Original Research Article

Reduction of pathogens in palisade grass seeds by contact with fertilizer

ABSTRACT

The objective of this study was to evaluate the effects of the contact of *Brachiaria* seeds with the fertilizer 05-25-15 on the sanitary quality of the seeds. Experiment was carried out in a completely randomized design, in a 2x5 factorial, with four replications. Treatments consisted of the contact times (0, 24, 48, 72 and 96 hours) of the fertilizer with seeds of ruziziensis grass and marandu grass. After the contact times with the fertilizer with *Brachiaria* seeds, the seeds were separated from each species and the analyzes were carried out: water content, germination, sanitation ("Blotter Test"), and an accelerated, with later germination test and sanitary analysis of seeds. Data were analyzed for variance and regression analysis at the significance level of 5%, and the descriptive analysis for the results of the sanitary analysis. Contact time reduces the incidence of pathogens in seeds of ruziziensis grass and marandu grass treated with fertilizer due to salinity and acid pH from the fertilizer. The fungi related to the reduction in germination were *Aspergillus* sp. and *Fusarium*sp. Disinfestation process increased the incidence of *Fusarium*sp., under high internal infestation of this fungus in palisade grass seeds.

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

1. INTRODUCTION

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

This system can be used by farmers with the succession of grain crop and annual forage, in rural properties where agriculture with annual summer crops predominates, mainly soybeans and maize. Besides, it can be used as an alternative to soil cover in the form of straw for the no-till system and as an income source in the off-season (Santa Fe System), or in properties where the main activity is the cattle raising [4].

Based on the implantation premises of the Santa Fe system, where the grain crop is intercropped annually with a forage crop, we highlight the corn and *Brachiaria* (Syn. *Urochloa*) *ruziziensis* cv. Kennedy (Ruziziensis palisade grass). To a deployment of the Barreirão system, 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 corn and *Brachiaria* (Syn. *Urochloa*) *brizantha* cv. Marandu (Marandu palisade grass) [5].

The corn culture is an alternative for the second crop in the Cerrado (Brazilian Savanna) due to its adaptability to the region edaphoclimatic conditions, with several commercial cultivars

for the production of grains or silage [5]. In addition, it stands out in integrated systems because it has competitiveness in intercropping, suppresses weeds, presents selective herbicides, increases the surface soil residue input and maximizes nutrient cycling, being a good economic-environmental option of production [6]. For the corn crop implantation, in MatoGrosso state, it is commonly used the fertilizer 05-25-15 (N-P_2O_5-K_2O).

Tropical forages of the genus *Brachiaria* (Syn. *Urochloa*) are known for their adaptation to tropical climate and soil conditions, with high potential for dry matter production under adequate temperature and soil moisture conditions. In addition, they present a broad range of grazing height, greater regrowth and soil cover capacity, as well as lower clumps formation and greater ease of desiccation [7]. The forage plants desiccation promotes root death, increasing soil porosity, due to the formation of canaliculi, and the organic matter content, nutrients source for soil microorganisms [8].

Marandu palisade grass use expanded in the central region of Brazil due to adaptation to edaphoclimatic conditions, dry mass production with medium soil fertility, and resistance to spittlebug [9], a major problem in the region. In contrast, ruziziensis palisade grass stands out for its rapid soil cover, chemical composition, palatability, excellent nutrient recycling, ease of desiccation and uniform seed production, since it only blooms once [10].

To minimize the 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, simultaneous sowing of the grain crop seeds with the forage seeds is commonly done, associating them to the fertilizer applied at the grain-producing crop seeding [11].

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

2. MATERIAL AND METHODS

The trial was carried out in a completely randomized design, with four replications. Treatments consisted of a 5x2 factorial, with five contact times (0, 24, 48, 72 and 96 hours) of the fertilizer 05-25-15 (N-P_2O_5-K_2O) to the *Brachiaria* (Syn. *Urochloa*) *ruziziensis* cv. Kennedy (ruziziensis palisade grass) and to the *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.

For each grass species, the associations of the seeds with the fertilizer were carried out, which were transferred to closed plastic packages and stored until the pre-established contact times. Then, the fertilizer was removed from the mixtures and the grasses seeds were submitted to the analyses of water content, germination, sanitation ("Blotter Test" with salt stress), and accelerated aging, with later germination test and sanitary conditions analysis of seeds.

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 the weighing was carried out in analytical scale (0.0001 g). The results were expressed as percentage [12].

The methodology used for the standard germination test was the one described in the Rules of Seed Analysis [12], in which four sub-samples of 50 seeds were used for each species and treatment. The seeds were placed equidistantly in plastic box (gerbox) on two sheets of blotter paper as substrate, moistening them 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). The 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 "Blotter Test" method [13] modified with water restriction [14]. For each treatment and species of grass, 100 pure and viable seeds and 100 pure, viable and disinfested seeds were used. The disinfestation process was performed in a laminar flow chamber by immersing the seeds in a 1% sodium hypochlorite soluton for three minutes, and then the seeds were washed with sterile distilled water [13].

Then, the seeds were distributed equidistantly in a gerbox containing three sheets of filter paper, previously moistened with sterile sodium chloride solution (-0.6 MPa) [14], in a proportion equivalent to two and a half times the dry mass of the substrate. During the analyses period the moisture of the substrate was maintained by the addition of sterile sodium chloride solution (-0.6 MPa) to restrict the seeds germination and ensure the accurate evaluation of the incident microorganisms.

The seeds were incubated in a BOD chamber under a constant temperature of 20 °C and a 12-hour photoperiod [13]. After seven days, the seeds individual evaluation was carried out with the aid of stereoscopic and biological microscopes. The fungi was identified by the morphological structures observation and with the aid of specialized literature. The results were expressed as percentage of fungi incidence [13].

The accelerated aging test was realized by the methodology proposed by the author cited in reference [15], in which the seeds of each treatment were distributed on aluminium screen attached to the gerbox with 40 mL of distilled water in the recipient. 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, the germination and the sanitation test (Blotter Test adapted) with seed evaluation were carried out at seven days.

Data were submitted to analysis of variance and regression analysis at a significance level of 5% of probability ($P < 0.05$) and descriptive analysis for the results of the sanitary analysis.

3. RESULTS

In the water content analysis, there was no effect of the contact time and neither interaction between forages and contact times. An average water content of 7.91% was observed.

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

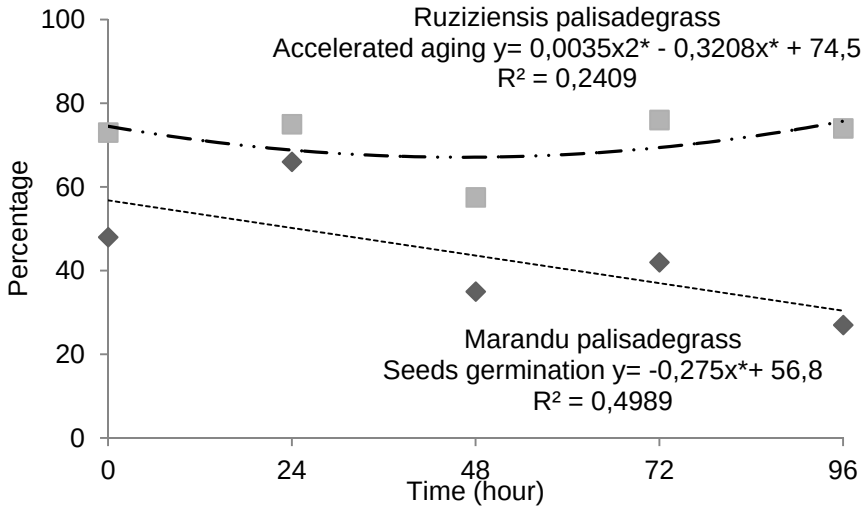

Fig. 1. Germination of marandu palisade grass seeds (A) and accelerated aging of ruziziensis palisade grass seeds (B) as a function of the contact time with the fertilizer 05-25-15. **Significant at 5% probability (P =.05).*

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

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

For the sanitary analysis of 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. e *Rhizopus* sp., and predominance of fungi *Aspergillus* sp. and *Fusarium* sp., in both analyses and forage species.

It was found a *Aspergillus* sp. incidence percentage of 6.36 and 5.05% without disinfestation, and 2.96 and 2.05% with disinfestation in the seeds treated with fertilizer of ruziziensis palisade grass and marandu palisade grass, respectively; and incidence 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 the accelerated aging of ruziziensis palisade grass and marandu palisade grass, respectively (Table 1).

Table 1. Average percentage of fungi incidence *Aspergillus* sp. and *Fusarium*sp. in seeds of the ruziziensis and marandu palisade grass, with and without disinfestation process, submitted to contact with fertilizer.

Grass	Treated with fertilizer				Treated with fertilizer and aged			
	Aspergillus sp.		*Fusarium* sp.		*Aspergillus* sp.		*Fusarium* sp.	
	WOD[1]	WD[2]	WOD	WD	WOD	WD	WOD	WD

Ruziziensis	6,36	2,96	78,66	73,33	71,08	5,40	63,42	35,20
Marandu	5,05	2,05	81,33	62,33	21,21	3,80	63,72	40,23

[1]*WOD: without disinfestation;* [2]*WD: with disinfestation.*

There was a high percentage of *Fusarium* sp. in seeds treated with fertilizer (78.66 and 81.33% in ruziziensis palisade grass and marandu palisade grass, respectively), and in seeds treated with fertilizer exposed to accelerated aging (63.42 and 63.72%, in ruziziensis palisade grass and marandu palisade grass, respectively) (Table 1).

The incidence of *Fusarium* sp. in seeds remained high in fertilized and disinfested seeds (73.33 and 62.33% in ruziziensis and marandu palisade grass seeds, respectively), and in seeds treated with fertilizer and exposed to accelerated and disinfested aging (35.20 and 40.23% in ruziziensis and marandu palisade grass seeds, respectively) (Table 1).

It was observed that the percentage of *Fusarium* sp. was higher in seeds treated with fertilizer than in seeds treated with fertilizer and subjected to accelerated aging, independent of the disinfestation process (Table 1).

The increase in contact time of the marandu and ruziziensis palisade grass seeds with the fertilizer decreased the incidence of fungi (Fig. 2). When comparing the incidence of fungi in the absence of contact with fertilizer (time zero) with the maximum time studied (96 h), it was observed a reduction of 18.67 and 27.38% in the seeds of ruziziensis palisade grass and marandu palisade grass, respectively.

In relation to the seeds of ruziziensis and marandu palisade grass treated with fertilizer and exposed to accelerated aging, when comparing the incidence of fungi in the absence of contact with fertilizer (time zero) with the maximum time studied (96 h), it was observed increase of 13.00% and reduction of 5.42% in the seeds of ruziziensis palisade grass and marandu palisade grass, respectively.

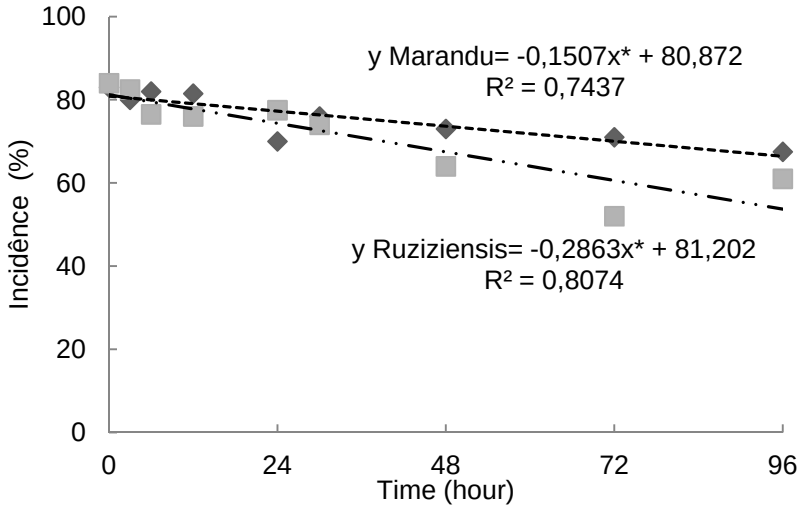

Fig. 2. Percentage of fungi incidence in ruziziensis and marandu palisade grass seeds as a function of the contact time with the fertilizer 05-25-15, after the treatments.**Significant at 5% probability (P =.05).*

When analysing the accelerated aging test, the germination percentage of the ruziziensis palisade grass seeds was reduced up to 42 hours of contact to the fertilizer, possibly due to the interference of the external pathogens intense sporulation after accelerated aging, confirmed by the sanitary analysis (Fig. 3).

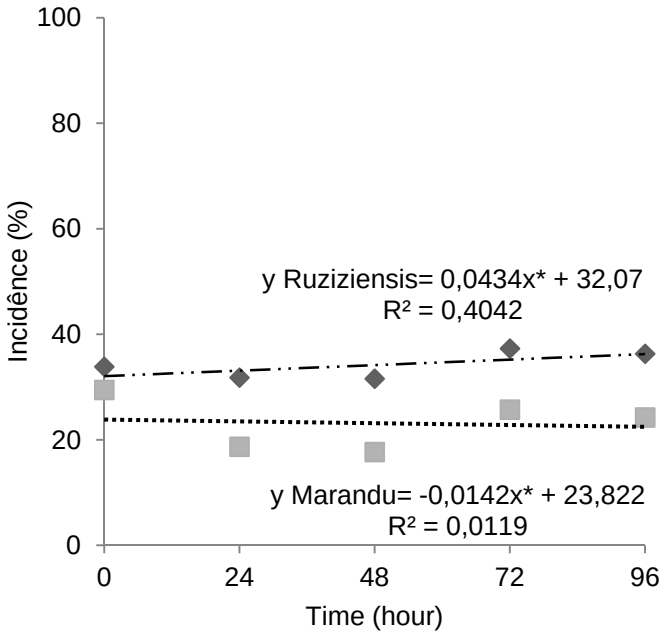

Fig. 3. Percentage of fungi incidence in aged seeds of ruziziensis and marandu palisade grass as a function of the contact time with the fertilizer 05-25-15.* *Significant at 5% probability (P =.05).*

4. DISCUSSION

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

Differently, the authors cited in the reference [16] studying ruziziensis palisade grass seeds submitted to contact with urea, obtained positive linear effect for the water content, as a consequence of the fertilizer high hygroscopicity. The authors cited in the reference [17, 18, 19, 20] observed an increase in the water content of marandu palisade grass seeds during the contact time to fertilizers due to the high urea hygroscopicity, the acid phosphate fertilizers obtainment, and the high saline index of potassium chloride.

In the evaluation of the ruziziensis palisade grass seeds germination percentage, it was verified that there was no effect of the seeds contact time to the fertilizer on the germination. However, a reduction was observed for the marandu palisade grass seeds as the contact time to the fertilizer increased (Fig. 1), due to tegument rupture and extravasation of electrolytes by saline effect [21].

The authors cited in the reference [17,18, 19] verified a reduction in the germination percentage of marandu palisade grass as the seeds contact time to the fertilizers was prolonged, corroborating with the present study.

The same phenomenon was not observed for the ruziziensis palisade grass 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 the tegument constitution of the species used in the present work are necessary.

There was no effect of the seeds contact time to the fertilizer on the germination of the marandu palisade grass seeds submitted to accelerated aging. The phenomenon may be related to reduced sporulation of external pathogens, as shown in Fig. 3, derived from the sanitary quality of the seeds.

Based on this, it is concluded on the importance of seed health, since the aging test predicts the behaviour of the seeds stored, and it was verified in this study the interaction between the germination decrease of aged seeds and the low sanitary quality of the seeds.

From the seeds sanitary analysis, it was verified that the results of fungi incidence obtained by the authors cited in the reference[22, 23, 24, 25] 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 [22] have similarly identified the fungi Alternariatenuis, *Aspergillus* sp., *Cladosporium*sp., *Fusarium* sp., *Penicillium* sp. and *Rhizopus*sp. in *Brachiaria* (Syn. *Urochloa*) *brizantha* seeds. Whereas, other authors cited in the reference [23] reported a similar incidence of *Bipolaris* sp. and *Cladosporium* sp. in the marandu palisade grass seeds produced in MatoGrosso, besides *Alternaria* sp. and *Fusarium* sp. In seeds of *Brachiaria* (Syn. *Urochloa*) sp. and *Panicum maximum*.

In studies carried out by the authors cited in thereference [24, 25] it was verified the presence of fungi *Aspergillusniger*, *Bipolaris*sp., *Fusarium*sp., *Penicillium*sp. e *Rhizopus*sp. in *Brachiaria* (Syn. *Urochloa*) *brizantha* cv. BRS Piatã (piatã palisade grass). In addition, the authors cited in the reference [25] observed the presence of *Alternaria* sp., *Aspergillusflavus*, *Aspergillusochraceus*and *Cladosporium* sp.

The main fungi evidenced in this work were *Aspergillus* sp. and *Fusarium* sp., in both forage species, and may be related to the reduction in the seeds germination [25], causing damage to the quality and establishment of forage plants [26, 24].

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 [27].

The fungi that can be transmitted by seeds, with infestation / infection capacity during storage and in the physiological maturity point, interfere in seed quality, reducing germination and forage production by compromising the establishment, mainly under development favourable conditions and inefficient control method [25, 28, 26]. In the transmission of seed pathogens to seedlings, the authors cited in the reference [25] reported the occurrence of pathogens *Aspergillus* sp. and *Fusarium* sp.

It is observed that the use of low quality sanitary seed results in unsuccessful pasture formation and seed lots commercialization, due to the presence of fungi and nematodes [23].

Based on the seeds treated with fertilizer and subjected to accelerated aging (Table 1), a high incidence of *Aspergillus* sp. on the surface of the *Brachiaria* seeds can occur, due to the high incidence of this pathogen after the process of disinfestation and accelerated aging on the seeds.

In accelerated aging, the seeds are submitted to high temperature and humidity, which are favourable conditions for sporulation and development of pathogens, mainly of the seeds outer layer. In this sense, it was observed that the percentage of *Aspergillus* sp. was higher in Brachiaria seeds treated with fertilizer and subjected to accelerated aging than in Brachiaria seeds treated with fertilizer, regardless of the disinfestation process (Table 1).

The contamination of the seeds by *Aspergillus* sp. can cause damage to the physiological quality of seed, reducing germination and vigor[27], causing a reduction of the planting stand, as well as being a inoculum source for the development of diseases and introducing pathogens in unaffected regions [29], such as the disease of burned grains in corn ears [30].

The incidence of *Fusarium* sp. in Brachiaria seeds (treated with fertilizer and treated with fertilizer and exposed to accelerated aging) remained high after the disinfestation process, evidencing the possibility of high incidence in the interior of the seeds (Table 1).

In addition, the incidence of *Fusarium* sp. was higher in Brachiaria seeds treated with fertilizer than in seeds treated with fertilizer and submitted to accelerated aging, regardless the disinfestation process (Table 1), confirming the high incidence of the pathogen inside the Brachiaria seeds, once that the accelerated aging test provides the proper conditions (high temperature and humidity) to the development and sporulation of fungi in the outer layer.

As a consequence of the 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 [30, 31].

By having alternative hosts (corn, sorghum, sugarcane, grass, among others), crop rotation is not a very efficient control practice in these cases. Among them, *Fusariummoniliforme*and *Fusariumgraminearum*, stand out for causing stalk and root rot to infected plants (Costa et al., 2009), and *Fusariumclamydosporium* for causing wilt symptom followed by death in forage plants such as *Stylosanthes*sp. [31].

It is observed that the contact of the marandu and ruziziensis palisade grass seeds to the fertilizer occasioned improves the seeds sanitization, since the contact time prolongation to the fertilizer decreased the fungi incidence.

The effect of dormancy overcoming due to the accelerated aging process was excluded, since the germination percentage of ruziziensis palisade grass seeds submitted to accelerated aging in the absence of contact (time zero) to 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 [32] observed a reduction in the germination percentage of marandu palisade grass seeds after 24 hours of aging at 43°C.

The increase in the contact time to the fertilizer of the Brachiaria seeds exposed to accelerated aging leads to an increase in the fungi incidence on ruziziensis palisade grass seeds and reduces the fungi incidence on marandu palisade grass seeds (Fig. 3). This effect can be attributed to the accelerated aging process of the seeds treated with fertilizer, which promotes the proliferation and development of fungi due to the optimal conditions provided (Fig. 3).

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., *Nigrospora* sp. in the seeds of ruziziensis and marandu palisade grass, treated with fertilizer and, treated with fertilizer and exposed to 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 to the fertilizer 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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