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Does the substrate affect the germination of soybean seeds treated with phytosanitary products?

O substrato afeta a avaliação da germinação de sementes de soja tratadas com produtos fitossanitários?

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ABSTRACT

The use of an appropriate method that accurately expresses physiological seed quality and minimizes possible phytotoxicity by chemical products in laboratory testing with soybean seeds is relevant. The objective of this work was to evaluate the influence and infer the method adequacy for the representative evaluation of physiological quality of soybean seeds treated with phytosanitary products. Two experiments were carried out in a completely randomized design, with trial 1 in a 9×5 factorial scheme involving nine seed treatments, including fungicides and insecticides, and solutions with five osmotic potentials (0, -0.25, -0.5, -0.75 and -1.0 MPa) for wetting a paper substrate in a germination experiment, with evaluation of the normal seedlings at five and eight days after sowing. The second experiment consisted of a 9×7 factorial, with nine seed treatments and seven methods for germination evaluation at 5 days (between paper - BP, between sand - BS, between paper with water restriction - BPWR, between paper with preconditioning - BPC, sand between paper - SBP and vermiculite between paper - VBP). Treatments with insecticides affect seedling germination and evaluation, with greater phytotoxicity in relation to fungicides. Methods with readily available water led to greater phytotoxicity than methods with low water availability. For soybean germination evaluation of seeds treated with phytosanitary products, the BPC, SBP and VBP methods were optimal.

Index terms: Seed analysis; toxicity; *Glycine max*; physiological quality; seed treatment.

RESUMO

O uso da metodologia adequada e que expresse a real qualidade fisiológica de sementes e que minimiza possível fitotoxidez por produtos químicos em experimentos laboratoriais com sementes de soja é relevante. O objetivo no trabalho foi avaliar a influência e inferir sobre a adequação de metodologias para a representativa avaliação da qualidade fisiológica de sementes de soja tratadas com produtos fitossanitários. Realizou-se dois experimentos em delineamento inteiramente casualizado, sendo o experimento um em esquema fatorial 9x5, envolvendo nove tratamentos de sementes, entre fungicidas e inseticidas, e cinco potenciais osmóticos (0, -0,25; -0,5; -0,75 e -1,0 MPa) na solução para molhamento do substrato papel no experimento de germinação, com avaliação das plântulas normais aos cinco e oito dias. O 2° experimento foi constituído por fatorial 9x7, com nove tratamentos de sementes e sete metodologias para avaliação da germinação aos cinco dias (entre papel – EP, entre areia – EA, entre papel com restrição hídrica – EPR, entre papel com pré-condicionamento – EPC, areia entre papel – AEP e vermiculita entre papel – VEP). Os tratamentos com moléculas inseticidas afetam a germinação e avaliação das plântulas, com maior fitotoxidez em relação à fungicidas. Metodologias com água prontamente disponível, ocasionam maior fitotoxidez. Para avaliação de germinação de soja tratadas com produtos fitossanitários as metodologias EPC, AEP e VEP são as mais apropriadas.

Termos para indexação: Análise de sementes; toxidez; *Glycine max*; qualidade fisiológica; tratamento de semente.

INTRODUCTION

Soybean is one of the most important cultivated crops in Brazilian agriculture, being one of the main sources of foreign exchange for the country since it contributes to Brazilian exports (Santos et al., 2018). In Brazil, soybean occupies the largest cultivated area of grains. In the 2018/19 harvest year, 35.87 million hectares of soybean were planted with a production 115.03 million tons (CONAB, 2019), consolidating soybean cultivation as a market of great relevance to national production. Due to the large areas occupied by this crop, the demand for soybean seeds is high, especially for those with better quality (Carvalho et al., 2016).

As with production of many other crops, soybean production can also be affected by different stresses. Among abiotic stresses, there is water deficit and salinity, and among biotic stresses, there are occurrences of diseases, pests and nematodes that can negatively affect germination, emergence and seedling establishment and, consequently, damage stand and crop productivity (Bradley, 2008; Silva et al., 2019).

Several management techniques have been adopted so that these factors cause the least possible damage to soybean crops, including chemical seed treatment (Brzezinski et al. 2015). This process consists of the application of compounds capable of protecting seeds against deleterious pathogen effects and protect them in the initial period of crop establishment, promoting emergence and development (Balardin et al., 2011). Seed treatment using fungicides, insecticides and nematicides has been widely used in soybean cultivation due to the perception of seed value and the importance of protecting and/or improving its performance (Dan et al., 2010). Seed chemical treatment is an important tool for the correct establishment of soybean stands in the field because it can protect seeds and seedlings from pests and pathogens (Conceição et al., 2014; Camilo et al., 2017).

A seed coating of insecticides and fungicides protects the seeds; however, it may also be responsible for damage to the physiological quality of many seeds, with these damages occurring immediately after coating treatment or after storage (Camilo et al., 2017). Several products are often used on the same seed, such as the combination of fungicides, insecticides, nematicides, micronutrients, biostimulants, polymers, dyes or pigments, powder driers and inoculants (*Bradyrhizobium*). Some products may cause phytotoxicity to seeds and seedlings, and this phytotoxic effect of some molecules tends to be enhanced by increasing storage time (Dan et al., 2010; Piccini et al., 2013; Brzezinski et al., 2015). The coating products and their concentration in the coating for soybean treatment have a direct influence on maintaining seed viability and vigour throughout storage. Therefore, appropriately choosing products will ensure physiological seed quality when seed storage is necessary (Santos et al., 2018). Another fact to be considered is the phytotoxicity that is sometimes shown in laboratory seed testing using treated soybean seeds, which may not represent the real physiological potential of the seeds in the field. More research about this subject and its correlation is needed.

Studies that help in the understanding, adjustment and proposition of new and appropriate methods for the evaluation of treated seeds, together with the economic and operational viability in routine seed analysis laboratories, are necessary. In this context, the information is still insufficient and often not discussed in scientific studies.

Thus, the objective of this work was to study the influence of substrates on the physiological quality evaluation of soybean seeds treated with phytosanitary products.

MATERIAL AND METHODS

This work was developed in a Seed Laboratory accredited by the Ministry of Agriculture and Supply (MAPA), Brazil. Seeds from the same batch of the SYN1366CIPRO crop were stored in commercial packaging under controlled conditions (20 ºC) with a water content of 9% and were divided into two kg subsamples registered and treated with eight phytosanitary products, including fungicides and/or insecticides, and the control treatment (water application). Treatment was carried out manually with the plastic bags in constant movement for better coverage. The coating volume was standardized for each treatment at 600 mL 100 kg^{-1} of seeds, which was the product dose plus water to achieve the final volume (Table 1). No polymer and powder drier were used.

After the two-day treatment procedure, the water contents in the seeds were measured by the laboratory oven method at 105 ºC ± 2 ºC for 24 hours, and the contents were expressed as percentages, according to Brasil (2009).

Then, seeds were submitted to two experiments: the first was carried out to select the osmotic potential in the paper substrate, and the second was carried out to evaluate the seed quality after treatment with different germination methods.

Experiment one

After seed treatment, the seeds were submitted to germination tests on paper substrates (Brasil, 2009). The seeds were seeded in germitest paper (two sheets) moistened with solutions containing mannitol (P.A. $C_6H_{14}O_6$) - P.M. 182.17) at the following osmotic potentials: 0, -0.25, -0.5, -0.75 and -1.0 MPa. The solution was added at an amount equivalent to 2.5 times the dry paper weight.

The mannitol concentrations were calculated by the Van't Hoff formula, i.e., Yos = -RTC, where Yos is the osmotic potential (atm), R is the ideal gas constant (8.32 J mol^{-1} K^{-1}), T is temperature (K), and C is the concentration in g L^{-1} of water (Morales et al., 2015, Maciel et al., 2017) used to obtain each level of osmotic potential. Moreover, 1 MPa = 10 bar, 1 bar = 0.987 atm, and T (K) = 273 + T (ºC). Mannitol quantities used in the conditions in this work to obtain each treatment are given in grams per litre of distilled water in Table 2.

Table 1: Active ingredients, commercial products, types and doses used for the soybean seeds treatment.

Active ingredient (i.a.)	Commercial name	Type[1]	Commercial product dose[2]	Dose water[3]
Imidacloprid + Thiodicarb	Cropstar®	I + I	600	0
Thiamethoxam	Cruiser 350 FS®	I	300	300
Acetamiprid	Pirâmide®	I	200	400
Cyantraniliprole	Fortenza 600 FS®	I	200	400
Carbendazin + Thiram	Derosal Plus®	F + F	200	400
Fludioxonil + Metalaxyl-M	Maxim XL®	F + F	100	500
Thiophanate-methyl +Fluazinam	Certeza®	F + F	200	400
Fipronil + Piraclostrobin + Methyl thiophanate	Standak Top®	I + F + F	200	400
Control	-	-	-	600

[1]Type: I: insecticide; F: fungicide; [2] Dose commercial product: mL 100 kg^{-1} seeds; [3] Dose of water: mL 100 kg^{-1} seeds; Coating volume: 600 mL 100 kg^{-1} seeds. Source: Own author.

Table 2: Mannitol concentration used to obtain the osmotic potentials, with temperature at 25 ºC.

Osmotic potential (MPa)	Concentration (g mannitol L H_2O^{-1})
0.00	0.000
-0.25	18.60
-0.50	37.15
-0.75	55.72
-1.00	74.30

Source: Own author.

The osmotic potential of zero (control) was achieved using only distilled water and was considered the standard germination test, according to the Brazilian Rules for Seed Analysis (Brasil, 2009). After assembly, the paper rolls were kept in a Mangelsdorf-type germinator at 25 °C. The evaluations of normal seedlings were carried out at five days and eight days after sowing, and the final results are expressed as the percentage of normal seedlings, analysed according to Brasil (2009).

The statistical design was completely randomized in a 9×5 factorial scheme involving nine seed treatments and five osmotic potentials, with four replicates of 50 seeds. Data were submitted to variance analysis using Sisvar® software (Ferreira, 2014) with 5% probability by the F test, and the averages were grouped by the Scott and Knott test ($p < 0.05$).

Experiment two

The seeds submitted to the phytosanitary treatments, according to Table 1, were also evaluated under different germination methods and kept in a Mangelsdorf-type germinator, except the sand substrate that the trays were kept were on shelves in a room with controlled conditions. In both environments, the lighting was constant, and the temperature used was 25 ºC with daily control and a tolerance of ± 2 ºC. The methods for germination were as follows:

Between papers (BP): germitest papers (two papers for each repetition) were moistened to 2.5 times the weight of the dry paper with distilled water as the standard test Brasil (2009).

Between sand (BS): seeds were placed on a white bottom tray with a transparent lid with internal dimensions of 307×132×115 mm and external dimensions of 353×178×121 mm^3. The trays were made of plastic, and a uniform layer of sand with medium granulometry was placed in the trays. The seeds were sown in the thin layer of loose medium sand (a layer of approximately one cm) and later covered with sand; after sowing, the trays were covered with the lids. Subsequently, irrigation was carried out in the sand at 60% of the holding capacity.

Between papers with water restriction (BPWR): seeds were seeded in germitest paper (two sheets), rolled, moistened with an aqueous solution of mannitol prepared at a water potential of -0.25 MPa, as described above and established according to the results of experiment one, and kept in a Mangelsdorf-type germinator.

Between papers with preconditioning (BPC): adapted gerboxes filled with 40 mL of water were used, seeds were deposited in a single layer on the stainless steel screen, capped and conditioned in a Mangelsdorf-type germinator at 25 ºC for 24 hours. After this period, seeded in germitest paper (two sheets) moistened with distilled water in an amount equivalent to 2.5 times the weight of the dry paper and kept in a Mangelsdorf-type germinator.

Between sand and paper (BSP): dry, medium, autoclaved sand (136 g) was distributed evenly on a sheet of germitest paper that had been previously moistened with distilled water in an amount equivalent to 2.5 times the weight of the dry paper. The seeds were sown on this thin layer of sand, covered with another sheet, rolled and kept in a Mangelsdorf-type germinator.

Between vermiculite and paper (BVP): fine, expanded, dry vermiculite (21 g) was distributed evenly on a sheet of germitest paper that had been previously moistened with distilled water in an amount equivalent to 2.5 times the weight of the dry paper. The seeds were sown on the thin layer of vermiculite, covered with another sheet of germitest, rolled and kept in a Mangelsdorf-type germinator.

Seedling analyses were carried out five days after sowing, considering the percentage of normal seedlings, according to the criteria of Rules for Seed Analysis (Brasil, 2009).

The statistical design was completely randomized in a 9×6 factorial structure involving nine seed treatments and six methods to evaluate soybean seed germination treated with phytosanitary products, with four replicates of 50 seeds. Statistical analyses were performed using variance analysis with the aid of Sisvar® software (Ferreira, 2014) at 5% probability by the F test ($p<0.05$). The averages were analysed using the Scott-Knott test at 5%.

RESULTS AND DISCUSSION

The moisture content of seeds after treatment oscillated in value close to 9%, with a difference between the lowest and the highest value of only 0.5% for the treatments with Crosptar® (9.09%), Cruiser 350 FS® (9.08%), Pirâmide® (9.13%), Fortenza 600 FS® (9.05%), Derosal Plus® (9.06%), Maxim XL® (9.31%), Certeza® (8.97%), Standak Top® (9.13%) and the control (8.87%). This confirms the efficacy of the inferences about the effects of phytosanitary products at the time of the physiological tests.

The seed moisture content directly influences several aspects of its physiological quality, so its determination is fundamental in official seed lot quality tests (Sarmento et al. 2015).

In Table 3, referring to the first germination count on paper substrates with different water potentials, it was verified that in comparison with pure water (0.00 MPa), the treatment of seeds with the insecticide Pyramid® provided a lower germination percentage. With water restriction, -0.25 MPa, products containing insecticides caused lower percentages of normal seedlings and exhibited damage by phytotoxicity (root thickening and seedlings with reduced growth).

Table 3: First germination count (%), conducted between paper with water potentials by mannitol, of soybean seeds treated with phytosanitary products - Treatment Products (TP).

TP*	Osmotic potential (MPa)**				
	0.00	-0.25	-0.5	-0.75	-1.00
Cropstar ® (I)	97.50Aa	62.00Bb	15.50Cc	6.00Da	0.00Da
Cruiser ® (I)	98.00Aa	47.00Bc	19.50Cc	3.50Da	0.00Da
Pirâmide® (I)	70.50Ab	48.00Bc	15.00Cc	0.00Da	0.00Da
Fortenza ® (I)	98.50Aa	59.50Bb	28.00Cb	3.00Da	0.00Da
Derosal Plus® (F)	97.50Aa	94.00Aa	26.75Bb	0.50Ca	0.00Ca
Maxim Xl® (F)	97.00Aa	96.00Aa	49.00Ba	0.00Ca	0.00Ca
Certeza® (F)	99.50Aa	97.50Aa	25.00Bb	0.00Ca	0.00Ca
Standak Top ® (IF)	98.00Aa	97.00Aa	22.00Bb	3.00Ca	0.00Ca
Control	95.50Aa	96.50Aa	16.50Bc	2.50Ca	0.00Ca

*I: insecticide, F: fungicide. **Averages followed by the same lowercase letter in the column and uppercase in the line do not differ by Scott-knott test at 5% probability.

The potential of -1.0 MPa was considered severe for *E. involucrata* seed germination (Inocente et al., 2019). Under higher water restriction, prolongation of the stationary phase of the imbibition process occurs (Pires et al., 2016).

It was possible to observe a reduction in the first seed germination count with water restriction since it was zero with a water potential of -1.0 MPa in all treatments. In the milder water restriction of -0.25 MPa, seeds treated with fungicide and the control did not differ in relation to germination in substrate paper with pure water (Table 4).

The count at eight days of germination attenuated the severe water restriction effects found at five days, especially at the potentials for which there was no germination (-0.75 and -1.0 MPa), characteristic of the high seed lot vigour used (Table 4).

With pure water (0.00 MPa) and moderate water restriction (-0.25 MPa), no differences were observed among the seed treatments used. With the lowest water potential, -0.50 MPa, treatments with insecticides provided lower germination counts than that of the control (Table 4), probably due to the complex relation between water stress and/or phytotoxicity of contact with the chemical product on paper substrates.

Thus, considering both germination evaluations at five and eight days, the water potential of -0.25 MPa presented potential for use in paper substrates and was therefore used in test two.

In test two, by observing the results of variance analysis, a significant effect was found for all sources of variation in isolation, treatment of seeds and substrate, as well as for interaction between these factors during germination evaluation. Thus, the germination of seeds treated with phytosanitary products depends and presents different behaviour depending on the substrate used.

Regarding the comparison of treatment methods, it was verified that two products, Cruiser® and Pyramid®, both insecticides, exhibited differences among the substrates, which reiterates the tendency of insecticides to affect germination. In general, for germination evaluation at eight days, all treatments presented high germination, above 92%, which was higher than the minimum considered for commercialization of soybean seeds in Brazil, 80% (Brasil, 2013).

For soybean seeds, there are reports about physiological quality reduction caused by insecticides, which increase with increasing storage time of the treated seeds (Dan et al., 2010; Ferreira et al., 2016). However, even before storage, atrophied seedlings, radicular thickening and seed death were observed, as well as the influence of insecticide treatments on soybean germination, depending on the substrates used.

The fungicidal formulations presented no problems in any of the evaluated substrates, with values similar to those found for the control treatment (Figure 1). Ferreira et al. (2016) reported that treatments with fungicides increase seed sanitary quality for short storage periods.

For the seeds treated with phytosanitary products between paper and water only (BP), three seed treatments were designated as inferior to the others, i.e., the insecticides Cropstar®, Pyramid® and Fortenza® (Figure 1a), which may be related to the greater phytotoxicity of these products in the presence of readily available water. Additionally, the between sand (BS) substrate showed similar results for the Cruiser® and Pyramid® insecticides (Figure 1b), for which a larger number of abnormal seedlings were observed.

Table 4: Germination at 8 days (%), conducted between paper with water potentials by mannitol, of soybean seeds treated with phytosanitary products - Treatment Products (TP).

TP *	Osmotic potential (MPa)**				
	0.00	-0.25	-0.50	-0.75	-1.00
Cropstar® (I)	97.50Aa	96.50Aa	95.50Aa	93.00Aa	88.00Bb
Cruiser® (I)	98.00Aa	99.00Aa	93.25Bb	91.50Ba	94.00Ba
Pirâmide® (I)	92.50Aa	97.00Aa	87.50Bc	87.50Bb	79.50Cc
Fortenza® (I)	98.50Aa	98.00Aa	92.00Bb	95.50Ba	90.50Bb
Derosal Plus® (F)	97.50Aa	94.00Aa	98.50Aa	95.50Aa	96.50Aa
Maxim Xl® (F)	97.00Aa	96.00Aa	96.00Aa	94.00Aa	88.00Bb
Certeza® (F)	99.50Aa	97.50Aa	98.00Aa	93.50Ba	91.50Bb
Standak Top® (IF)	98.00Aa	97.00Aa	97.00Aa	94.00Aa	93.50Aa
Control	95.50Aa	96.50Aa	96.00Aa	95.00Aa	89.50Bb

*I: insecticide, F: fungicide. **Averages followed by the same lowercase letter in the column and uppercase in the line do not differ by Scott-knott test, 5% probability.

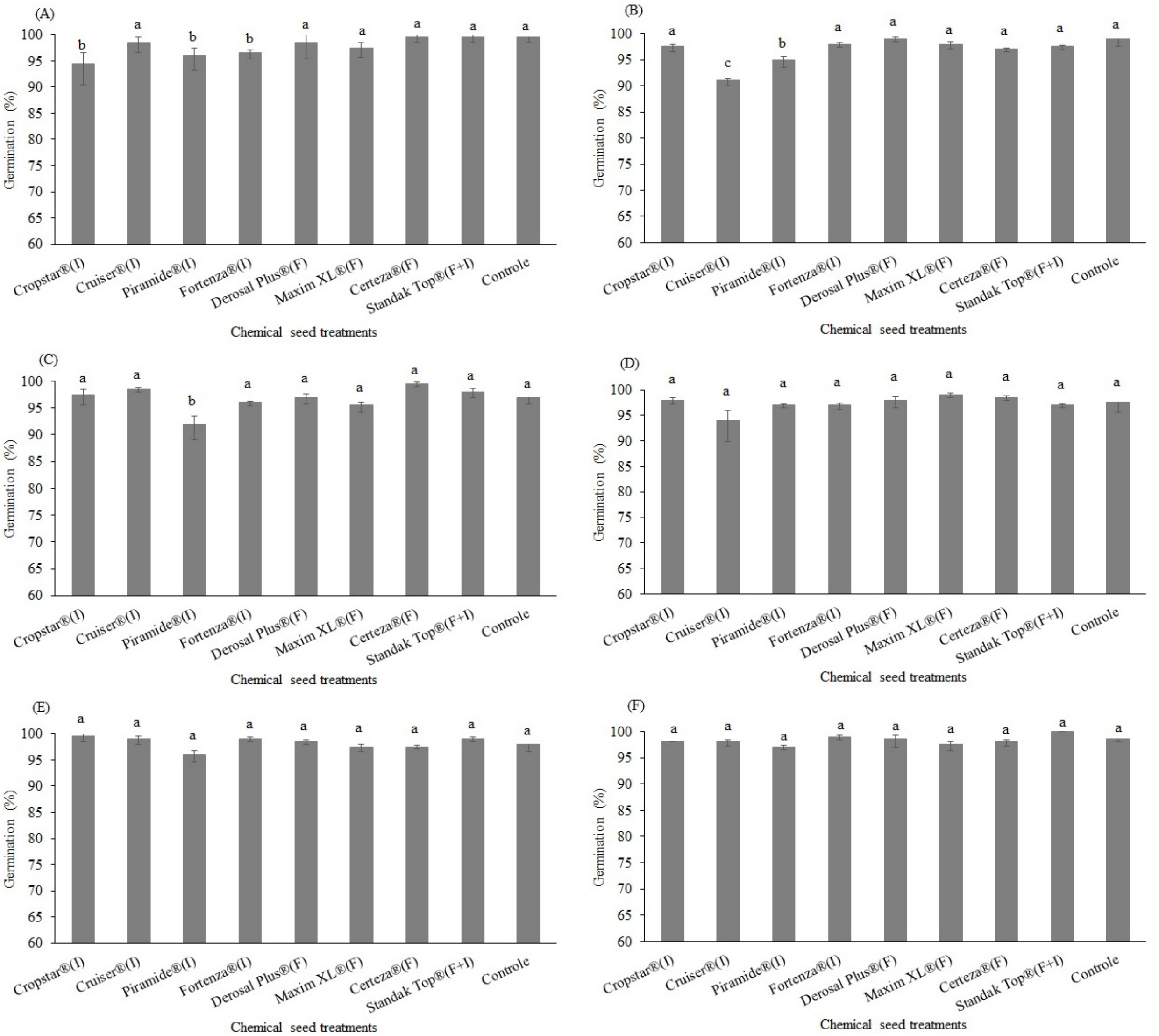

Figure 1: Soybean seeds germination (%) at 8 days after sowing, treated with phytosanitary products and conducted under different methodologies A. Between paper (BP), B. Between sand (BS), C. Between paper with water restriction (BPWR), D. Between paper with preconditioning (BPC), E. Between sand and paper (BSP), F. Between vermiculite and paper (BVP). *Averages followed by the same letter, in each methodology, do not differ among themselves by the Scott-knott test, at 5% probability. Vertical bars represent the sample standard deviation.

Seedling abnormalities caused by phytotoxic effects in this study were not uniform for all treatments but generally showed delayed development of secondary roots and plumule reduction, especially in treatments with insecticides.

With water restriction of -0.25 MPa, only seeds treated with the Pyramid® insecticide presented lower germination values than the control (Figure 1c). The lower immediate water availability and, consequently, slower imbibition resulted in a lower phytotoxic effect of the products on the seeds and seedlings compared to the previous methods. For the seeds germinated between paper with preconditioning (BPC), there was no difference in seed germination counts for seeds treated with different phytosanitary products, as all of them presented high values, likely due to the lower water imbibition rate and, consequently, lower penetration and/or absorption of the chemical product into seeds and seedlings, especially in the initial imbibition stage since the seeds had low water contents at the test time (close to 9%).

Bahry et al. (2017) evaluates the seed sensitivity of soybean cultivars to damage during imbibition in a germination test and verified that some cultivars responded positively to preconditioning, while others did not present positive responses. The water content in soybean seeds is an important characteristic that can affect the results obtained in germination tests using the standard paper procedure (paper roll). If the initial seed moisture content is low (<11-13%), imbibition damage is observed, while contents above 15% do not affect seed quality in the standard germination test (Toledo et al., 2010). Therefore, since the seeds in this study had a low water content, preconditioning provided a lower rate of initial water imbibition and, consequently, a lower rate of phytosanitary product uptake.

Despite the good results of the preconditioning analysis, in the laboratory routine, this method presents limitations: the necessity of box-type germinators with stainless screens, which raises both purchase and operation costs, and increases the analysis time required in one day.

Germination tests using sand between paper (SBP) (Figure 1e) and vermiculite between paper (VBP) (Figure 1f) showed that all treatments also had high germinations and did not differ statistically. In both methods, the use of dry materials between the sheets of paper affects the water imbibition rate of the seeds and, consequently, their direct contact with the chemical products; however, in this sense, more studies are necessary. Moreover, the use of additional substrates provided germination without the influence of the chemical product that was closer to the behaviour of seeds in the soil of production fields. Thus, this seed analysis utilized treatments that are environmentally relevant in terms of technical parameters, operability and standardization despite the inconveniences of day-to-day laboratory seed analysis regarding space, organization, cleaning, and, in this case, sand weight (Oliveira et al., 2016).

CONCLUSIONS

Soybean seed treatments with insecticides affected seedling germination and evaluation and exhibited higher phytotoxicity than fungicides. The phytotoxicity to seeds and seedlings of phytosanitary products in germination methods with readily available water, between paper (BP) and between sand (BS), were higher than in the other germination methods. For the soybean seeds treated with phytosanitary products, the germination methods between paper with preconditioning (BPC), sand between paper (SBP) and vermiculite between paper (VBP) were optimal.

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