7.7		
-----Cold %-----			
Insecticide/fungicide	With fungicide	Without fungicide	
With insecticide	81.7 bB*	91.2 aA	
Without insecticide	93.0 aA	87.2 aA	
Mean	88.3		
CV%	8.5		

\* Significant by the Scott-Knott test ( $\alpha \leq 0.05$ ); lowercase letters differ in the column, uppercase in the row.

For the RL of the TBIO Sinuelo cultivar, in both interactions, the fungicide acted as an inhibitor (Table 5). On the other hand, seeds treated with insecticide showed positive effects on root length. This result confirms the hypothesis that thiamethoxam acts to synthesize and activate the enzymes related to plant growth, altering the production of the amino acid precursors of plant hormones. Higher hormone production leads to greater root development (CASTRO et al., 2008). Seedlings with a developed root system tend to have an increased the area of water and nutrient uptake.

In addition to the negative effect on RL, difenoconazole did not have a good interaction with the bacterium for the TBIO Sinuelo cultivar (Table 5). When the seed was inoculated with *A. brasilense* and without the use of the fungicide, an increase of

1.8 cm was observed for RL. Some fungicides may lead to deleterious effects four hours after contact with bacteria (ALCÂNTARA NETO et al., 2014) or cause the death of up to 100% of bacteria (CAMPO et al., 2009); however, the viability of the bacteria has not been evaluated in this study. It is assumed that, in addition to the active ingredient, the pH (SIQUEIRA, FRANCO, 1988) is responsible for the reduction in the number of viable cells (BASHAN et al., 2004) or bacterial death (ARAÚJO; ARAÚJO, 2006) caused by direct contact with fungicides.

The percentage of seedlings germinated in the cold test decreased for the TBIO Sinuelo cultivar when the seeds were treated with fungicide and insecticide together (Table 5). The values found in the cold test when compared to the vigor test increased from 80 to 93% with fungicide and from 77 to 91%



with insecticide. This increase in vigor is probably a direct effect of the different active ingredients used in seed treatment (MARCOS FILHO, 2015), and the cold may have accelerated seed metabolism and induced embryonic axis formation, leading to rapid seedling development.

During plant development, the initial stages are considered critical, because the highest mortality rates occur between seed germination and seedling establishment. Thus, vigorous seedlings can compete efficiently, especially under conditions of light, nutrient and/or water stress, resulting in the establishment of the population and grain production (FAROOQ et al., 2006). The results of the experiment show that the treatment of seeds with *A. brasilense* can help in the development of more vigorous seedlings, providing uniform plant stands and greater yields for wheat crops due to the benefits of the association of bacteria with the crop. During the initial stage of development, inoculation of seeds with *A. brasilense* can promote increases in seedling biomass, which is related to the development of the embryo induced by the growth regulators produced by the bacterium, which penetrate the seed coat together with water during the imbibition process, accelerating the growth of the radicle and thus increasing the uptake capacity (CASSÁN et al., 2009).

In conclusion, the physiological quality of wheat cultivars is maintained with the use of thiamethoxam, difenoconazole and *A. brasilense* in an isolated or associated way. In addition, the thiamethoxam insecticide is compatible with inoculation with *A. brasilense*, increasing the root length. In contrast, the fungicide difenoconazole is incompatible with the bacterium, reducing the root area.

## Acknowledgements

To the National Council for Scientific and Technological Development (CNPq) for granting the PQ2 scholarship to the second author.

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