Distinct levels of quality of treated soybean seeds evaluated in alternative substrates to the germination test

Níveis distintos de qualidade de sementes de soja tratadas avaliadas em substratos alternativos ao teste de germinação

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ABSTRACT
The aim was to investigate alternative substrates to the germination test with treated soybean seeds, considering distinct seed quality. Seeds with distinct levels of quality, the fungicide fludioxonil+metalaxyl-M+thiabendazole and the insecticides imidacloprid+thiodicarb, bifenthrin+imidacloprid and thiamethoxam, associated or not, were used to compose the treatments. The standard methods of the Rules for Seed Analysis, paper rolls and sand, and alternative methods, vermiculite between paper and sand between paper, were used as substrates. The variables evaluated were: first count, abnormal seedlings and germination. For the first count there were interactions among the chemical treatment and the substrate, the chemical treatment and the quality level, and among the substrate and the quality level. For the variables abnormal seedlings and germination, both with triple interactions, T1 and T3 stood out in the distinct substrates and levels of quality with the greater abnormalities, and sand with the best percentages of normal seedlings between different treatments and levels of quality. The sand substrate presented greater stability of the results, being the most suitable for treatments considered a problem for the standard paper roll test. The grater the seed quality, the lower the problems with the germination test with treated seeds.

Keywords: chemical treatment, RAS, Glycine max.

RESUMO
O objetivo foi investigar substratos alternativos ao teste de germinação com sementes de soja tratadas, considerando qualidade distinta da semente. Sementes com níveis distintos de qualidade, o fungicida fludioxonil + metalaxil-M + tiabendazol e os inseticidas imidacloprid + tiodicarbe, bifentrina + imidaclopride e tiametoxam, associados ou não, foram utilizados para compor os tratamentos. Os métodos padrão das Regras para Análise de Sementes, rolos de papel e areia, e os métodos alternativos, vermiculita entre papel e areia entre papel, foram utilizados como substratos. As variáveis avaliadas foram: primeira contagem, plântulas anormais e germinação. Para a primeira contagem, houve interações entre o tratamento químico e o substrato, o tratamento químico e o nível de qualidade, e entre o substrato e o nível de qualidade. Para as variáveis plântulas anormais e germinação, ambas com interação tripla, T1 e T3 destacaram-se nos distintos substratos e níveis de qualidade com as maiores anormalidades e areia com as melhores porcentagens de plântulas normais entre diferentes tratamentos e níveis de qualidade. O substrato areia apresentou maior estabilidade dos resultados, sendo o mais adequado para os tratamentos considerados problemáticos para o teste padrão do rolo de papel. Quanto maior a qualidade das sementes, menor os problemas com o teste de germinação das sementes tratadas.

Palavras-chave: tratamento químico, RAS, Glycine max.

1 INTRODUCTION
Soybean is one of the most significant crops for the Brazilian agribusiness sector and, therefore, for the Brazilian economy. Such level of importance was reached due to great technological advances the crop was subjected in the last decades, initiated with the use of high quality seeds which is, without a doubt, one of the major factors for a successful crop, ensuring vigorous and healthy plants, which will have superior development in the field (França-Neto et al., 2016).
Soybean seeds, to be considered of high quality, must present high rates of vigor, germination and health, besides physical and genetic purity, and absence of weed seeds (Krzyzanowski et al., 2018). High vigor seeds promote rapid germination and seedling emergence (França-Neto et al., 2016) while seed health is characterized as the absence of pathogens, which can be transported to the field through the seeds, causing great losses, besides acting as focus of infection (Maciel et al., 2005), the physical integrity is paramount for field performance, being an important quality requisite (Krzyzanowski, 2004), and the genetic purity is fundamental to allow the cultivar to express its agronomic attributes (Krzyzanowski et al., 2018).

These factors are responsible for field performance, where the association of high-quality seeds and treatment with various products, which markedly reduce the need for reseeding, culminate with the establishment of the required population for the cultivar, contributing to high yield levels (França Neto et al., 2010; Krzyzanowski et al., 2018).

Yet, in Brazil, even with the high indices of the recent years, slightly higher for yield in the 2017/2018 growing season than the greater soybean producer, with 3,333 kg ha\(^{-1}\) (Conab, 2018) compared to 3,299 kg ha\(^{-1}\) for the United States of America (Usda, 2018), several climatic factors can limit the obtainment of satisfactory physiological and sanitary quality in some regions of the Country (Minuzzi et al., 2010), wherein soybean seed production is one of the responsible factors for the increase in grain production and, especially, in crop yield, due to a fast and efficient technology transfer (Abrass, 2015).

Considering that soybean seeds are especially sensitive to environmental variations, seed production, with quality, in the central region, for example, becomes a constant challenge (Meneghello & Peske, 2013), such fact can be verified observing the relation with the rate of purchased seed use (PSU) in each Brazilian State, where for Mato Grosso, the greatest soybean producer in the Country, the PSU is 75% compared to 35% in the State of Rio Grande do Sul (Abresem, 2015), which presents more favorable conditions for storage, and therefore, for saving seeds.

The state of Tocantins, during the off-season (May-June), presents an absence of rainfall associated with low air relative humidity and low night temperatures, these conditions favor the obtainment of good quality seeds and make the region highly attractive for seed producers, due to the higher returns for seeds (Pelúzio et al., 2008).

To meet this demand, with seed lots of distinct levels of quality, the tests that infer seed physiological quality, as the germination test which is standardized by the Rules for Seed Analysis and is indispensable for legal commercialization of seeds, must be sufficiently robust to evaluate
the maximum potential of a seed lot, providing ideal conditions of temperature, moisture and substrate, allowing the comparison and distinction of the quality of different lots, thus estimating the value for sowing in the field (Brasil, 2009).

Therefore, considering that more than 95% of the soybean seeds in Brazil are treated with fungicides and insecticides, either industrially or on farm (Nunes, 2016), that seeds with distinct levels of quality are produced and marketed in the Country, from seeds with high germination percentages to those with the allowed minimum limit (80% germination for soybean seeds) and that the tests used for physiological quality evaluation were not established based in treated seeds, using paper rolls and sand as substrates, which frequently are not equivalent when seeds are treated with chemical products, the scientific investigation for alternative substrates for the germination test becomes necessary, taking into account the differences in seed quality, these reasons motivated the development of this study.

2 MATERIALS AND METHODS

The study was performed at the Laboratory of Seed Analysis of the Phytotechny Department of the “Eliseu Maciel” College of Agronomy (FAEM) at the Federal University of Pelotas (UFPel).

Soybean seeds from the cultivar TMG 7062 IPRO were used, whereas the fungicide fludioxonil+metalaxyl-M+thiabendazole and the insecticides imidacloprid+thiodicarb, bifenthrin+imidacloprid and thiamethoxam at the doses of 125, 700, 700 and 300 mL 100 kg of seeds\(^1\), respectively, associated or not, composed the following chemical treatments: CT0 – without treatment; CT1 – fludioxonil+metalaxyl-M+thiabendazole + imidacloprid+thiodicarb; CT2 – fludioxonil+metalaxyl-M+thiabendazole + bifenthrin+imidacloprid; CT3 – imidacloprid+thiodicarb; CT4 –bifenthrin+imidacloprid and CT5 – thiamethoxam.

The treatments were performed using a seed treating machine, model TRATEC LAB (MECMAQ\(^a\), Piracicaba – Brazil) designed for research purposes and with up to 2 kg of capacity, following the manufacturer’s recommendations and, to satisfy any questions related to phytotoxicity, using the maximum dose recommended.

The seed lots were selected according to physiological quality analyzes and classified into the levels high (above 90% germination), medium (90-85% germination) and low (85-80% germination).

As substrates, the standard methods of the Rules for Seed Analysis, which are the paper roll and sand, and alternative methods, that were vermiculite between paper and sand between paper, were used. The methods of germination in the paper roll, vermiculite between paper and sand
between paper only differed due to the addition of vermiculite and sand into the paper roll, in contact with the seeds. Each paper roll was composed by three sheets of Germitest paper moistened with distilled water in a ratio of 2.5 times the weight of the dry paper. For rolls with vermiculite or sand added, two sheets of the paper received 50 mL of moisten medium size vermiculite (vermiculite between paper) or medium size sand (sand between paper). The vermiculite was previously moistened in a bucket containing distilled water for approximately 16 hours, removing the excess water for usage. Sand was moistened according to the water retention test, where an amount of 165 mL of water kg\(^{-1}\) of sand was determined, weighting enough quantity for usage. After all substrates were prepared, 50 seeds were disposed into each roll, which were carefully assembled, where four rolls composed one experimental unit.

For the germination test on sand, plastic trays with dimensions 7 cm (height) x 21 cm (width) x 29.5 cm (length) were used. The trays were filled with 2 Kg of sand, with particle sizes varying from 0.05 to 0.08, which was moistened with 330 mL of distilled water (165 mL kg\(^{-1}\) of sand), sowing 50 seeds per tray, where four trays composed one experimental unit.

All paper rolls and trays were kept into germination chambers containing a water blade for moisture maintenance, for eight days (until the final counting), at 25ºC\(\pm\)1ºC, on a regime of 12 hours of light.

The experiment was performed under a completely randomized design in 6x4x3 scheme with four repetitions. The factor A corresponded to the chemical treatments used (CT0, CT1, CT2, CT3, CT4 and CT5), factor B to the substrates (paper, sand, vermiculite between paper and sand between paper) and factor C to the quality levels of the seeds used (high, medium and low). Each experimental unit was composed of four rolls or four trays.

The evaluations examined the variables: first count of normal seedlings, abnormal seedlings and germination (sum of normal seedlings in two counts). Dead seeds were not presented due to their low incidence and there were no hard seeds. Data were analyzed for normality by the Shapiro Wilk test; for homoscedasticity using the Hartley test; and for independence of the residues through a graphical analysis. Data were subjected to the analysis of variance through the F test (p<0.05). For all variables, the effect of the chemical treatments, substrates and levels of seed quality were compared by the Tukey test (p<0.05).

### 3 RESULTS AND DISCUSSION

In the present study, for the variable first count of seedlings, interactions between the factors chemical treatment and substrate, chemical treatment and level of quality and between substrate and...
level of quality were observed. The three-way interaction between factors was not significant. Considering the behavior of the chemical treatments in each substrate, it is possible to observe that for sand there were no significant variations, on the other hand, for the substrates vermiculite between paper and sand between paper the smaller percentages of normal seedlings in the first count were observed in CT1, while the greater were verified in CT0, which is the control (untreated seeds) and was superior to the other treatments, except for vermiculite between paper, where CT5 was not significantly different (Table 1). For the paper substrate, the smaller percentage of normal seedlings in the first count was observed in CT3, which did not differ from CT1, with percentages of 80 and 85%, respectively, compared to 92% for CT0 (Table 1). Comparing substrates in each chemical treatment, sand presented greater percentages of normal seedlings for all chemical treatments, however, not differing from the other substrates in CT2, from paper and sand between paper in CT4, and vermiculite between paper and sand between paper in CT5 and CT0 (Table 1). In treatments CT3, CT5 and CT0, the paper substrate presented the lower percentages of normal seedlings, not differing from vermiculite between paper in CT4, considered the worst substrate for this treatment (Table 1).

Table 1: First count of normal seedlings (%) derived from seeds with distinct levels of quality, chemically treated and subjected to the germination test in the standards of the Rules for Seed Analysis or in alternative substrates. FAEM/UFPel, Capão do Leão/RS, 2018.

| Chemical treatment | Substrate          | Vermiculite between paper | Sand between paper | Level of quality |
|--------------------|--------------------|----------------------------|--------------------|------------------|
|                    | Paper              | Sand                       | High               | Medium           | Low             |
| CT0                | 92 Ab^1/           | 96 Aa                      | 92 Aab             | 94 Aab           | 92 Ab           | 96 Aa           |
| CT1                | 85 BCb             | 90 Aa                      | 77 Cc              | 81 Dbc           | 84 Ba           | 82 Ca           | 83 Da           |
| CT2                | 88 ABa             | 90 Aa                      | 86 Ba              | 88 Ba            | 89 ABa          | 87 ABCa         | 89 BCa          |
| CT3                | 80 Cc              | 92 Aa                      | 83 Bbc             | 85 Cb            | 85 Ba           | 83 BCa          | 87 CDa          |
| CT4                | 88 ABab            | 93 Aa                      | 86 Bb              | 90 Bab           | 88 Ba           | 88 ABCa         | 92 ABa          |
| CT5                | 88 ABb             | 93 Aa                      | 91 Aab             | 90 Bab           | 94 Aa           | 88 ABb          | 90 BCb          |

^1/Averages followed by the same uppercase letter in the column (comparing treatments for each substrate and for each level of quality) and averages followed by the same lowercase letter in the line (comparing substrates and quality levels for each treatment; comparing substrates for each level of quality), did not differ between each other by the Tukey test (p<0.05).
Considering that the first count of seedlings is an indicative of seed vigor, as well was the speed of emergence test, a study where seeds treated with thiamethoxam were sown in raised beds filled with sand did not observe differences from untreated seeds, however, seeds treated with imidacloprid+thiodicarb were inferior (Dan et al., 2011). These results partially support the results of the present study, where CT5 (thiamethoxam) consistently presents similar performance to CT0 (untreated seeds) while CT1 (fludioxonil+metalaxyl-M+thiabendazole + imidacloprid+thiodicarb) and CT3 (imidacloprid+thiodicarb) were inferior to CT0 only for the substrate paper, not differing when sand was used as the substrate.

For the interaction between chemical treatment and quality level, for high quality seeds, small variations were observed, with greater percentages of normal seedlings for the first count in CT0 and CT5, which did not differ from CT2, on the other hand, for the medium level, CT2, CT4 and CT5 did not differ from CT0, with the greater percentage, and for the low level only CT4 did not differ from CT0, with greater variations between treatments, where only in CT5 and CT0 there was statistical difference between seed quality levels (Table 1). According to Fossati (2004), soybean seeds with high physiological and sanitary quality can dismiss seed treatment with fungicides while Menten (1991) states that seed vigor affects seed response to fungicide treatment.

For the interaction between substrate and level of quality, statistical differences were observed for quality levels only for paper (Table 1). On the other hand, observing substrates in each level of quality, the sand substrate presented the greater percentages of normal seedlings in the first count for all levels, not differing from sand between paper in the medium level and from paper and sand between paper in the low quality level (Table 1).

For the variable abnormal seedlings, an interaction between the factors chemical treatment, substrate and level of quality was found. Analyzing the behavior of the treatments in each substrate and level of quality, it is possible to observe that when high quality seeds were used there were variations between treatments for the substrates vermiculite between paper and sand between paper, with greater number of abnormal seedlings in CT3 for both substrates, which did not differ between each other, and differences for substrates only in CT1, where paper rolls presented more abnormal seedlings than sand and sand between paper, that had lower values for this variable (Table 2). For the medium quality level, there were no variations between chemical treatments for the sand substrate, whereas for the others, the smaller values of abnormal seedlings were observed in CT0, also in CT5 for the substrate vermiculite between paper. All treatments differed regarding the substrate used, with smaller values of abnormal seedlings for the sand substrate, except for CT0 where sand between paper had the best result, while the greater abnormalities were found for the
paper substrate in CT2, CT3, CT5 and CT0, and for vermiculite between paper in CT1 and CT4 which did not differ from the paper (Table 2). For the low-quality level there were variations between treatments in all substrates, with the greater number of abnormal seedlings for paper in CT3, for sand in CT1 and CT5, and for vermiculite between paper and sand between paper in CT1, also varying for substrates in this level the treatments CT1, CT3 and CT0 (Table 2). The variations for quality level occurred in CT2, CT3 and CT5 for paper, in CT1, CT4 and CT0 for vermiculite between paper and in CT1 for sand between paper (Table 2).

Dan et al. (2010) while studying the effect of different insecticides on the physiological quality of soybean seeds, observed comparable results and considered that treated seeds presented adequate germination percentages, despite of the fact that the treatments with imidacloprid+thiodicarb, acephate and carbofuran resulted in greater percentages of abnormal seedlings, differing from seeds treated with thiamethoxam, which did not differ from the untreated control. CT3 (imidacloprid+thiodicarb) presented the greater number of abnormal seedlings, especially when the paper substrate was used, independently of the quality level of the seed.

Regarding the substrate used, Pereira et al. (2000) found that, in general, germination in sand was superior to the results obtained for paper rolls, when evaluating different cultivars and sowing dates. It is presumed that, depending of the seed treatment, the quality of seeds germinated in paper can be hindered compared to those germinated in sand, due to the presence of an aggravating factor for such an event, as observed in this study, which leads to the observation by some authors, that the substrate most suitable for the germination test depends on the type of product and active ingredient in seed treatment (Tunes et al., 2020). Unlike sand, on paper the active ingredient remains concentrated around the seeds, which can lead to the known phytotoxic effect.

The exiguous understanding of the influence of chemical products used for seed treatment on the physiological quality has, for some years, leading researchers to explore the subject and these studies demonstrated that some products applied on seeds can, under specific circumstances, negatively affect seed germination (Oliveira & Cruz, 1986), cause phytotoxic effects (Cruz, 1996) and reduce seedling root development (Silveira et al., 2001).

Germination, as the previous variable, demonstrated a three-way interaction between the factors studied. Considering the high-quality level, it is possible to observe that for the paper and sand substrates there were no differences between the treatments, while for the substrates vermiculite between paper and sand between paper, CT3 differed with the lower percentage of germination and, between the substrates used, only CT1 varied, with paper negatively differing from the other substrates (Table 3). For the medium quality level, the chemical treatments also did not
differ between each other in the substrates paper and sand while in the substrates vermiculite between paper CT1 and CT4 presented smaller percentages, not differing from CT3 and for sand between paper CT1, CT2 and CT3 resulted in the smaller values and did not differ from CT4 and CT5 (Table 3). For the factor substrate, all chemical treatments differed, presenting greater percentages of germination for sand, except for CT5 and CT0, and smaller for paper, except for CT1 and CT4 (Table 3). For low-quality seeds, there were no statistical differences for the chemical treatments for the sand substrate while in the others the smaller percentages of germination were observed in CT3 for paper, and in CT1 for vermiculite between paper and sand between paper, whereas the greater value for this variable in these substrates was observed in CT0, these last treatments (CT0 and CT1) were the only ones that varied according to the substrate (Table 3). The factor level of quality only varied for the treatments CT4 and CT5 in the paper substrate, CT1 and CT0 in sand, CT1 and CT4 in vermiculite between paper and for CT1 and CT2 in sand between paper (Table 3).
Table 2: Abnormal seedlings (%) derived from seeds with distinct levels of quality, chemically treated and subjected to the germination test in the standards of the Rules for Seed Analysis or in alternative substrates. FAEM/UFPEl, Capão do Leão/RS, 2018.

| Chemical treatment | Substrate                                      | Paper | Medium | Low | Sand | Medium | Low | Vermiculite between paper | Medium | Low | Sand between paper | Medium | Low |
|--------------------|------------------------------------------------|-------|--------|-----|------|--------|-----|---------------------------|--------|-----|--------------------|--------|-----|
| CT0                |                                                | 3     | Aaa2   | 6   | Bac  | 4      | Bac | 2                          | Aaa    | 3   | Aba                | 0      | Baa |
| CT1                |                                                | 9     | Aaa    | 9   | Aaa  | 1      | Aaa | 5                          | Aba    | 7   | Aaa                | 6      | Aaa |
| CT2                |                                                | 5     | Aaβ    | 10  | Baβ  | 3      | Aaa | 4                          | Aaa    | 2   | Aaa                | 2      | Aaa |
| CT3                |                                                | 5     | Aaβ    | 14  | Aaa  | 4      | Aaa | 2                          | Aaa    | 2   | Aaa                | 9      | Aaa |
| CT4                |                                                | 7     | Aaa    | 9   | Aaa  | 5      | Aaa | 2                          | Aaa    | 5   | Aaa                | 3      | Aaa |
| CT5                |                                                | 4     | Aaβ    | 10  | Aaa  | 7      | Aaa | 3                          | Aaa    | 6   | Aaa                | 9      | Aaa |

2 Averages followed by the same uppercase letter in the column (comparing chemical treatments for each substrate, in the quality levels high, medium and low), averages followed by the same lowercase letter in the line (comparing substrates for each chemical treatment, in the quality levels high, medium and low) and averages followed by the same Greek alphabet letter in the line (comparing the levels of quality for each chemical treatment, in the substrates paper, sand, vermiculite between paper and sand between paper), did not differ between each other by the Tukey test (p≤0.05).

Table 3: Seedling germination (%) derived from seeds with distinct levels of quality, chemically treated and subjected to the germination test in the standards of the Rules for Seed Analysis or in alternative substrates. FAEM/UFPEl, Capão do Leão/RS, 2018.

| Chemical treatment | Substrate                                      | Paper | Medium | Low | Sand | Medium | Low | Vermiculite between paper | Medium | Low | Sand between paper | Medium | Low |
|--------------------|------------------------------------------------|-------|--------|-----|------|--------|-----|---------------------------|--------|-----|--------------------|--------|-----|
| CT0                |                                                | 9     | Aaa2   | 9   | Abα  | 9      | Abα | 9                          | Aaa    | 9   | Aaa                | 9      | Aaa |
| CT1                |                                                | 9     | Aaa    | 9   | Abα  | 9      | Abα | 8                          | Aaa    | 8   | Abα                | 9      | Aaa |
| CT2                |                                                | 9     | Aaα    | 8   | Aab  | 9      | Aab | 9                          | Aab    | 9   | Aab                | 8      | Aab |
| CT3                |                                                | 9     | Aaα    | 8   | Aab  | 9      | Aab | 8                          | Aab    | 9   | Aab                | 8      | Aab |
| CT4                |                                                | 6     | Aaα    | 2   | Aaα  | 5      | Aaα | 6                          | Aaα    | 6   | Aaα                | 3      | Aaα |
| CT5                |                                                | 5     | Aaα    | 8   | Aaα  | 9      | Aaα | 5                          | Aaα    | 9   | Aaα                | 3      | Aaα |

2 Averages followed by the same uppercase letter in the column (comparing chemical treatments for each substrate, in the quality levels high, medium and low), averages followed by the same lowercase letter in the line (comparing substrates for each chemical treatment, in the quality levels high, medium and low) and averages followed by the same Greek alphabet letter in the line (comparing the levels of quality for each chemical treatment, in the substrates paper, sand, vermiculite between paper and sand between paper), did not differ between each other by the Tukey test (p≤0.05).
According to Gomes et al. (2009), the substrate used for germination tests has major influence on the results, taking into consideration the level of seed vigor and the presence of any chemical treatment and, despite noting that in their study the results from sand did not differ from paper in most of the treatments evaluated, it is possible to observe the superiority of the sand substrate compared to paper, with 74% and 65% of germination, respectively, corroborating with the results here reported.

4 CONCLUSIONS

The sand substrate is the most suitable for germination tests of treated soybean seeds and with distinct levels of physiological quality.

The alternative substrates are more robust than paper rolls and less laborious than sand, being easier to handle for use with treated soybean seeds.

The treatments CT1 (fludioxonil+metalaxyl-M+thiabendazole + imidacloprid+thiodicarb) and CT3 (imidacloprid+thiodicarb) favor the increase of abnormal seedlings.

Treated soybean seeds and with low physiological quality require greater care in the germination test.

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