Original Research Article

Effect of fertilizer on palisadegrass seeds pathogens

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ABSTRACT

This study aimed to evaluate the effects of the contact of *Brachiaria* seeds with the fertilizer 05-25-15 on the sanitary quality of the seeds. The experimental design was completely randomized, in a (2x5) factorial design, with four replications. The treatments consisted of five contact times (0, 24, 48, 72 and 96 hours) of the fertilizer with seeds of ruziziensis grass and marandu grass. After the contact times of the *Brachiaria* seeds with the fertilizer, the seeds from each species were separated and the following analyses were performed: water content, germination, seed health ("Blotter Test"), and accelerated aging, with later germination test and sanitary analysis of seeds. Data were analysed for variance and regression analysis at the significance level of 5% and descriptive analysis was used for the results of the sanitary analysis. The contact time reduces the incidence of pathogens in the seeds of ruziziensis grass and marandu grass treated with the fertilizer due to the salinity and acid pH of the fertilizer. The fungi related to the reduction in germination were *Aspergillus* sp. and *Fusarium* sp. The disinfestation process increased the incidence of *Fusarium* sp., under high internal infestation of this fungus in palisadegrass seeds.

Keywords: accelerated aging; Aspergillus sp.; blotter test; Brachiaria sp.; Fusarium sp.; germination.

1. INTRODUCTION

Integrated crop-livestock systems are a feasible alternative in order to promote socioeconomic and sustainable development before a growing food demand and the need to reduce deforestation [1], increasing production system efficiency [2]. In addition to the financial benefits [3,1], there is synergism between pastures and annual crops, such as: improvement of physical, chemical and biological properties of soil, control of diseases, pests and weeds [4].

Based on the implantation premises of the Santa Fé system, where the grain crop is annually intercropped with a forage crop, stand out maize and *Brachiaria* (Syn. *Urochloa*) *ruziziensis* cv. Kennedy (Ruziziensis palisadegrass). For the deployment of the Barreirão system, in which a grain crop is intercropped and/or rotated with a forage crop that acts in the area as a perennial culture, the most used species are maize and *Brachiaria* (Syn. *Urochloa*) *brizantha* cv. Marandu (Marandu palisadegrass) [5]. For the maize crop implantation in Mato Grosso state, Brazil, the fertilizer 05-25-15 (N-P_2O_5-K_2O) is commonly used.

In order to minimize problems arising from the forage sowing in integrated systems, such as the lack of uniformity in the initial stand due to the reduced size and low weight of the seeds, the simultaneous sowing of grain and forage seeds is commonly performed, associating them to the fertilizer applied at the grain crop sowing [6].

However, the contact of the seeds with the fertilizer can influence their sanitary quality, altering the incidence of pathogens associated to the seeds due to fertilizer salinity and pH. Therefore, this study aimed to evaluate the effect of the contact time of fertilizer 05-25-15 (N-P_2O_5-K_2O) on the sanitary quality of palisade grass seeds.

2. MATERIAL AND METHODS

The experiment was conducted in a completely randomized design, with four replications. Treatments consisted of a 5x2 factorial design, with five contact times (0, 24, 48, 72 and 96 hours) of the fertilizer 05-25-15 (N-P_2O_5-K_2O) on *Brachiaria* (Syn. *Urochloa*) *ruziziensis* cv. Kennedy (ruziziensis palisade grass) and *Brachiaria* (Syn. *Urochloa*) *brizantha* cv. Marandu (Marandu palisade grass). The fertilizer consisted of monoammonium phosphate, single superphosphate, triple superphosphate and potassium chloride, with a saline index of 70.59% and pH 4.83.

The association of the seeds with the fertilizer was performed for both grass species, with the seeds being then transferred to sealed plastic packages and stored until reaching the pre-established contact times. Afterwards, the fertilizer was removed from the mixtures and the grass seeds were subjected to the tests of water content, germination, seed health ("Blotter Test" with salt stress) and accelerated aging analyses, with later germination test and analysis of sanitary conditions.

In order to determine the water content, three 4.0 g samples were placed in a drying oven for 24 hours at a temperature of 105 ± 1 °C, for each species and treatment. After the drying process, the samples were placed in desiccators to promote the cooling and then weighed in analytical balance (0.0001 g). The results were expressed as percentage, according to the methodology of the authors cited in the reference [7].

The methodology used for the standard germination test followed the described by the authors cited in the reference [7], in which four sub-samples of 50 seeds were used for each species and treatment. The seeds were placed equidistantly in a polystyrene box (gerbox) on two sheets of blotter paper as substrate, and moistened with distilled water in the ratio of two and a half times the dry mass of the paper.

Subsequently, the boxes were sealed with film paper to reduce moisture loss, and taken to the BOD (Biochemical Oxygen Demand) chamber, with photoperiod and temperature regulation (12 hours of light at 35 °C and 12 hours in the absence of light at 20 °C). Moisture inside the gerbox was maintained with the addition of distilled water. At 21 days the germinated seeds were evaluated, considering germinated the seeds that had emitted 2 mm of root. The results were expressed as percentage.

Seed sanitary analysis was performed according to the methodology of the authors cited in the reference [8] modified for water restriction, according to the methodology of the authors cited in the reference [9]. For each treatment and grass species, 100 pure and viable seeds and 100 pure, viable and disinfested seeds were used. The disinfestation process was performed in a laminar flow cabinet by immersing the seeds in a 1% sodium hypochlorite solution for three minutes. The seeds were then washed with sterile distilled water, according to the described by the authors cited in the reference [8].

Afterwards, the seeds were distributed equidistantly in a gerbox containing three sheets of filter paper, previously moistened with a sterile sodium chloride solution (-0.6 MPa), according to the described by the authors cited in the reference [9], in a proportion equivalent to two and a half times the dry mass of the substrate. During the analyses period, substrate moisture was maintained by the addition of a sterile sodium chloride solution (-0.6 MPa) to restrict seed germination and to ensure the accurate evaluation of microorganisms incidence.

The seeds were incubated in a BOD chamber under the constant temperature of 20 °C and a 12-hour photoperiod. Seven days later, the individual evaluation of the seeds was performed with the aid of stereoscopic and biological microscopes. The fungi were identified by the observation of morphological structures, with the aid of specialized literature. Results were expressed as percentage of fungi incidence, according to the methodology of the authors cited in the reference [8].

The accelerated aging test was performed according to the methodology proposed by the author cited in reference [10], in which the seeds of each treatment were distributed on an aluminium screen attached to a gerbox containing 40 mL of distilled water. The boxes were then capped, forming a wet chamber, and placed in a BOD chamber for a period of 36 hours at a temperature of 42 °C. After the accelerated aging, germination and seed health tests, along with seed evaluation, were performed seven days later.

Data were subjected to analysis of variance (ANOVA) and regression analysis at a significance level of 5% probability (P=0.05), and descriptive analysis for the results of the total sanitary analysis (high variability data).

3. RESULTS

In the water content analysis, there was no effect of the contact time and no interaction between forages and contact times. A mean water content of 7.91% was observed.

In the germination standard test there was an interaction effect of contact time x forage species. The contact time with the fertilizer did not affect the germination of the ruziziensis palisadegrass seeds, with mean value of 75.10%. However, the evaluation of the marandu palisadegrass seeds showed a reduction in germination percentage as the contact time of the seeds with the fertilizer was prolonged (Fig. 1).

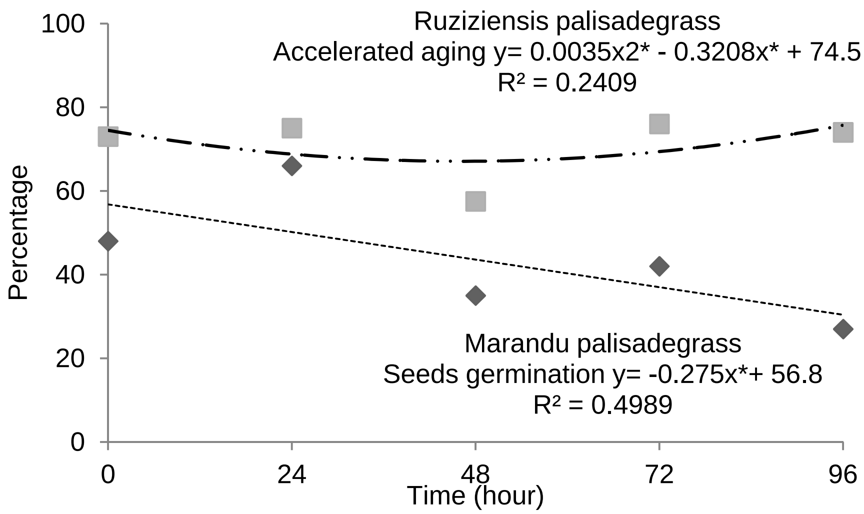

Fig. 1. Germination of marandu palisade grass seeds (A) and accelerated aging of ruziziensis palisade grass seeds (B) as a function of the contact time with the fertilizer 05-25-15. **Significant at 5% probability (P = .05).*

An interaction effect of contact time x forage species on the percentage of seed germination after the accelerated aging was observed. There was a quadratic effect in the contact time of ruziziensis palisade grass seeds with the fertilizer on accelerated aging (Fig. 1), with a decrease in the germination percentage of aged seeds as the contact time with the fertilizer increased up to 42 hours, with minimum germination value of 66.50%.

In the present study, the contact time with the fertilizer did not affect the germination of the marandu palisade grass seeds subjected to accelerated aging, with a mean germination value of 49.80%.

For the sanitary analysis of the seeds treated with fertilizer and for those treated with fertilizer and exposed to the accelerated aging, there was an interaction effect of the contact time x forage species on the percentage of fungi incidence, with the occurrence of the fungi *Alternaria* sp., *Aspergillus* sp., *Bipolaris* sp., *Cladosporium* sp., *Cercospora* sp., *Fusarium* sp., *Nigrospora* sp., *Penicillium* sp.,*Rhizoctonia* sp. and *Rhizopus* sp., and predominance of fungi *Aspergillus* sp. and *Fusarium* sp. (Fig. 2), in both analyses and forage species.

Fig. 2. Fungi *Aspergillus* sp. (A, B) and *Fusarium* sp. (C) found in the grass seeds.

Fungal incidence data reflect the average incidence of fungi from the experimental treatments, presenting, therefore, high variability. Because of this, descriptive analysis was adopted.

For *Aspergillus* sp., incidence percentages of 6.36 and 5.05% without disinfestation, and 2.96 and 2.05% with disinfestation were observed in the fertilizer-treated seeds of ruziziensis palisadegrass and marandu palisadegrass, respectively; and incidence percentages of 71.08% and 21.21% without disinfestation and 5.40% and 3.80% with disinfestation in the seeds treated with fertilizer and exposed to accelerated aging of ruziziensis palisadegrass and marandu palisadegrass, respectively.

There was a high percentage of *Fusarium* sp. in the seeds treated with fertilizer (78.66% and 81.33% in ruziziensis palisadegrass and marandu palisadegrass, respectively), and in the seeds treated with fertilizer exposed to accelerated aging (63.42% and 63.72%, in ruziziensis palisadegrass and marandu palisadegrass, respectively).

The incidence of *Fusarium* sp. in the seeds remained high both in fertilized-disinfested seeds (73.33% and 62.33% in ruziziensis and marandu palisadegrass seeds, respectively), as in the seeds treated with fertilizer and exposed to accelerated and disinfested aging (35.20% and 40.23% in ruziziensis and marandu palisadegrass seeds, respectively).

The increase in the contact time of the marandu and ruziziensis palisadegrass seeds with the fertilizer decreased the incidence of fungi (Fig. 3). When comparing the incidence of fungi in the absence of contact with the fertilizer (time zero) with the maximum studied time (96 h), a reduction of 18.67% and 27.38% was observed in the seeds of ruziziensis palisadegrass and marandu palisadegrass, respectively.

With regard to the seeds of ruziziensis and marandu palisadegrass treated with fertilizer and exposed to accelerated aging, when comparing to the incidence of fungi in the absence of contact with the fertilizer (time zero) in the maximum studied time (96 h), an increase of 13.00% and a reduction of 5.42% were observed in the seeds of ruziziensis palisadegrass and marandu palisadegrass, respectively.

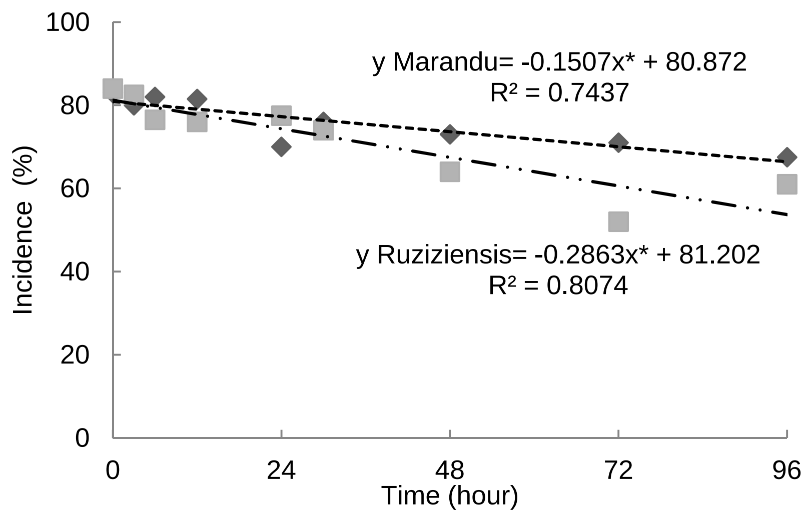

Fig. 3. Percentage of fungi incidence in ruziziensis and marandu palisadegrass seeds after the treatments as a function of the contact time with the fertilizer 05-25-15
**Significant at 5% probability (P =.05).*

When analysing the accelerated aging test, the germination percentage of the ruziziensis palisadegrass seeds was reduced with contact time up to 42 hours, possibly due to interference of the intense sporulation of external pathogens after the accelerated aging, confirmed by the sanitary analysis (Fig. 4).

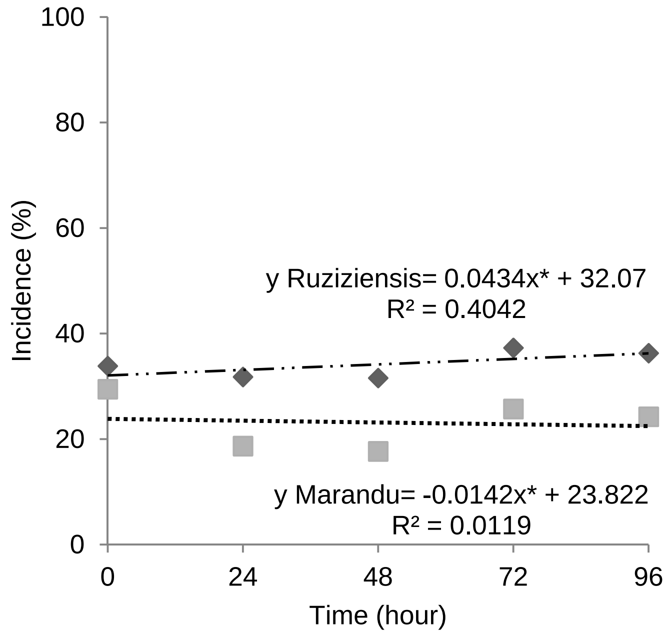

Fig. 4. Percentage of fungi incidence in aged seeds of ruziziensis and marandu palisadegrass as a function of the contact time with the fertilizer 05-25-15.* *Significant at 5% probability (P = .05).*

4. DISCUSSION

As to the water content analysis, seed contact time with the fertilizer does not interfere in their water content, although the utilized fertilizer consists of single and triple superphosphate and potassium chloride, besides having a high saline index (70.59%) and acidic pH (4.83).

Conversely, the authors cited in the reference [11], when studying ruziziensis palisadegrass seeds subjected to contact with urea, obtained positive linear effect for the water content as a consequence of the high hygroscopicity of the fertilizer. The authors cited in the references [12, 13, 14, 15] observed an increase in the water content of marandu palisadegrass seeds during the contact time with fertilizers due to the high urea hygroscopicity, to the acid phosphate fertilizers obtainment, and to the high saline index of potassium chloride.

In the evaluation of the germination percentage of ruziziensis palisadegrass seeds, it was verified that there was no effect of the seeds contact time with the fertilizer on germination. However, a reduction in germination was observed for the marandu palisadegrass seeds as the contact time with the fertilizer increased (Fig. 1), due to tegument rupture and extravasation of electrolytes by saline effect [16].

The authors cited in the references [12, 13, 14] verified a reduction in the germination percentage of marandu palisadegrass as the seeds contact time with the fertilizers was prolonged, corroborating with the present study.

The same phenomenon was not observed for the ruziziensis palisadegrass seeds, probably due to the tegument being less susceptible to damage by intrinsic factors to the fertilizer, such as acid pH and saline effect. Further studies on tegument constitution of the species used in the present study are necessary.

There was no effect of seeds contact time with the fertilizer on the germination of the marandu palisadegrass seeds subjected to accelerated aging. This phenomenon can be related to the low sporulation of external pathogens, as presented in Fig. 4, derived from the sanitary quality of the seeds.

Based on this, the importance of seed health can be endorsed, since the aging test predicts the behaviour of stored seeds; furthermore, an interaction between germination decrease of aged seeds and low sanitary quality of seeds was verified.

From the seeds sanitary analysis, it was verified that the results of fungi incidence obtained by the authors cited in the reference [17, 18, 19, 20] corroborate with those found in the present study (*Alternaria* sp., *Aspergillus* sp., *Bipolaris* sp., *Cladosporium* sp., *Cercospora* sp., *Fusarium* sp., *Nigrospora* sp., *Penicillium* sp., *Rhizoctonia* sp. e *Rhizopus* sp.).

The authors cited in the reference [17] have similarly identified the fungi *Alternaria tenuis*, *Aspergillus* sp., *Cladosporium* sp., *Fusarium* sp., *Penicillium* sp. and *Rhizopus*sp. in *Brachiaria* (Syn. *Urochloa*) *brizantha* seeds, whereas other authors, cited in the reference [18], reported a similar incidence of *Bipolaris* sp. and *Cladosporium* sp. in marandu palisadegrass seeds produced in Mato Grosso, besides *Alternaria* sp. and *Fusarium* sp. in seeds of *Brachiaria* (Syn. *Urochloa*) sp. and *Panicum maximum*.

In studies performed by the authors cited in the reference [19, 20], they verified the presence of the fungi *Aspergillus niger*, *Bipolaris* sp., *Fusarium* sp., *Penicillium* sp. and *Rhizopus* sp. in *Brachiaria* (Syn. *Urochloa*) *brizantha* cv. BRS Piatã (piatã palisadegrass). In addition, the authors cited in the reference [20] observed the presence of *Alternaria* sp., *Aspergillus flavus*, *Aspergillus ochraceus* and *Cladosporium* sp.

The main fungi evidenced in this work were *Aspergillus* sp. and *Fusarium* sp. in both forage species, and can be related to the reduction in seed germination [20], causing damage to the quality and establishment of forage crops [21, 19].

In addition to the physiological damage caused to *Brachiaria* seeds, both fungi can produce mycotoxins under low humidity conditions, which can lead to intoxication, cancer and death if ingested by animals [22].

The fungi that can be transmitted by seeds, with infestation/infection capacity during storage and in the physiological maturity point, may interfere in seed quality, reducing germination and forage production by compromising plant establishment, especially under favourable development conditions and with inefficient control methods [20, 23, 21]. In the transmission of seed pathogens to seedlings, the authors cited in the reference [20] reported the occurrence of pathogens *Aspergillus* sp. and *Fusarium* sp.

It is observed that the use of low sanitary quality seed results in unsuccessful pasture formation and seed lots commercialization, due to the presence of fungi [18].

Based on the seeds treated with fertilizer and subjected to accelerated aging, a high incidence of *Aspergillus* sp. on the surface of the *Brachiaria* seeds may occur due to the high incidence of this pathogen after the processes of disinfestation and accelerated aging on the seeds (ruziziensis palisadegrass presented 71.08 and 5.40 %, before and after disinfestation, respectively; marandu palisadegrass presented 21.01 and 3.80 %, before and after disinfestation, respectively).

In the accelerated aging, seeds are subjected to high temperature and humidity, which are favourable conditions for sporulation and development of pathogens, especially in the outer layer of seeds.

The contamination of the seeds by *Aspergillus* sp. can cause damage to the their physiological quality, reducing germination and vigor [22], causing a reduction of the planting stand, as well as being an inoculum source for the development of diseases and introducing pathogens in unaffected regions [24], such as the kernel rot disease in corn ears [25].

The incidence of *Fusarium* sp. in *Brachiaria* seeds (treated with fertilizer and treated with fertilizer and exposed to the accelerated aging) remained high after the disinfestation process (ruziziensis palisadegrass presented 78.66 and 73.33 %, before and after disinfestation, respectively; marandu palisadegrass presented 81.33 and 62.33 %, before and after disinfestation, respectively), evidencing the possibility of high incidence within the interior of the seeds. As a consequence of fungi incidence there may be a reduction in the viability percentage of the lots or death of the *Brachiaria* seeds, once *Fusarium* sp. is a soil fungus that can be associated with the seeds [25, 26].

Since it presents alternative hosts (maize, sorghum, sugarcane, grass, among others), crop rotation is not a very efficient control practice in these cases. Among them, *Fusarium moniliforme* and *Fusarium graminearum* stand out for causing stalk and root rot to infected plants [25], and *Fusarium clamydosporium* for causing wilt disease symptoms followed by death in forage plants such as *Stylosanthes* sp. [26].

The effect of seed dormancy breaking due to the accelerated aging process was excluded, since the germination percentage of ruziziensis palisadegrass seeds subjected to accelerated aging in the absence of contact (time zero) with the fertilizer was lower (74.90%) than the germination percentage obtained in the standard germination test (75.10%) (Fig. 1). The authors cited in the reference [27] observed a reduction in the germination percentage of marandu palisadegrass seeds after 24 hours of aging at 43 °C.

It is observed that the contact of the marandu and ruziziensis palisadegrass seeds with the fertilizer improved seed sanitization, since the prolongation of the contact time with the fertilizer decreased the incidence of fungi (Fig. 3).

The increase in the contact time of the *Brachiaria* seeds exposed to accelerated aging leads to an increase in the fungi incidence on ruziziensis palisadegrass seeds and reduces the fungi incidence on marandu palisadegrass seeds (Fig. 4). This effect can be attributed to the accelerated aging process of the seeds treated with fertilizer, which promotes proliferation and development of fungi due to the optimal conditions provided (Fig. 4).

4. CONCLUSION

It is concluded that there is presence of the fungi *Bipolaris* sp., *Fusarium* sp., *Rhizoctonia* sp., *Cercospora* sp., *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Penicillium* sp., *Rhizopus* sp. and *Nigrospora* sp. in the seeds of ruziziensis and marandu palisadegrass treated with fertilizer and in the seeds treated with fertilizer and exposed to the accelerated aging.

The fungi related to the reduction in the germination of *Brachiaria* seeds are *Aspergillus* sp. and *Fusarium* sp., with a high internal infestation of *Fusarium* sp.

It is verified that the increase in the contact time of the *Brachiaria* seeds treated with fertilizer reduces the incidence of fungi, improving the sanitary quality of the seeds.

COMPETING INTERESTS

We declare that no competing interests exist.

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