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Quality of soybean seeds under application of herbicides or growth regulators

Caroline Santana Marchi[1], Alfredo Junior Paiola Albrecht[1], Leandro Paiola Albrecht[1], Fabiula Patricia Novakoski[1], André Felipe Moreira Silva[2]*, Tamara Thaís Mundt[3]

[1] Universidade Federal do Paraná, Palotina, PR, Brasil. E-mail: cmarchi16@gmail.com; ajpalbrecht@yahoo.com.br; lpalbrecht@yahoo.com.br; fabiula.novakoski@gmail.com
[2] Crop Science Pesquisa e Consultoria Agronomica LTDA, Palotina, PR, Brasil. E-mail: afmoreirasilva@hotmail.com
[3] Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu, SP, Brasil. E-mail: tamarathaism@gmail.com

ABSTRACT: Seeds may undergo interference from chemical products that are applied to the mother-plant, either impairing or improving their features, and to determine if there was interference in the seeds, germination and vigor tests are carried out. This study aimed to assess the quality of soybean seeds subjected to pre- and post-emergence herbicides and post-emergence growth regulators. At experiment 1, pre-emergence soybean herbicides were applied, and seeds were sown at the same day of application, at experiment 2, herbicides and growth regulators were applied at post-emergence soybean (V4). Seeds were harvested and subjected to the necessary tests, namely 1,000-seed weight, moisture content, germination, seedling length, tetrazolium and electrical conductivity. In general, herbicides and growth regulators did not cause an adverse impact on soybean seeds quality. Application of flumioxazin or mepiquat chloride caused minimal effects on the soybean seeds quality, with minor reductions in vigor, and no effect on germination.

Key words: *Glycine max* L.; physiological quality; viability; vigor

Qualidade de sementes de soja sob aplicação de herbicidas ou reguladores de crescimento

RESUMO: A aplicação de produtos químicos à planta-mãe pode interferir na qualidade da semente, prejudicando ou melhoramento suas características, de forma que para analisar se houve interferência na semente, são realizados teste de germinação e vigor. Objetivou-se avaliar a qualidade das sementes de soja submetidas a aplicação de herbicidas em pré e pós-emergência e reguladores de crescimento em pós-emergência. No experimento 1 foram aplicados herbicidas em pré-emergência da soja, com semeadura no mesmo dia da aplicação, enquanto no experimento 2 foram aplicados herbicidas e reguladores de crescimento em pós-emergência da soja (V4). As sementes foram colhidas e submetidas a determinação do grau umidade, massa de 1.000 sementes, testes de germinação, comprimento de plântula, tetrazólio e condutividade elétrica. Os herbicidas e reguladores de crescimentos não afetam negativamente a qualidade das sementes de soja. A aplicação de flumioxazin ou mepiquat chloride causou mínimo efeito na qualidade de sementes de soja, com pequenas reduções no vigor, sem efeito sobre a germinação.

Palavras-chave: *Glycine max* L.; qualidade fisiológica; viabilidade; vigor

* André Felipe Moreira Silva - E-mail: afmoreirasilva@hotmail.com (Corresponding author)
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Introduction

Growth regulators are synthetic chemical substances that influence plant metabolism and cause plant physiological responses. Most plant regulators used as growth retardants act by inhibiting gibberellin biosynthesis, hormones that, among other actions, promote cell elongation. They have similar actions to the already-known groups of plant hormones such as cytokines, gibberellins, auxins and ethylene. Reduced plant size, resulting from the action of regulators, makes that metabolites migrate to the reproductive structures where the products of economic importance are, as well as facilitate crop management and harvesting (Kappes et al., 2012).

Some studies suggest that herbicides such as glyphosate and ALS-inhibitors, when applied at low rates, may act as plant growth regulators, stimulating dry matter accumulation (Cedergreen, 2008). Herbicides could also be used to reduce the height and size of soybean plants, considering that some herbicides may cause symptoms of injury and/or plant height reduction without reducing yields. Thus, we can cite lactofen (Silva et al., 2018a), cloransulam (Silva et al., 2018b), chlorimuron (Belfry et al., 2016), diclosulam and sulfentrazone (Fornazza et al., 2018), among other herbicides.

Although application of herbicides and growth regulators does not cause yield losses and accomplishes their role of controlling weeds and reducing plants height, any stress may cause an adverse effect on normal growth and development of plant species (Albrecht & Ávila, 2010). Stressors that change soybean physiology may interfere with the processes that lead to seeds formation and development, affecting the viability and vigor of these seeds (Albrecht et al., 2012a).

Studies advise that herbicides may cause damages to soybean development due to its phytotoxicity (Albrecht et al., 2018), with adverse effects on the nutritional balance, water use efficiency, photosynthesis, biomass accumulation, among other processes involved in plant physiology and metabolism (Krenchinski et al., 2017a). These deleterious effects may also appear in the product harvested, that is, in the performance of soybean seeds (Albrecht et al., 2012b). Applications of some herbicides may cause damaging effects on seeds chemical composition, potentially altering protein and/or nutrient contents (Henry et al., 2011).

Seeds quality comprises a set of features that determine their value for sowing, indicating that the potential of seeds performance can be consistently identified when the interaction of genetic, physical, physiological and health attributes is considered. The performance potential must consider the seeds capacity to generate normal seedlings, the speed and uniformity of seedlings emergence and growth in the field, the storage potential and preservation of the physiological potential (Marcos Filho, 2015).

According to Krenchinski et al. (2017b), the product's quality is associated with the management system adopted for cultivation, including pre-harvest desiccation. Also, the herbicide application timing and the product used may negatively affect the productive parameters and the seeds physiological potential.

Knowing that there are ways to reduce the plant size in the field by using herbicides and/or growth regulators, it is necessary to investigate whether such management will not be detrimental to the physiological performance of the seeds produced. Thus, the present work aimed to assess the soybean seeds quality with pre- and post-emergence application of herbicides and post-emergence application of growth regulators.

Materials and Methods

Experimental design and field conditions

Two experiments were conducted during the 2017/18 growing season, in a cropping area in Palotina, PR, Brazil, climate in the region is Cfa, according to Köppen classification. Soil in the area of experiment 1 was classified as of clayey texture (68.75% clay, 15% silt and 16.25% sand), with the following chemical characteristics at 0-20 depth: pH ($CaCl_2$) of 5.3 and O.M. of 2.49%. Experiment 2 was also conducted in soil with clayey texture (69% of clay, 15% silt and 16% sand), and at the 0-20 cm layer, pH ($CaCl_2$) was 4.7 and O.M. was 2.86%.

For experiment 1, pre-emergence herbicides (diclosulam, sulfentrazone, chlorimuron, and flumioxazin) were applied, and sowing was done in the same day of application, with treatments as described in Table 1. In experiment 2, herbicides (cloransulam and lactofen) and growth regulators (trinexapac and mepiquat chloride) were applied at post-emergence of soybean plants, at V4 stage, with treatments as described in Table 2.

For booth experiment, soybean cultivars M 6210 IPRO (Monsoy® - Monsanto Co. do Brasil, São Paulo, SP, Brazil) and TMG 7062 IPRO (Tropical Melhoramento & Genética S.A., Cambé, PR, Brasil) were used, which belong to the maturation

Table 1. Treatments composed by pre-emergence application of herbicides at soybean plants (experiment 1). 2017/18 season, Palotina, PR, Brazil.

Treatments	Commercial products	Rate (g a.i. ha^{-1})
Control (without application)	-	-
Diclosulam	Spider® 840 WG	33.6
Sulfentrazone	Boral® 500 SC	600
Chlorimuron	Classic®	37.5
Flumioxazin	Flumyzin® 500	60

Table 2. Treatments composed by post-emergence application of herbicides or growth regulators at soybean plants (experiment 2). 2017/18 season, Palotina, PR, Brazil.

Treatments	Commercial products	Rate (g a.i. ha^{-1})
Control (without application)	-	-
Trinexapac	Moddus®	125
Mepiquat chloride	Pix® HC	100
Cloransulam	Pacto®	33.6
Lactofen	Cobra®	36

group 6.2 and are moderately resistant to lodging. The cultivar M 6210 IPRO has a 125-day cycle and undetermined growth habit, while cultivar TMG 7062 IPRO has a 115-day cycle and semi-determined growth habit.

The experimental design consisted of randomized blocks with four replications for all experiments. Herbicides applications were done using a backpack sprayer, CO_2 pressurized, at 2 bar of constant pressure and flow rate of 0.45 L min^{-1}, 150 L ha^{-1} of liquid volume, with a 3-m spraying boom with 6 nozzles (XR 110.015) and at a speed of 1 m s^{-1}. At the harvest time, the two central rows of each field plot measuring four meters long were harvested. Seed samples from each field plot were placed into paper bags for assessment in laboratory.

Laboratory assessments

To determine the soybean seeds quality, analyses were performed according to the Seed Analysis Rules (Brasil, 2009). The following aspects were assessed: 1,000-seed weight, germination, seedling length (shoot and root), vigor and viability by the tetrazolium and electrical conductivity tests. For 1,000-seed weight, the weight of 100 seeds from eight subsamples of each experimental plot was determined. The mean value of 100 seeds from each plot was multiplied by ten, and moisture corrected to 13%.

For the germination test, four replications per plot were done, it was carried out using four subsamples of 50 seeds per field replication of each treatment, and placed to germinate between three sheets of filter paper moistened with demineralized water at a ratio of three times the dry paper weight. The papers containing the seeds were rolled up and placed in a germinator that was set to a constant temperature of 25 °C, under 12 hours of light per day. The first count was done after five days, when all germinated seeds, whether normal or abnormal, were removed from the paper rolls, and the seeds that have not germinated remained in the roll and returned to the germinator for a second count, which occurred eight days after installation. The germination rate was determined by the sum of both counts.

The average seedling lengths were determined by the germination test, and during the first count ten seedlings were selected from each of the four rolls of each field plot. The lengths of shoots and roots were measured using a millimeter ruler, the seeds vigor and viability were determined by the tetrazolium test. Two subsamples with 50 seeds from each field plot were assessed. The seeds were placed into plastic cups and covered with 75 mL of deionized water and taken to a germinator at 25 °C, where they remained for 16 hours in the dark to hydrate the seeds. After this period, the water was removed from the cups using a sieve, and the seeds were immersed in 40 mL of colorless tetrazolium bromide solution, which is used as an indicator to reveal the reduction process that occurs inside living cells. The seeds remained in this solution for an additional period 4 hours in the germinator, at 35 °C in the dark, and then were rinsed before assessment. The seeds were divided into eight classes (Krzyzanowski et al., 1999):

Class 1: higher vigor, all embryo structures intact, coat uniformly and superficially colored, normal and turgid embryo tissues. *Class 2:* high vigor, including seeds with minor damages on the outer surface; *Class 3:* medium vigor, seeds that exhibit bright crimson streaks on the coat, or white areas on the outer surface of cotyledons. *Class 4:* seeds exhibit dark red-crimson colored areas or milky white areas (dead tissue); damages are visible on the inner surface of cotyledons. *Class 5:* seeds exhibit severely damaged cotyledons, but 50% or more of the reserve tissue remains viable and functional. *Class 6:* they are characterized by the presence of injuries similar to those described in class 5, but larger and deeper, making the seed unviable. *Class 7:* deep damage in the central cylinder; more than 50% of the reserve tissue is deteriorated. *Class 8:* all embryo structures of the seeds are whitish, necrotic (dead), with flaccid and torn tissues. Seeds that fall into classes 1, 2 and 3 are considered viable and vigorous, and seeds of classes 4 and 5 are considered viable only, while seeds of other classes are considered non-viable. Results of vigor and viability are expressed in percentage.

For determination of electrical conductivity, two subsamples of 50 seeds each from each field plot were utilized. The seeds were weighed and placed into plastic cups and then covered with 75 mL of deionized water. The cups were kept at 25 °C during 24 h in a germinator for a photoperiod of 12 h, and then the electrical conductivity was determined using a conductivity meter. The value obtained was divided by the seeds weight to obtain the value in μ cm^{-1} g^{-1} (Vieira & Krzyzanowski, 1999).

Statistical analysis

The resulting data were subjected to analysis of variance ($p < 0.05$), with the average values of the treatments compared by the Tukey test ($p < 0.05$), according to Pimentel-Gomes & Garcia (2002), using the statistical software Sisvar 5.6.

Results

Herbicides applied in soybean pre-emergence (experiment 1)

For the cultivar M 6210 IPRO, differences between the treatments were only found for electrical conductivity. When herbicide flumioxazin was applied, the soybean seeds exhibited more conductivity than when sulfentrazone and chlorimuron were used, and for the control (without application of herbicide) (Table 3). For cultivar TMG 7062 IPRO, no differences were observed for all variables examined (Table 4).

Herbicides or growth regulators applied in soybean post-emergence (experiment 2)

In the analysis of cultivar M 6210 IPRO, differences between the treatments were observed again for electrical conductivity. Also, for shoots length, a reduction was found with application of trinexapac, when compared to cloransulam, but without difference for the control treatment (untreated) (Table 5).

Table 3. Seed quality of soybean cultivar M 6210 IPRO under pre-emergence application of herbicides (experiment 1). 2017/18 season, Palotina, PR, Brazil.

Treatments	SW	G	EC	SHOOT	ROOT	VIG	VIAB
	(g)	(%)	(µ cm^{-1} g^{-1})	(cm)		(%)	
Control	137.6	95	120.9 ab	3.9	8.5	77.3	92
Diclosulam	138.3	92	139.8 bc	4.1	9.4	74.8	90
Sulfentrazone	140.3	95	118.6 ab	4.6	8.4	74.5	93
Chlorimuron	139.0	95	111.9 a	4.0	8.3	78.5	92
Flumioxazin	139.6	94	147.4 c	4.5	8.7	68.0	86
Mean	139.0	94	127.7	4.2	8.7	74.6	91
CV (%)	2.4	2.4	7.9	14.1	7.9	10.5	4.7
F	NS	NS	*	NS	NS	NS	NS

SW: 1,000-seed weight; G: germination; EC: electrical conductivity; seedling length of SHOOT; seedling of ROOT; VIG: vigor, and VIAB: viability (tetrazolium test).
* Means followed by the same letter in column are not different by Tukey test, at 5% probability.
NS Not significant, means do not differ by F-test, at 5% probability.

Table 4. Seed quality of soybean cultivar TMG 7062 IPRO under pre-emergence application of herbicides (experiment 1). 2017/18 season, Palotina, PR, Brazil.

Treatments	SW	G	EC	SHOOT	ROOT	VIG	VIAB
	(g)	(%)	(µ cm^{-1} g^{-1})	(cm)		(%)	
Control	174.3	76	80.0	3.3	4.6	47.5	84
Diclosulam	176.8	79	82.0	3.2	5.5	45.5	84
Sulfentrazone	173.6	79	81.5	2.9	5.7	53.8	78
Chlorimuron	171.7	79	81.3	3.6	5.1	52.8	83
Flumioxazin	172.9	75	80.0	3.3	4.9	52.0	77
Mean	173.8	77	81.0	3.3	5.2	50.3	81
CV (%)	2.6	7.6	7.8	10.2	14.2	19.4	12.6
F	NS	NS	NS	NS	NS	NS	NS

SW: 1,000-seed weight; G: germination; EC: electrical conductivity; seedling length of SHOOT; seedling of ROOT; VIG: vigor, and VIAB: viability (tetrazolium test).
NS Not significant, means do not differ by F-test, at 5% probability.

Table 5. Seed quality of soybean cultivar M 6210 IPRO under post-emergence application of herbicides and growth regulators (experiment 2). 2017/18 season, Palotina, PR, Brazil.

Treatments	SW	G	EC	SHOOT	ROOT	VIG	VIAB
	(g)	(%)	(µ cm^{-1} g^{-1})	(cm)		(%)	
Control	141.0	93	132.3 b	4.8 ab	9.3	72.5	90
Trinexapac	136.8	95	99.3 a	4.0 b	9.5	74.5	93
Mepiquat chloride	139.2	92	113.1 ab	4.5 ab	9.6	64.8	92
Cloransulam	135.5	91	102.1 a	4.9 a	8.7	70.3	93
Lactofen	133.2	95	112.5 ab	4.4 ab	9.5	74.5	94
Mean	137.1	93	111.9	4.5	9.3	71.3	92
CV (%)	3.1	3.5	8.9	8.0	13.1	6.7	4.3
F	NS	NS	*	*	NS	NS	NS

SW: 1,000-seed weight; G: germination; EC: electrical conductivity; seedling length of SHOOT; seedling of ROOT; VIG: vigor, and VIAB: viability (tetrazolium test).
* Means followed by the same letter in column are not different by Tukey test, at 5% probability.
NS Not significant, means do not differ by F-test, at 5% probability.

Table 6. Seed quality of soybean cultivar TMG 7062 IPRO under post-emergence application of herbicides and growth regulators (experiment 2). 2017/18 season, Palotina, PR, Brazil.

Treatments	SW	G	EC	SHOOT	ROOT	VIG	VIAB
	(g)	(%)	(µ cm^{-1} g^{-1})	(cm)		(%)	
Control	177.9	80	147.5 a	3.2	5.7	43.5 bc	83 a
Trinexapac	176.4	75	153.6 ab	3.2	5.7	61.0 a	91 a
Mepiquat chloride	177.3	77	181.1 b	3.1	5.8	35.0 c	67 b
Cloransulam	178.2	84	173.3 ab	3.3	5.5	56.3 ab	90 a
Lactofen	175.6	78	157.9 ab	3.5	6.3	51.3 ab	90 a
Mean	177.1	79	162.7	3.3	5.8	49.4	84
CV (%)	4.8	6.4	8.6	12.6	10.1	12.5	7.0
F	NS	NS	*	NS	NS	*	*

SW: 1,000-seed weight; G: germination; EC: electrical conductivity; seedling length of SHOOT; seedling of ROOT; VIG: vigor, and VIAB: viability (tetrazolium test).
* Means followed by the same letter in column are not different by Tukey test, at 5% probability.
NS Not significant, means do not differ by F-test, at 5% probability.

Differences for electrical conductivity were also found for the cultivar TMG 7062 IPRO, where application of mepiquat chloride caused an increase when compared to the control (untreated). For this cultivar, differences between the treatments were also observed for the variables viability and vigor (by the tetrazolium test), with decreased percent rates with application of mepiquat chloride (Table 6).

Discussion

None of the products applied (herbicides or growth regulators) interfered in the 1,000-seed weight, germination, and root length of the seedlings. For shoots length, some differences were observed, but no product reduced shoots length when compared with the control treatment. Thus, it is clear that the product has a minimal effect on the quality parameters of soybean seeds.

Application of chlorimuron (25 g a.i. ha^{-1}) at soybean post-emergence, combined with glyphosate, did not reduce germination of the seeds produced (Albrecht et al., 2012b). In the present study, the herbicide chlorimuron did not interfere with germination and other seeds quality-related variables, and it should be noted that chlorimuron in this study was applied at pre-emergence of soybean, but at a higher rate (37.5 g a.i. ha^{-1}). Similarly, the herbicides cloransulam and lactofen, for application at soybean post-emergence, did not affect the quality of the seeds produced (Albrecht et al., 2011; Silva et al., 2018a), the results of our study corroborate the ones found by these authors.

Application of sulfentrazone (168 g a.i. ha^{-1}) at soybean pre-emergence, followed by application of other herbicides at pre- or post-emergence did not affect the biochemical composition of the soybean seeds produced (Duke et al., 2003). In other studies, soybean yields did not decrease with application of sulfentrazone (Belfry et al., 2015). For application of diclosulam and flumioxazin, there is lack of information about the effects of these products on the quality of soybean seeds. In general, studies indicate minimal effects on soybean agronomic performance with application of diclosulam (31.9 g a.i. ha^{-1}) (Braz et al., 2017) or flumioxazin (63.3 g a.i. ha^{-1}) (Sarangi & Jhala, 2019). The results of the present study corroborate those found by these authors, only for flumioxazin, reduced seeds vigor was observed in one cultivar, according to the electrical conductivity test, but for the other variables no deleterious effect was found.

In the analysis of all products used, there was a trend of reduction in vigor and viability with application of mepiquat chloride. The electrical conductivity and tetrazolium tests showed that this growth regulator reduced the soybean seeds vigor and viability, as indicated in the tetrazolium test for cultivar TMG7062 IPRO. However, in both cultivars, the product did not affect seeds germination. This disagrees with the results of Jaidka et al. (2018), who did not observe any deleterious effect on vigor, germination, and lengths of soybean seedlings with application of post-emergence mepiquat chloride (300 ppm). In regard to application of trinexapac, in the present study, no deleterious effects on the seed quality were observed, studies that assess the effect of this product on the quality of seeds produced are scarce. To assess soybean agronomic performance, Correia & Leite (2012) did not find adverse effects with trinexapac application (up to 400 g a.i. ha^{-1}).

According to Marcos Filho (2015), through the electrical conductivity test, current metabolic condition of the seeds is indirectly assessed, considering that the larger the amount of exudates (electrolytes) released, the higher is the electrical conductivity of the solution, corresponding to the lowest vigor value of the seeds lot, as a result of the degree of membranes breakdown. Readings below 70 μ cm^{-1} g^{-1} identify samples of high vigor soybean seeds, and those above 100 μ cm^{-1} g^{-1}, as of low vigor seeds; therefore, the values found in the experiments correspond to low vigor seeds. However, França Neto et al. (1998) state that a high emergence rate of soybean seeds in the field can be achieved with conductivity up to 110 μ cm^{-1} g^{-1}, as long as there are appropriate field conditions for germination and emergence of seedlings. On the other hand, under small limitations for germination, conductivity cannot exceed 90 μ cm^{-1} g^{-1}.

The results obtained can be affected by seed damages, given that damaged seeds tend to eliminate a larger amount of exudates. In this case, it is recommended to remove all seeds with possible damages, considering that in a 25-seed sample containing two damaged seeds, conductivity is already affected. It can also be affected by the seed size and/or genotype, and moisture content in the seeds (França Neto et al., 1998). The tetrazolium test is a fast method used to estimate seeds viability, based on altered color of living tissues, reflecting the activity of the dehydrogenase enzyme system which is closely related to the seeds viability (Marcos Filho, 2015). The values of viability derive from the tetrazolium test, according to the seeds class.

Conclusion

In general, herbicides and growth regulators did not cause an adverse impact on soybean seeds quality. Application of flumioxazin or mepiquat chloride caused minimal effects on the soybean seeds quality, with minor reductions in vigor, and no effect on germination. For application of mepiquat chloride or flumioxazin, there is lack of information about the effects of these products on the quality of soybean seeds. Other studies, like this one, are required to elucidate the effects of these products.

Compliance with Ethical Standards

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Conflict of interest: There is no conflict of interest.

Author contribution: Conceptualization: CSM, AJPA, LPA; Data curation: CSM, AJP; Formal analysis: CSM, AJP, AFMS; Investigation: CSM, FPN, TTM; Supervision: AJPA, LPA; Writing – original draft: CSM, AFMS; Writing – review & editing: CSM, AJPA, LPA, FPN, AFMS, TTM.

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