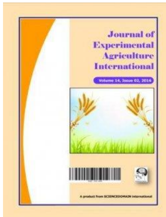


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# Reduction of the Forage Seeds Physiological Quality by the Contact with Fertilizer in the Crop-Livestock Integration

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author ACDA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DAF and CEAC managed the analyses of the study. Authors ECC and ARMS managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

The aim of this study was to evaluate the effects of *Brachiaria* seed contact time with 05-25-15 fertilizer ($N$-$P_2O_5$-$K_2O$) on the seed's physiological quality. The experimental design was a completely randomized design with four replicates. Treatments were arranged in a 5x4 factorial, being: five seeds contact times with fertilizer (0, 24, 48, 72 and 96 hours); four forages: Marandu palisadegrass, Piatã palisadegrass, Xaraés palisadegrass and Ruziziensis grass. The performed tests were: water content, germination (first count and germination percentage), tetrazolium (viability), electrical conductivity, accelerated aging, sand emergency and emergence speed index. Contact time of 05-25-15 fertilizer with *Brachiaria* seeds reduces linearly: Germination, emergence

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and emergence speed index of *Brachiaria* seeds; Piatã and Xaraés palisadegrass seeds viability; vigor (electrical conductivity), except in Ruziziensis grass seeds; and seeds germination of Piatã palisadegrass submitted to accelerated aging test (vigor). Piatã palisadegrass is the least recommended, among the analyzed grasses, for intercropping in simultaneous sowing.

*Keywords: Brachiaria; emergence; germination; grass; vigor.*

## 1. INTRODUCTION

The Central-West region has maintained monoculture and conventional agriculture for decades, as well as livestock activity with pastures in an advanced degradation stage. Reduction in fertility and low soil water retention in degraded pasture areas reduce the quantity and quality of feed offered to animals [1], compromising cattle potential productive, as well as negatively impacting the environment. In addition to the problems of monoculture and area degradation, the cattle potential productivities is reduced due to the forage production seasonality.

Also, there is a need to increase the income of agriculture, due to the increasing food demand, the need to reduce opening of new areas by existing laws, and reduce the greenhouse gases emission [2,3]. An alternative to mitigate these impacts and promote socioeconomic and sustainable development is the integrated crop-livestock system (ICLS), through the consortium of grain crops, such as maize, with forage plants, mainly the *Brachiaria* (Syn. *Urochloa*) genus, increasing the production system efficiency [3].

In the ICLS, in case of the consortium of grain crops, such as maize, with forage plants, such as *Brachiaria*, the simultaneous sowing can be used to minimize problems such as lack of uniformity and reduced initial forage stand due to the reduced size and low weight of *Brachiaria* seeds. *Brachiaria* seeds are mixed with fertilizer recommended for sowing the grain crop, being distributed at the same depth. Thus, the fertilizer will be used partially by the forage, which will show slow development until the grain harvest.

However, there’s a possibility of damage to the forage seeds physiological quality [4], resulting in economics losses due the lower initial stand and consequent lower forage production, due to the fertilizer harmful effects on seeds. Results shows that *Brachiaria brizantha* [5] and *Brachiaria ruziziensis* [6] contact time with fertilizer, decreased the seeds germination and vigor. Therefore, it was aimed to evaluate the effects of *Brachiaria* seed contact time with 05-25-15 (N-$P_2O_5$-$K_2O$) fertilizer on the forage seed physiological quality.

## 2. MATERIALS AND METHODS

The experimental design was a completely randomized design with four replicates. The treatments were arranged in a 5x4 factorial, being: five *Brachiaria* seeds contact times (0, 24, 48, 72 and 96 hours) with 05-25-15 (N-$P_2O_5$-$K_2O$) fertilizer, commonly used in maize crop sowing; four forages: *Brachiaria brizantha* (cv. Marandu, Piatã e Xaraés), (Marandu palisadegrass, piatã palisadegrass and xaraés palisadegrass, respectivily) and *Brachiaria ruziziensis* (cv. Kennedy), (Ruziziensis grass).

Based on the cultural value of the forage seed lots, the sowing rate of 4 kg of pure viable seeds per hectare and the fertilizer quantity of 100 kg $P_2O_5$ $ha^{-1}$, required for sowing corn, the ratio between seeds and fertilizer was adjusted.

Elapsed pre-established times, fertilizer and seeds were separated, and the following tests were carried out: water content, germination (first germination count and germination percentage), tetrazolium (viability), electrical conductivity, accelerated aging, emergence in sand and emergence speed index.

To quantify water content, three samples of 4.0 g were used for each treatment, which were placed in an oven for 24 hours at a temperature of 105 ± 1°C. After the drying process the samples were placed in desiccators to promote the cooling and then the weight was obtained using an analytical balance (0.0001 g) [7].

The methodology used for the germination test was described to the methodology of the authors cited in the reference [7], using four subsamples of 50 seeds of each forage species and fertilizer contact time with the forage seeds. The forage seeds were equidistantly distributed in "gerbox" type germination boxes, on two sheets of blotting paper as substrate, and moistened with distilled water in the proportion of two and a half times of the paper dry mass.

Subsequently, they were taken to the biochemical oxygen demand (BOD) chamber with photoperiod regulation of 12 hours and alternating temperature of 35/20°C. Forage seeds were analyzed at seven and 21 days, considering that the seeds that had emitted 2 mm of primary root germinated.

For tetrazolium test, four subsamples of 50 seeds were used for each forage species and the fertilizer contact time with forage seeds. The seeds were pre-wetted between paper sheet for germination, conditioned in a BOD chamber with a temperature of 30°C for 18 hours without light. Then, the longitudinal sectioning of the seeds was performed, and one part of the seeds was immersed in 0.5% tetrazolium salt solution (2,3,5-triphenyl chloride-tetrazolium). It was placed in a BOD chamber at a temperature of 30°C for 3 hours for staining the living tissue of forage seeds. In the sequence it was classified into viable and non-viable seeds, according to the methodology of the authors cited in the reference [7].

In electrical conductivity analysis, four subsamples of 50 seeds were used for each treatment, which were weighed in an analytical balance (0.0001 g), placed in a container containing 75 mL of distilled water and conditioned in a BOD chamber with a temperature of 25°C for 24 hours, without light. Then, the exudates released through the conductivity meter were measured, according to the methodology of the authors cited in the reference [8].

The methodology of the authors cited in the reference [9] was adopted for accelerated aging analysis, in which the seeds of each treatment were distributed on aluminum screen coupled to gerboxes containing 40 mL of distilled water. The boxes were then capped, forming a wet chamber, and placed in a BOD chamber for 36 hours at 42°C, without light. Finally, the germination test was carried out with the aged forage seeds, according to the methodology of the authors cited in the reference [7].

For emergency sand test, sowing was carried out in trays (29.1 x 23.0 x 5.3 cm) with 2.5 kg of sterilized sand at 105 ± 1ºC for 24 hours. Seeds were seeded for each treatment, in 10 mm depth, maintained at 12 hours of light incidence and humidity around 65% of the field capacity, for daily maintenance of the initial weight [9]. Daily analysis of the seeds was performed up to 21 days, considering seedlings with 2 mm above the substrate level emerged at least. Subsequently, emergency speed index (ESI) was calculated using to the methodology of the authors cited in the reference [10].

In variance analysis was considered the forage species effect, contact time and the interaction between them. For contact time analysis, a linear regression test was performed for each forage, and the coefficients were submitted to the t test. For all tests it was considered a 5% probability level. Was used the SANEST software [11].

## 3. RESULTS AND DISCUSSION

It was verified that there was contact time effect and no interaction between forages and contact times, which shows that seeds contact time with 05-25-15 fertilizer does not interfere in water content. An average water content of 11.42% was observed.

The absence of a change in water content may have been due to the fact that the seed mixture with fertilizer was stored in semi permeable packaging (plastic bags), making it difficult to absorb air moisture by both components, as well as the establishment of hygroscopic balance inside the package.

Another factor is that the seeds hygroscopicity varies according to the chemical constitution, especially the proteins, which have high affinity for water [12], and fatty acids that present a hydrophobic characteristic, with an antagonistic relation to the seed's contents [13], besides to being influenced by genetics, environmental conditions and plant traits [14]. Based on this, it is inferred that the hydrophilic substances content of the species studied in this work may be low.

In contrast to what was observed in this work, the authors cited in the reference [5] observed an increase in water content of marandu palisadegrass with contact time with 04-14-08fertilizer ($N_2$-$P_2O_5$-$K_2O$; urea, triple superphosphate and potassium chloride), attributing this effect to the high hygroscopicity of urea, which may have transferred air moisture to seeds. One of the negative effects on the water content increase is the increase in seed's metabolic activity, providing lower physiological quality [6].

Corroborating the above results, the authors cited in the references [15,16,17] verified an

**Table 1. First (1E) and final evaluation (FE) of seeds germination (%) of Marandu palisadegrass, Piatã palisadegrass, Xaraés palisadegrass and ruziziensis grass submitted to contact with fertilizer and linear regression (L)**

| Analysis | Contact time (hours) | | | | | L |
|---|---|---|---|---|---|---|
| | 0 | 24 | 48 | 72 | 96 | |
| 1E | 55.43 | 57.48 | 50.94 | 51.13 | 44.97 | 0.011* |
| FE | 59.80 | 64.21 | 54.96 | 55.83 | 47.46 | 0.001* |

** Significant at 5% probability*

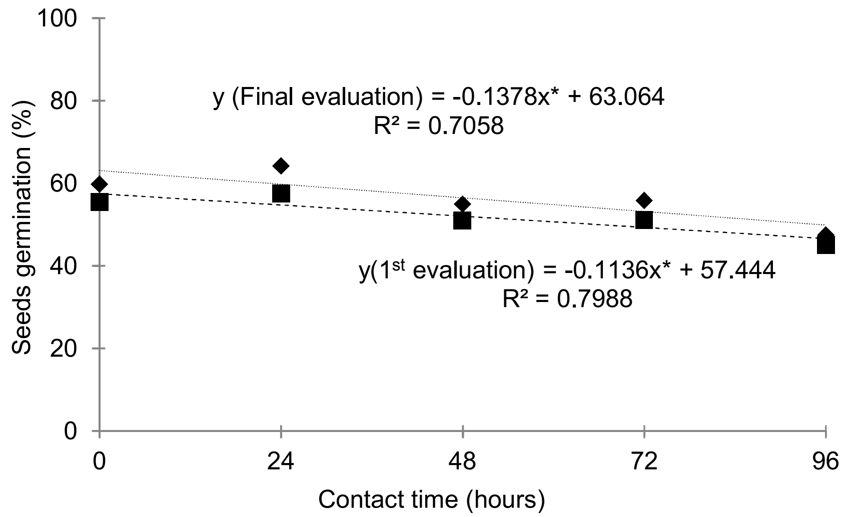


**Fig. 1. First and final evaluation of seeds germination (%) of marandu palisadegrass, piatã palisadegrass, xaraés palisadegrass and ruziziensis grass submitted to contact with fertilizer and linear regression (L)**

**Significant at 5% probability*

**Table 2. Seeds viability (%) of marandu palisadegrass, piatã palisadegrass, xaraés palisadegrass and ruziziensis grass submitted to contact with fertilizer and linear regression (L)**

| Forages | Contact time (hours) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 24 | 48 | 72 | 96 | L |
| Marandu | 76.00 | 70.00 | 70.50 | 73.50 | 72.00 | 0.390 ns |
| Piatã | 80.00 | 62.5 | 57.50 | 71.00 | 57.00 | 0.000* |
| Xaraés | 73.00 | 56.00 | 59.50 | 55.50 | 36.50 | 0.000* |
| Ruziziensis | 62.83 | 65.33 | 65.00 | 65.17 | 62.83 | 0.978 ns |

** Significant at 5% probability (P = .05); ns: not significant at 5% probability (P = .05)*

increase in seeds water content of marandu palisadegrass as the contact time with triple superphosphate, single superphosphate and potassium chloride, respectively. The authors justify these results due to the seed coat rupture and absorption of environment moisture, as a consequence of obtaining the acid phosphate fertilizers, and the high salinity index of potassium chloride. Although the fertilizer used in this work consists of single and triple superphosphate and potassium chloride, which constitutes high salt content and acid pH, the effect described above was not evidenced.

There was no effect of contact time and forage interaction on the first and final evaluation of the germination percentage of *Brachiaria* seeds (P = .05), (Table 1).

There was a linear decreasing effect of forage seeds contact time with fertilizer on the first and final evaluation of seed's germination, regardless of the forage (Fig. 1). Comparing the 96-hour treatment with the absence of contact, there was an average reduction of 18.87 and 20.63% in the first and final evaluation of the germination, respectively.

Probably, the effect was justified by the tegument rupture and electrolytes extravasation, since the contact with fertilizer can act negatively on the seed coat and consequently inhibit the germination by the saline effect [18], besides the

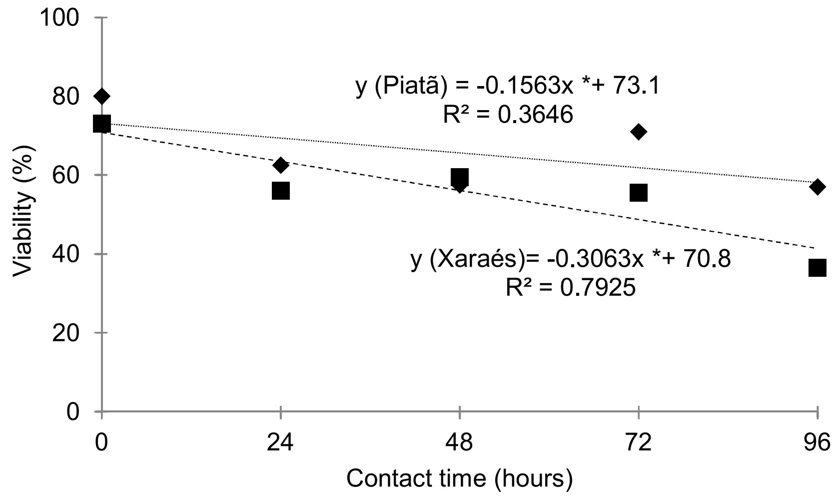


**Fig. 2. Seeds viability (%) of piatã and xaraés palisadegrass submitted to contact with fertilizer and linear regression (L)**
**Significant at 5% probability (P = .05)*

**Table 3. Electrical conductivity ($\mu^{-1}$ $cm^{-1}$ $g^{-1}$) of seeds of marandu palisadegrass, piatã palisadegrass, xaraés palisadegrass and ruziziensis grass submitted to contact with fertilizer and linear regression (L)**

| Forages | Contact time (hours) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 24 | 48 | 72 | 96 | L |
| Marandu | 22.74 | 70.30 | 77.92 | 104.57 | 66.42 | < 0.0001 |
| Piatã | 51.53 | 75.02 | 79.13 | 102.76 | 99.47 | < 0.0001 |
| Xaraés | 63.78 | 87.72 | 89.16 | 95.21 | 82.39 | < 0.0001 |
| Ruziziensis | 57.44 | 91.76 | 53.51 | 55.31 | 67.64 | 0.284 ns |

** Significant at 5% probability (P = .05); ns: not significant at 5% probability (P = .05)*

**Table 4. Seeds germination (%) of marandu palisadegrass, piatã palisadegrass, xaraés palisadegrass and ruziziensis grass submitted to contact with fertilizer after accelerated aging and linear regression (L)**

| Forages | Contact time (hours) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 24 | 48 | 72 | 96 | L |
| Marandu | 76,00 | 70,00 | 70,50 | 73,50 | 72,00 | 0,390 ns |
| Piatã | 80,00 | 62,5 | 57,50 | 71,00 | 57,00 | 0,000* |
| Xaraés | 73,00 | 56,00 | 59,50 | 55,50 | 36,50 | 0,000* |
| Ruziziensis | 62,83 | 65,33 | 65,00 | 65,17 | 62,83 | 0,978 ns |

** Significant at 5% probability (P = .05); ns: not significant at 5% probability (P = .05)*

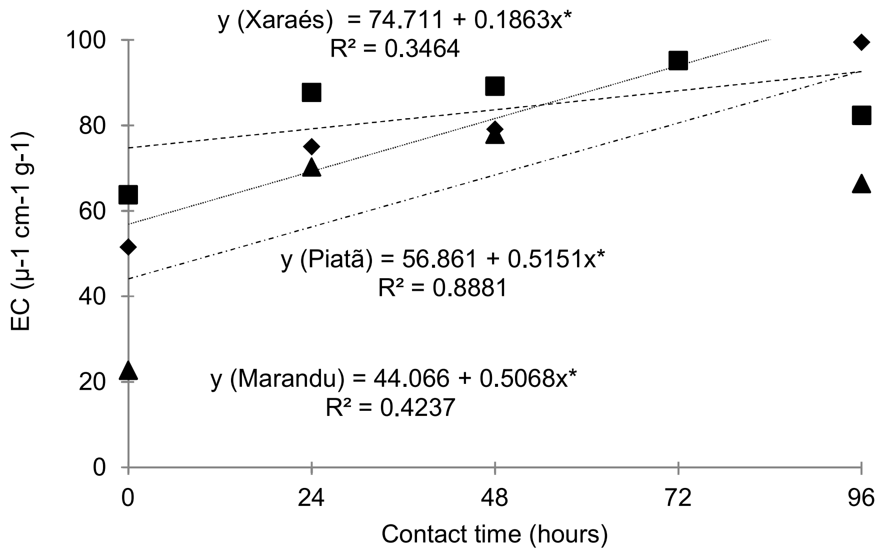


**Fig. 3. Electrical conductivity (EC; $\mu^{-1}$ $cm^{-1}$ $g^{-1}$) of seeds of marandu, piatã and xaraés palisadegrass and linear regression (L)**

**Significant at 5% probability (P = .05)*

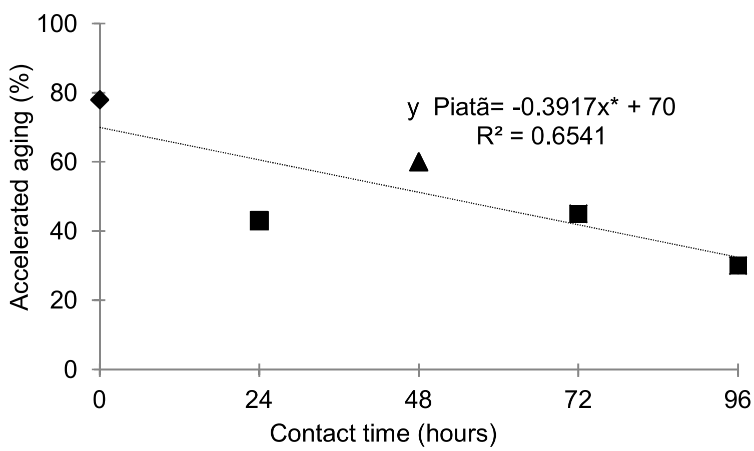


**Fig. 4. Seeds germination (%) of marandu palisadegrass, piatã palisadegrass, xaraés palisadegrass and ruziziensis grass submitted to contact with fertilizer after accelerated aging and linear regression (L)**

**Significant at 5% probability (P = .05)*

**Table 5. First (1E) and Final Evaluation (FE) of seeds emergence (%) of marandu palisadegrass, piatã palisadegrass, xaraés palisadegrass and ruziziensis grass and linear regression (L)**

| Analysis | Contact time (hours) | | | | | |
|---|---|---|---|---|---|---|
| | **0** | **24** | **48** | **72** | **96** | **L** |
| **1E** | 50.00 | 48.41 | 47.90 | 48.00 | 42.20 | 0.006* |
| **FE** | 63.53 | 64.41 | 61.43 | 62.97 | 54.77 | 0.001* |

** Significant at 5% probability (P = .05)*

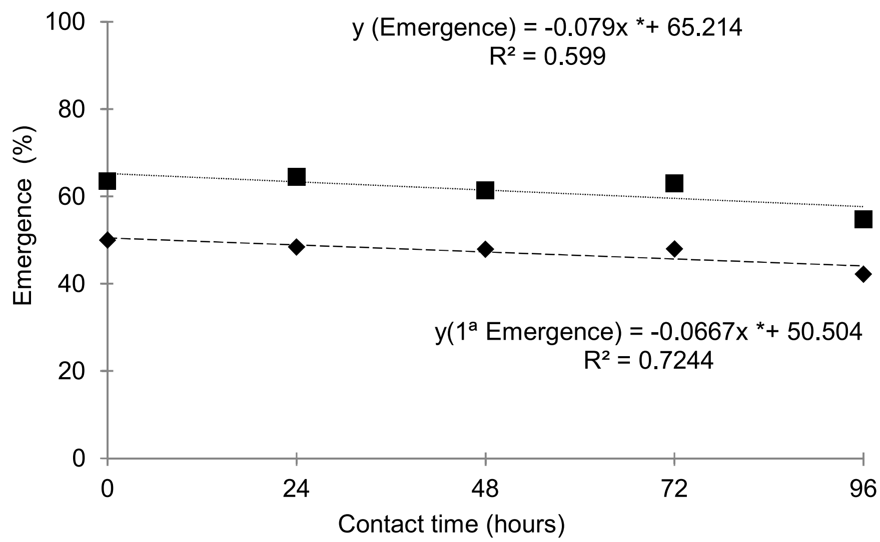


**Fig. 5. First and final evaluation of seeds emergence (%) of marandu palisadegrass, piatã palisadegrass, xaraés palisadegrass and ruziziensis grass and linear regression (L)**
**Significant at 5% probability (P = .05)*

**Table 6. Emergence Speed Index (ESI) of marandu palisadegrass, piatã palisadegrass, xaraés palisadegrass and ruziziensis grass and linear regression (L)**

| Analysis | Contact time (hours) | | | | | |
|---|---|---|---|---|---|---|
| | **0** | **24** | **48** | **72** | **96** | **L** |
| **ESI** | 46.03 | 45.69 | 43.21 | 44.06 | 37.15 | 0.001* |

** Significant at 5% probability (P = .05)*

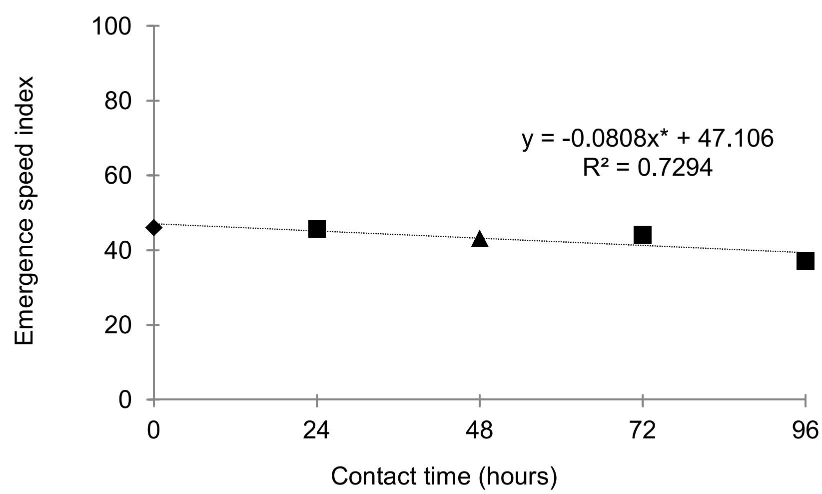


**Fig. 6. Emergence speed index of marandu, piatã, xaraés and ruziziensis grasses and linear regression (L)**
**Significant at 5% probability (P = .05)*

acid residues from the process of obtaining phosphate fertilizers [5]. Another influencing factor is probably the rapid water absorption by the seed during the imbibition phase, causing damage to seed tissues [19,20].

The decrease in germination percentages obtained in first and final evaluation of germination test of forage seeds was also verified by the authors cited in the references [15,5], indicating deleterious effect of marandu palisadegrass seeds contact time with superphosphate fertilizers triple and formulated 04-14-08 on seeds vigor, with reductions of 95.74 and 82.41%, respectively, in germination obtained with the first evaluation of the germination test. In addition, the authors also observed a reduction in seed germination percentage in the final evaluation of the germination test after being conditioned for a period superior to three and 36 hours, respectively.

There was interaction effect of contact time and forage on viability (P = .05), (Table 2).

There was a linear decreasing effect of seeds contact time with fertilizer on the seed's viability of the Piatã and Xaraés palisadegrasses (Fig. 2).

Comparing the 96-hour treatment with the absence of contact, there was an average reduction of 28.75 and 50% in the seed's viability of the species, respectively. In addition, when analyzing the germination percentage at zero contact time and viability at the same time, the dormancy phenomenon is observed, as reported in studies of the authors cited in the references [21,22] (Fig. 2).

In Piatã and Xaraés palisadegrasses the seeds contact with fertilizer caused damage to seed embryos. Probably, due to the intrinsic factors of the fertilizer (high salinity, pH and electrical conductivity) causing tegument ruptured and injured the seed embryo. Based on the eminent effect only on the forage described previously, it is inferred that they have a tegument more susceptible to damages than the other forages studied.

More vigorous seeds have higher soluble proteins amounts than those with less vigor [23]. Therefore, it is necessary to study the constitution of seed cover of *Brachiaria* seeds and the use of Piatã and Xaraés palisadegrasses in integrated systems under a simultaneous sowing system.

In a tetrazolium test carried out on marandu palisadegrass seeds, submitted to contact with granulated mono-ammonium phosphate, granular superphosphate and superphosphate powder, the authors reported in the reference [16] observed a linear decreasing effect of the contact time of all fertilizers with the seeds on viability.

Interaction (P = .05) was observed between the forages and the contact time on the electrical conductivity, which shows that there was an effect of the fertilizer contact time for each *Brachiaria* cultivars. There was a linear effect of contact time with fertilizer on the electrical conductivity of all cultivars, except for Ruziziensis grass (Table 3).

As the seeds of Marandu, Piatã and Xaraés palisadegrasses contact time with fertilizer increased, there was an increase in the electrical conductivity (Fig. 3), which shows a reduction in seed vigor. When comparing the absence of contact with the maximum time studied in this work, it was verified that there was an average increase of 192, 93 and 29% in the electrical conductivity of the cultivars Marandu, Piatã, and Xaraés palisadegrass, respectively.

The results were because the 05-25-15 fertilizer consisted of mono-ammonium phosphate, single superphosphate, triple superphosphate and potassium chloride, and some of these fertilizers present acid residues or high salt content, which probably caused seed coat rupture and electrolytes release contained in the reserve substances [16] and is indicative of reduced vigor. In addition, in the electrical conductivity results, the fertilizer residue effect on the seeds outer layer can occur, since they were not washed before the beginning of the test.

Similar results were evidenced by authors cited in the references [16,17], who studied the contact of marandu palisadegrass seeds with simple superphosphate and ammonium monophosphate in granulated, ground and powder forms; and potassium chloride, respectively, observed a proportional increase in the seeds electrical conductivity values.

The absence of effect of 05-25-15 fertilizer contact time on the electrical conductivity of Ruziziensis grass seeds should be studied, since this indicates a higher resistance of this species to physiological damages. Thus, it can be seen that up to 96 contact hours, there was no reduction in Ruziziensis grass seeds vigor, which allows the producer greater flexibility in sowing activities, being able to carry out a mixture of seeds and fertilizers in advance.

There was an interaction effect (contact time versus forage grass) on seeds germination submitted to accelerated aging, therefore, an individual effect was evidenced for each forage tested. There was no effect of the contact time with fertilizer for the forage tested, except for Piatã palisadegrass, in which a significant linear effect was observed (Table 4).

Comparing the 96-hour treatment with the absence of contact, there was an average reduction of 38.46% in seeds germination of Piatã palisadegrass submitted to aging. The effect was not evidenced in the other studied species studied (Fig. 4).

The high temperature at which the seeds were submitted during aging may accelerate deterioration process, as it causes degenerative changes in seed metabolism, such as protein denaturation, besides to causing rapid dry seeds imbibition, which favors injuries, and / or mobilization of reserves and energy release through respiration, culminating in reduced germination after accelerated aging [24,25].

Conversely, in some cases, there is an increase in germination after accelerated aging, which may be associated with dormancy [26] or even with pathogen control.

The authors cited in the reference [26] evaluating seeds of *Brachiaria brizantha* cv. MG-5 Vitória, concluded that the accelerated aging method overcomes dormancy and favors seed germination. However, the effect was only evidenced when accelerated aging was carried out at 41°C for a 96 hours period.

From the viability results (Table 2), electrical conductivity (Table 3) and accelerated aging (Table 4), it is observed that Piatã palisadegrass stands out from the other forages studied to the effect of contact with fertilizer, presenting physiological damage more severe, intensified by the increase in fertilizer contact time with the seeds. Therefore, precaution is recommended in the use of Piatã palisadegrass in detriment of the other forages studied for the crop-livestock integration in simultaneous sowing system.

For the first and final evaluation of the emergence percentage, the effect of the seeds contact time with fertilizer was observed. There was no interaction effect between forages and contact time, therefore, for all the forage species analyzed, a similar effect was observed on the first count and emergence percentage (Table 5).

It was observed a reduction in the first and final evaluation of the *Brachiaria* seeds emergence with the increase of the contact time with the 05-25-15 fertilizer (Fig. 5).

Comparing the 96-hour treatment with the absence of contact, there was a mean reduction of 15.60 and 13.79% in the first and final evaluation of the emergence percentage, respectively (Fig. 5).

Studying emerged seedlings percentage of *Brachiaria brizantha* cv. MG-5 and the emergence speed, the authors cited in the reference [4] observed damages by application of the 08-28-16fertilizer next to the seed, regardless of the seeding depth. In this study, the authors reported a decrease in emergence percentage in Marandu palisadegrass subjected to contact with 04-14-08 fertilizer and potassium chloride, respectively.

Besides to direct damage caused by the contact of the fertilizer with the seeds, substances exuded from the seeds (mainly sugars) due to the tegument rupture (caused by high acidity, electrical conductivity and fertilizer pH) may have stimulated the development of microorganisms causing damage to seedlings establishment.

There was no interaction effect of contact time and forage on emergency speed index (ESI), (P = .05), (Table 6).

There was a linear decreasing effect of the seeds contact time with fertilizer on the ESI, independent of the forage species/cultivar (Fig. 6). Comparing the 96-hour treatment with the absence of contact, there was an average reduction of 19.29% in ESI.

The ESI reduction is a consequence of the interaction of seeds physiological potential with environmental conditions. Seed lots of forage species may have declined in physiological potential due to being subject to environmental changes, under conditions of temperature far from ideal, not withstand stress.

Besides that, substances exuded from seeds (mainly sugars) due to tegument rupture (high acidity, electrical conductivity and fertilizer pH) may have stimulated the development of microorganisms, causing damage to seedling establishment.

The ESI decrease of marandu palisadegrass seeds with the advance of the fertilizer contact time was also verified by the authors cited in the references [15,5] when using the superphosphate triple fertilizers and 04-14-08, respectively. The authors verified effect from 12 and 3 hours of contact with fertilizers, respectively.

## 4. CONCLUSION

Water content of *Brachiaria* seeds is not altered by the contact time with fertilizer 05-25-15.

Contact time of 05-25-15fertilizer with *Brachiaria* seeds reduces linearly: germination, emergence and emergence speed index of brachiaria seeds; Piatã and Xaraés palisadegrass seeds viability; vigor (electrical conductivity), except in Ruziziensis grass seeds; and seeds germination of Piatã palisadegrass submitted to accelerated aging test (vigor).

Piatã palisadegrass is the least recommended, among the analyzed grasses, for intercropping in simultaneous sowing.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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