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PHYSIOLOGICAL QUALITY OF *Brachiaria brizantha* SEEDS TREATED WITH FUNGICIDE AND INSECTICIDE¹

ERIC FABIANO SERAGUZI², CARLOS HENRIQUE QUEIROZ REGO², FERNANDA BRITO CARDOSO², ANA CARINA DA SILVA CÂNDIDO², CHARLINE ZARATIN ALVES^{2*}

ABSTRACT – This study aimed to evaluate the effects of chemical treatment of seeds with fungicide and insecticide on the seed physiological quality of *Brachiaria brizantha* cultivar MG5. Two experiments with four replicates were carried out in a completely randomized design. In the first experiment, the seeds were treated with the fungicide pyraclostrobin + fipronil + thiophanate-methyl and in the second, with the insecticide thiamethoxam, both at doses of 0, 150, 300, 450, and 600 mL of commercial product (CP)/100 kg of seeds. Physiological characterization was done on the basis of the first germination count, germination (%), emergence (%), emergence speed index, and length and dry mass of shoot and root. The treatment of *B. brizantha* seeds with the fungicide pyraclostrobin + fipronil + thiophanate-methyl benefits the physiological quality of seeds, improving germination and root development, with no phytotoxic effect up to the dose of 600 mL of CP/100 kg of seeds. The insecticide thiamethoxam has a biostimulating effect on *B. brizantha* cultivar MG5 up to the dose of 270 mL of CP/100 kg of seeds, but is phytotoxic in larger doses.

Keywords: Biostimulant. Pyraclostrobin + fipronil + thiophanate-methyl. Thiamethoxan. Vigor.

QUALIDADE FISIOLÓGICA DE SEMENTES DE *BRACHIARIA BRIZANTHA* TRATADAS COM FUNGICIDA E INSETICIDA

RESUMO – Objetivou-se avaliar o tratamento químico de sementes com fungicida e inseticida na qualidade fisiológica de sementes de *Brachiaria brizantha* cv. MG5. Foram realizados dois experimentos em delineamento inteiramente casualizado, com quatro repetições. No primeiro experimento, as sementes foram tratadas com o fungicida piraclostrobina+fipronil+tiofanato metílico e no segundo com o inseticida tiametoxam, ambos nas doses 0; 150; 300; 450 e 600 mL do p.c. 100 kg de sementes⁻¹. A caracterização fisiológica foi realizada por meio da primeira contagem de germinação, germinação, emergência, índice de velocidade de emergência e comprimento e massa seca de raiz e parte aérea. O tratamento de sementes de *B. brizantha* com o fungicida piraclostrobina+fipronil+tiofanato metílico beneficia a qualidade fisiológica das sementes, incrementando a germinação e o desenvolvimento radicular, não apresentando efeito fitotóxico até a dose de 600 mL p.c. 100 kg sementes⁻¹. O inseticida tiametoxam apresenta efeito bioestimulante em sementes de *B. brizantha* cv. MG5 até a dose 270 mL p.c. 100 kg de sementes⁻¹, sendo fitotóxico para as doses maiores.

Palavras-chave: Bioestimulante. Piraclostrobina+fipronil+tiofanato metílico. Tiametoxan. Vigor.

*Corresponding author

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²Department of Agronomy, Universidade Federal de Mato Grosso do Sul, Chapadão do Sul, MS, Brazil; eric_seraguzy@hotmail.com – ORCID: 0000-0002-9457-9062, carlosqueirozagro@gmail.com – ORCID: 0000-0001-5483-9264, fernandabcardoso@hotmail.com – ORCID: 0000-0002-0370-261X, ana.candido@ufms.br – ORCID: 0000-0002-9230-4807, charline.alves@ufms.br – ORCID: 0000-0001-6228-078X.

INTRODUCTION

Pasture areas cultivated with *Brachiaria* species have been increasing in Brazil, compared to other forages, mainly due to their rusticity, which allows them to adapt to the most diverse climatic and soil conditions (COSTA et al., 2008). For the production of quality pastures, adequate management alone is not enough, but the use of seeds with high germinative power and vigor should also be included (LIMA et al., 2016). Thus, research has sought to improve the sanitary and physiological quality of forage seeds, with the aid of techniques that help achieve higher expression of vigor.

Fungicides and insecticides are used to control fungi and pests, respectively. However, some of them have been used in the treatment of seeds because they act physiologically in plants, tending to result in vigorous growth and improved productive potential (CASTRO et al., 2008), an effect known as phytotonic effect.

In agriculture, fungicides have always focused on the control of fungal diseases, but this perspective changed with the evolution of the strobilurin group and its consequent physiological action. These actions involve plant growth inhibition, decreased elongation, increased chlorophyll levels, increased chloroplast numbers, thicker leaf tissue, increased root/shoot ratio, delayed senescence, increased antioxidant activity, alkaloid production (ZHANG et al., 2010), and effects on hormone synthesizing and nitrate-reducing enzymes (VENÂNCIO et al., 2003).

The pyraclostrobin + fipronil + thiophanate-methyl mixture consists of two fungicides (pyraclostrobin and thiophanate-methyl) and an insecticide (fipronil), of which pyraclostrobin is known to have beneficial physiological effects (VENÂNCIO et al., 2003). Pyraclostrobin is associated with inhibition of fungal mitochondrial respiration by blocking electron transfer in the complex III (bc₁) of the respiratory chain (BARTLETT et al., 2002). However, its physiological action in plants is associated with the delay in senescence, increase in photosynthetic rate, and better response to stresses, due to hormonal changes and its action as a precursor of enzymes such as nitrate reductase (VENÂNCIO et al., 2003).

Studies have demonstrated the ability of some molecules to improve the physiological potential of seeds, such as thiamethoxam (SOARES et al., 2012). This molecule has brought new possibilities to agriculture, considering that it increases germination, emergence, seedling length, seedling performance under stress conditions, and the total levels of proteins and enzymes in plants. Thiamethoxam is a neonicotinoid insecticide, which acts on a specific receptor in the insect nervous system; in plants, however, it acts on physiological functions such as amino acid synthesis for plant hormones, for

example, cytokinin, which is responsible for cell multiplication and elongation (CASTRO et al., 2008).

Thus, we aimed to evaluate whether the chemical treatment with fungicide and insecticide influence the seed physiological quality of *Brachiaria brizantha* cultivar MG5.

MATERIAL AND METHODS

In this study, we used *Brachiaria brizantha* cultivar MG5 seeds (*Urochloa brizantha* cultivar MG5). Two completely randomized experiments with four replicates were carried out. In the first experiment, seeds were treated with the fungicide pyraclostrobin + fipronil + thiophanate-methyl (Standak Top®) and in the second experiment, with the insecticide Thiamethoxam (Cruiser® 350 FS), both at doses of 0, 150, 300, 450, and 600 mL of commercial product (CP)/100 kg of seeds. Samples of 100 g seeds per treatment were placed in plastic bags, and a volume of 600 mL/100 kg of seeds was added, with subsequent mixing for 3 min until the seeds were completely coated. The control received only a spray of distilled water.

The following parameters were used to evaluate the physiological quality of the seeds: germination, first germination count, emergence, emergence speed index, length and dry mass of shoot and root.

The germination test was performed with four replicates of 50 seeds per treatment, arranged in "Gerbox" boxes on blotting paper previously moistened with 2.5 times the mass of the dry paper, and kept in a germinator set at 20-35 °C. Scoring of normal seedlings was performed 7 and 21 days after the test started (BRASIL, 2009). The first germination count was performed together with the germination test, with the evaluation of normal seedlings on the seventh day after the test started (BRASIL, 2009).

In the emergence test, four replicates of 50 seeds per treatment were used. They were conditioned in 200-cell expanded polystyrene trays containing commercial substrate, and the normal seedlings were counted daily. The emergence speed index was determined together with the emergence test, in which scoring started on the seventh day and was performed daily up to 21 days after the test started, and it was calculated according to the formula proposed by Maguire (1962).

For root and shoot length measurements, four replicates of 20 seeds were used for each treatment. These were arranged in "Gerbox" boxes on blotting paper previously moistened with 2.5 times the mass of the paper, and kept in a germinator set to 20-35 °C. Root and shoot lengths were measured after 21 days using a millimeter ruler, and values were expressed in cm/seedling (NAKAGAWA,

1999). Simultaneously, root and shoot dry mass were evaluated. Roots and shoots were dried a forced air circulation oven at 65 °C for 48 h. After 48 h, we assessed the dry mass, expressed in mg/seedling (NAKAGAWA, 1999).

Data were submitted to variance analysis, and the means were compared by polynomial regression at 5% probability.

RESULTS AND DISCUSSION

The effects of the chemical treatment with the fungicide pyraclostrobin + fipronil + thiophanate-methyl on the seeds of *B. brizantha* cultivar MG5 are shown in Figure 1. We found that increasing the fungicide dose showed a positive linear fit for germination (Figure 1A), root length (Figure 1B) and root dry mass (Figure 1C).

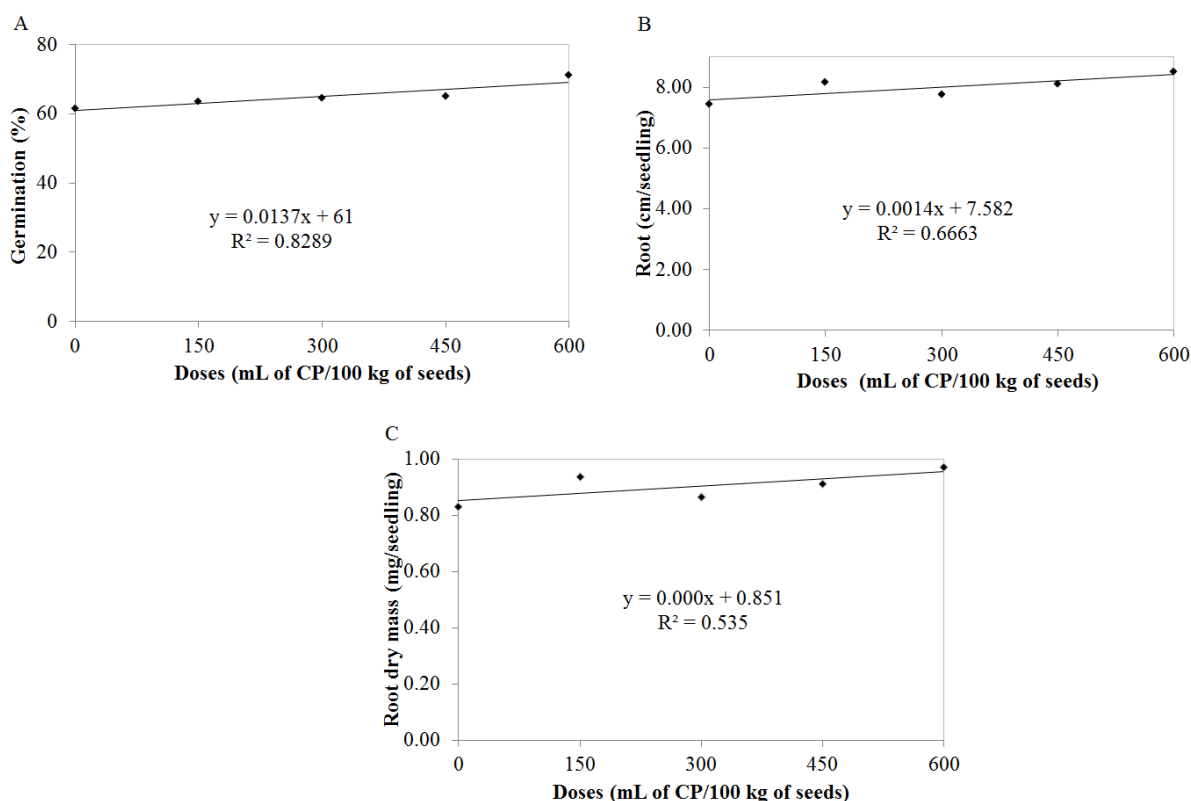


Figure 1. Germination (A), root length (B), and root dry mass (C) of *Brachiaria brizantha* cultivar MG5 seedlings, as a result of the treatment of seeds with different doses of a commercial product (CP) based on pyraclostrobin + fipronil + thiophanate-methyl.

It is known that the physiological effect of strobilurin fungicides, such as pyraclostrobin, can be attributed to the increase in nitrate reductase enzyme activity (VENÂNCIO et al., 2003) and to a decrease in ethylene synthesis (GROSSMANN; RETZLAFF, 1997). Moreover, strobilurins increase the photosynthetic rate and decrease cellular respiration (BRYSON; LEANDRO; JONES, 2000). They are also associated with increased tolerance of plants to abiotic stresses owing to their action on the metabolism of abscisic acid and antioxidant enzymes (GROSSMANN; KWIATKOWSKI; CASPAR, 1999).

The results showed a beneficial effect of pyraclostrobin + fipronil + thiophanate-methyl in the treatment of *B. brizantha* seeds, with an increase in

germination and root length. These results are satisfactory, as the increase in initial stand and root volume could lead to a better establishment of the crop, and consequently, better production. Studies on the treatment of soybean seeds with different commercial products containing pyraclostrobin + fipronil + thiophanate-methyl also observed increases in root length and dry mass, as well as other developmental aspects such as root volume, plant height, and leaf area (BALARDIN et al., 2011). Similarly, Oliveira et al. (2015) observed a positive effect on the performance of cowpea, and Migliorini et al. (2012) reported an increase in hypocotyl length and primary root in canola seeds.

In the experiment with the insecticide thiamethoxan (Figure 2), data were adjusted for

emergence, emergence speed index (ESI), length and root dry mass. Emergence (Figure 2A) and ESI (Figure 2B) were fitted to quadratic equations; an increase was observed up to the doses of 247 and

232 mL of CP/100 kg of seeds, respectively, after which a decrease was observed, and the highest doses showed lower results than the untreated seeds, indicating possible phytotoxicity.

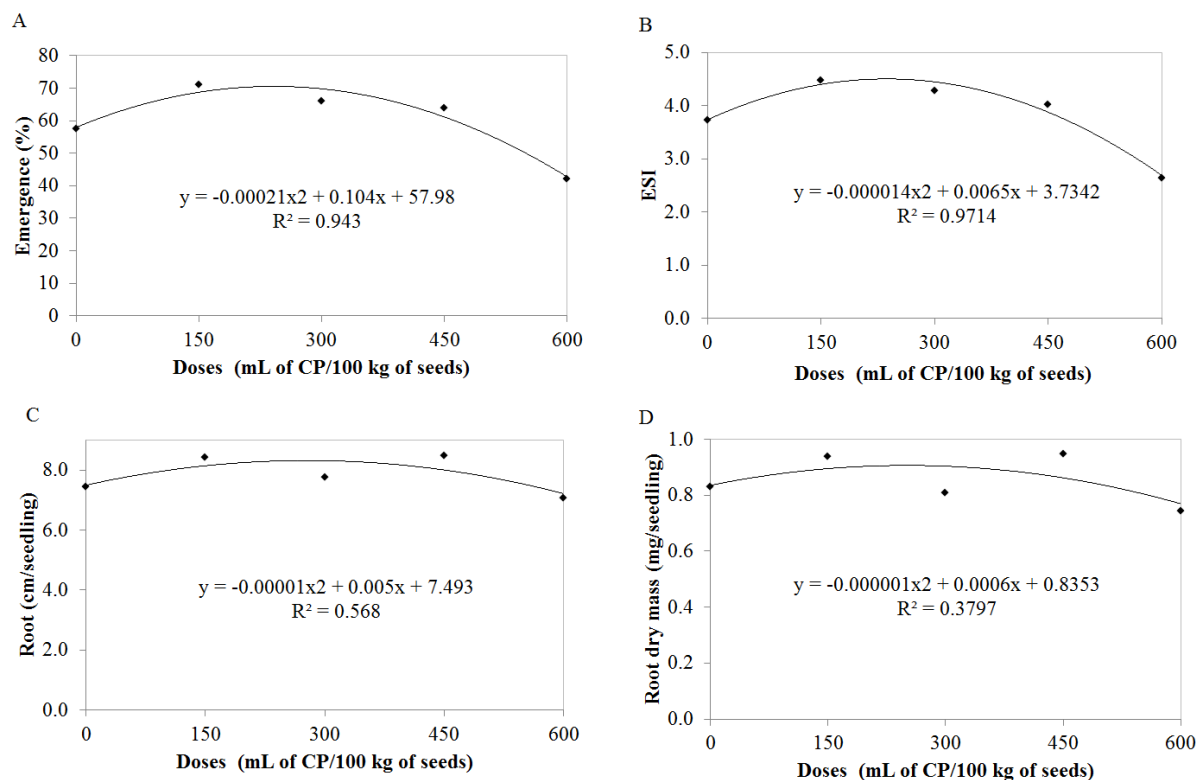


Figure 2. Emergence (A), emergence speed index (ESI) (B), root length (C) and root dry mass (D) of *Brachiaria brizantha* cultivar MG5 seedlings as a result of the treatment of seeds with different doses of a commercial product (CP) based on thiamethoxam.

The beneficial effect of thiamethoxam may be explained by the stimulation of enzyme activities, because this insecticide is transported into the plant, where it activates various physiological reactions, such as the expression of membrane proteins (ALMEIDA et al., 2011). It also acts on the pentose phosphate metabolic pathway, helping in the degradation of reserves and increasing the energetic supply for and, consequently, the speed of the germination and emergence processes (HORII; MCCUE; SHETTY, 2007). Castro et al. (2008) observed that soybean seeds treated with thiamethoxam showed an accelerated germination process owing to enzyme activity stimulation, and also reached a uniform emergence and adequate stand establishment. It has also been observed that thiamethoxam does not affect the quality of soybean seeds treated and stored for up to 2 months (FERREIRA et al., 2016). However, high doses of products to treat seeds might cause negative effects on seed physiological quality (SOARES; MACHADO, 2007).

Thiamethoxam is transported into the plant through the cells in which various physiological reactions such as protein expression

are activated. These proteins, in turn, interact with the stress-defense mechanisms of the plant, allowing it to better cope with adverse conditions such as drought, low pH, high soil salinity, free radicals, high-temperature stress, toxic effects of elevated aluminum levels, pest-induced injuries, winds, hail, virus attack, and nutrient deficiency, leading to the phytotonic effect, i.e., faster plant development with better vigor expression. In soybean, Clavijo (2008) observed increased vigor, productivity, and leaf and root area; uniformity in emergence; and better initial development of seedlings.

Root length (Figure 2C) and root dry mass (Figure 2D) were affected by the doses of thiamethoxam; the data were fitted to quadratic equations, with increased parameter values observed up to the doses of 268 and 272 mL of CP/100 kg of seeds, respectively.

The effect of thiamethoxam is indirect, acting on the expression of genes responsible for the synthesis and activation of metabolic enzymes related to plant growth, altering the production of amino acid precursors of plant hormones, which may reflect in a better vigor expression and root development (TAVARES et al., 2007). Several

studies have also found increased root elongation and volume after thiamethoxam use on soybean seeds (BALARDIN et al., 2011; MACEDO; CASTRO, 2011; DAN et al., 2012).

When used as a treatment for soybean seeds, thiamethoxam accelerates germination by stimulating peroxidase activity and preventing oxidative stress and induces the development of the embryonic axis, minimizing the negative effects in the presence of aluminum, salinity, and water deficiency (CATANEO, 2008). In this sense, there is a reduction in the time required for crop establishment in the field, reducing the negative effects of competition with weeds or for essential nutrients present in the soil (CASTRO et al., 2007). Under natural field conditions, plants are exposed to various stressors that may reduce their ability to express and achieve the full genetic potential for productivity. Under suboptimal conditions, plants derived from seeds treated with thiamethoxam are more tolerant to these stress factors and, consequently, develop better than plants derived from untreated seeds, with a higher probability of achieving their productive potential.

Pyraclostrobin and thiamethoxam are considered enhancers of seed germination; however, the response may vary depending on the species, cultivar, initial physiological quality, dose of product and storage time. They induce positive effects in some crops by altering physiological and morphological characteristics, but not in others, acting only in the initial control of pests and fungi, respectively, during the establishment of plants in the field.

The results obtained from the set of reported tests demonstrate that seed treatments with compounds containing pyraclostrobin and thiamethoxam are a viable alternative for improving the seed physiological quality of *Brachiaria brizantha* cultivar MG5. It is known that under natural field conditions, these species may present low, slow, and irregular germination, with uneven emergence. Therefore, these products could act as enhancers, allowing the expression of seed germination potential, accelerating root growth and increasing plant nutrient uptake. These characteristics, along with seeds of high genetic and physiological quality, could improve the productive capacity of the crop.

CONCLUSIONS

The chemical treatment of *Brachiaria brizantha* cultivar MG5 with the fungicide pyraclostrobin + fipronil + thiophanate-methyl benefits the physiological quality of seeds, improving germination and root development, without phytotoxic effects up to the dose of 600 mL of CP/100 kg of seeds. Thiamethoxam

shows a biostimulating effect on *Brachiaria brizantha* cultivar MG5 seeds up to the dose of 270 mL of CP/100 kg of seeds, but is phytotoxic in larger doses.

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