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Productivity and physiological quality of seeds with burn down herbicides at the pre harvest of bean crops[1]

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ABSTRACT - The physiological quality of bean seeds is influenced by the production environment and management practices employed during cultivation. The objective of this study was to evaluate the productivity and physiological quality of bean seeds after desiccant applications at different times at pre-harvest. A randomized block design was used, with a factorial (3x3) + 1 arrangement, where the application of three desiccants was tested: *glyphosate* (960 g a.i. ha^{-1}), *paraquat dichloride* (240 g a.i. ha^{-1}) and *glufosinate ammonium* (400 g a.i. ha^{-1}) + mineral oil, applied three times (days after flowering - DAF), and a control without herbicide in four replications. Seed quality was determined by the following tests: germination, first count, seedling emergence speed rate, accelerated aging, electrical conductivity and seedling dry biomass. Seed yield was not influenced by either herbicides or time of application. Regardless of time of application, the lowest percentage of normal seedlings after the germination, first count and accelerated aging tests was observed in seeds desiccated with the herbicide *glufosinate ammonium*. The herbicide *glyphosate* reduces seedling vigor. The times of application of the product *paraquat dichloride* did not affect the germination and vigor of bean seeds.

Index terms: *Phaseolus vulgaris* L.*,* herbicide, germination.

Produtividade e qualidade fisiológica das sementes com dessecação na pré-colheita do feijoeiro

RESUMO - A qualidade fisiológica de sementes de feijão é influenciada pelo ambiente de produção e por práticas de manejo empregadas durante o cultivo. O objetivo do trabalho foi avaliar a produtividade e qualidade fisiológica de sementes de feijão, após aplicações de herbicidas dessecantes em diferentes épocas na pré-colheita. Utilizou-se o delineamento de blocos ao acaso, em esquema fatorial (3x3) + 1, onde se testou a aplicação de três herbicidas dessecantes: *glyphosate* (960 g de ia.ha^{-1}), *dicloreto de paraquat* (240 g de ia.ha^{-1}) e *glufosinato de amônio* (400 g de ia.ha^{-1}) + óleo mineral, aplicados em três épocas (dias após o florescimento - DAF), mais uma testemunha sem aplicação de herbicida, em quatro repetições. A qualidade fisiológica das sementes foi determinada por meio dos testes de germinação, primeira contagem, índice de velocidade de emergência de plântulas, envelhecimento acelerado, condutividade elétrica e biomassa seca de plântulas. A produtividade de sementes não é influenciada pelos herbicidas e épocas de aplicação. Independente da época de aplicação, a menor percentagem de plântulas normais no teste de germinação, envelhecimento acelerado e primeira contagem, é de sementes dessecadas com o herbicida *glufosinato de amônio*. O herbicida *glyphosate* reduz o vigor de plântulas. As épocas de aplicação para o produto *dicloreto de paraquat* não afetam a germinação e o vigor das sementes de feijão.

Termos de indexação: *Phaseolus vulgaris* L.*,* herbicida, germinação.

Introduction

Advances in genetic breeding and irrigation systems, and the use of mechanical harvesting have increased yield and quality of bean crops. However, physiological characteristics of bean plants, added to unfavorable environmental conditions, make the time of harvest a very delicate operation, because the physiological maturity of seeds occurs with high water content; basal internodes of the plants are short; and height of pod insertion is low. Additionally, delayed harvesting exposes the seeds to deterioration by microorganisms, fluctuations in temperature and relative humidity, rainfall and natural pod

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shattering, causing loss of yield and physiological quality at pre-harvest (Lacerda et al., 2005; Kamikoga et al., 2009; Kappes et al., 2009; Botelho et al., 2010).

The use of desiccants has been observed in various crops in order to maintain productivity and mitigate problems such as the reduction of germination percentage and seed vigor (Caierão and Acosta, 2007; Kappes et al., 2008; Kappes et al., 2009; Daltro et al., 2010; Foloni et al., 2011). In bean plants, the use of these products is relatively recent; however, interest in this practice is increasing in producing regions, and related advantages include the possibility of harvest planning, increased efficiency of machines, faster release of the area for subsequent crops and reduction of damage caused by pests and fungi that may attack crops at the end of the cycle.

Among the products available in the market, the herbicides *paraquat dichloride*, *glufosinate ammonium* and *glyphosate* have proven efficient as desiccants in some crops, and they are registered by the Ministry of Agriculture, except for *glyphosate*. *Paraquat dichloride* and *glufosinate ammonium* are contact herbicides that act quickly upon plants, causing toxicity a few hours after application. However, translocation of *glufosinato ammonium* in plants, although limited, is higher than that of *paraquat dichloride*; therefore, there is more damage to the plant tissues when the former is applied early (Lacerda et al., 2005). In contrast, *glyphosate* is a post-emergent, non-selective, systemic herbicide for broad-spectrum control. It belongs to the chemical group of N-substituted glycines (Vargas et al., 2007). Although it is not registered, as above mentioned, it has been widely used in various regions of Brazil, mainly because of its good cost-benefit ratio compared with other herbicides (França-Neto et al., 2007).

Given the increasing number of crops in agricultural areas, aimed at greater optimization, the cycles of each crop are becoming shorter, and the beans end up with uneven physiological maturity. The use of desiccants to accelerate the release of the area for the next crop has increased continuously. Knowledge of the effect of desiccants on seed quality, therefore, is extremely important to avoid any loss of quality during cultivation. Thus, the aim of this study was to evaluate yield and physiological quality of seeds in different positions in the plant after the use of pre-harvest desiccants on bean plants in the off-season in northwestern Rio Grande do Sul.

Material and Methods

The study was carried out in two stages: the field stage was conducted at 27º36’25”S (latitude), 53º21’19”W (longitude); average altitude is 558 m, and the soil is a typical eutrophic red oxisol (Embrapa, 2006). Cultivar IPR Gralha, commercial black beans (type I) was used. Mechanical sowing was performed with 19 seeds per meter of furrow at a depth of 0.05 m and 0.45 m spacing between rows. The plots were comprised of 10 rows of 4.5 m length, separated by 0.50 m in the row and 1 m between rows. Seedling emergence occurred six days after seeding, with an estimated population of 360,000 plants.ha^{-1}. During the experiment, there was no water restriction to the normal development of the crop.

By the time of desiccant applications, the first application time (39 DAF) was considered to be the one where 50% of the pods had physiological maturity as indicated by their color (Vieira et al., 2006). The other application times were determined as days after the first application; they were performed on the fifth (44 DAF) and tenth (49 DAF) days. The applications were performed in the morning, with a CO_2 pressurized backpack sprayer calibrated to a volume of 200 L.ha^{-1}. The plots were harvested when the plants were visually dry. Grain yield, hundred seed weight (HSW) and seed quality were evaluated. For the evaluations of these variables, the eight central rows of each plot were considered as useful area, while 0.5 m was disregarded in the extremities (12.6 m²).

The harvest was manual and the track was made using a stationary harvester. The yield for each plot was measured by weighing the seeds on a precision balance, and the values were converted into kg.ha^{-1}. Hundred seed weight (HSW) was measured with the methodology proposed by Brasil (2009). Moisture was adjusted to 13% for both variables.

The second stage of the experiment, relative to assessments of physiological seed quality, was held with ten plants randomly collected within the useful plot. Seeds were collected at two locations in the plant: in the lower portion (base) and the upperportion (apex). The seeds of each portion were analyzed individually. After the study sample was set up, it was taken to the laboratory, and the physiological quality of seeds was determined by the following tests:

Germination (GT)- eight replicates of 50 seeds per treatment were arranged in rolls of paper, moistened with distilled water corresponding to 2.5 times the weight of the dry paper. The rolls were stored in sealed plastic bags and kept in a B.O.D. incubator at 25 °C for 9 days. The evaluation was conducted at five and nine days after sowing; the percentage of normal seedlings was measured;

First count (FC) - performed together with the germination test; the number of normal seedlings was assessed on the 5th day after the start of the test. The results were expressed as percentage of normal seedlings;

Seedling emergence speed rate (ESR) - evaluated in a greenhouse by sowing four replicates of 25 seeds per treatment, in plastic trays containing substrate for ornamental plants. The trays were irrigated and counts were performed daily after the emergence of the first seedling. Calculation of ESR (eq. 1) was based on the methodology proposed by Maguire (1962).

Accelerated aging (AA) - four replicates of 50 seeds of each treatment were laid out in a single layer on a screen inside gerboxes, avoiding contact with 40 mL of distilled water placed at the bottom. The gerboxes were sealed and kept inside an oven at 42 °C for 72 hours. Immediately after the end of the aging period, each replicate was placed in paper towel rolls, and exposed to the same conditions described for the germination test, but the percentage of normal seedlings was assessed at five days after sowing.

Electrical conductivity (EC) - conducted through the center-of-mass system with four replicates of 50 seeds per treatment. The seeds were accurately weighed to two decimal places after the decimal point, and then placed in 200 mL plastic cups filled with 75 mL distilled water, and kept in an incubator at a constant temperature of 25 °C. After 24 hours of soaking, electrical conductivity was measured in the soaking solution, using a digital conductivity meter whose results were expressed in $\mu S.\ cm^{-1}.g^{-1}$;

Seedling dry biomass (DB) -measured in parallel to the germination test by randomly selecting four replicates of 10 normal seedlings per treatment. They were fully packed in paper bags and taken to dry in a forced air circulation oven at 60 ± 2 °C for 48 hours. The material was then weighed for the dry biomass of 10 seedlings, with an accuracy of 0.001 g.

The experiment used a randomized block design, with a factorial (3x3) + 1 scheme, comprised of three desiccants - *glyphosate* (960 g a.i. ha^{-1}), *paraquat dichloride* (240 g a.i. ha^{-1}) and *glufosinate ammonium* (400 g a.i. ha^{-1}) + mineral oil, applied at three times (days after flowering - DAF), and a control without herbicide application, in four replications.

The results were subjected to analysis of variance by the F-test; thus, the significance of the treatments (desiccants and application times) and of the evaluation were checked for position in the plant. For statistical differences, Tukey's test ($p < 0.05$) was used for the treatment (desiccants) and the evaluation (position in the plant). In the evaluation of application times, the polynomial regression method was used for obtaining statistical differences. The control was compared individually with each treatment by Dunnett's test ($p < 0.05$). Statistical analysis was performed with the aid of the statistical software *Statistical Analysis System* – SAS 8.0 (SAS Institute, 2003).

Results and Discussion

The results in Table 1 show that the desiccant had no influence on yield and hundred seed weight of the crop, whereas hundred seed weight was influenced by time of application. The seeds of plants desiccated at 39 DAF had the lowest weight (Figure 1). These results are similar to those described by Kamikoga et al. (2009) and Coelho et al. (2012), who observed a linear and growing relationship in seed weight when the number of applications increased from 28 to 43 DAF. No direct influence of hundred seed weight was observed on yield. This is contrary to the results obtained by Penckowski, 2005; Kappes et al. (2012), who reported the occurrence of a positive linear effect on yield as desiccation was delayed. It should be noted that yield is dependent on genotype, crop and environment, as well as the interaction between these factors, achieved through assessments of adaptability and stability (Rocha et al., 2010).

Table 1. Average values of hundred seed weight (HSW) and yield (Y) of off-season beans, Gralha cultivar, desiccated with different herbicides and days after flowering (DAF).

Treatments	HSW (g)	Y ($kg.ha^{-1}$)
Herbicides– H		
Glyphosate	24.45	2265.84
P. dichloride	23.88	2186.60
G. ammonium	23.89	2312.10
Control	24.44	2039.01
F value$^{(1)}$	1.67^{ns}	0.25^{ns}
Application times - M		
39 DAF	23.57	2228.00
44 DAF	24.07	2307.69
49 DAF	24.59	2228.84
F value	4.06*	0.13^{ns}
H x M interaction		
F value	1.87^{ns}	0.44^{ns}
Mean	24.13	2224.01
CV (%)	3.66	19.34

[1]F-Test. *significant ($p < 0.05$). ^{ns} non-significant ($p < 0.05$). DAF: Days after flowering. P. dichloride: Paraquat dichloride. - G. ammonium: Glufosinate ammonium.

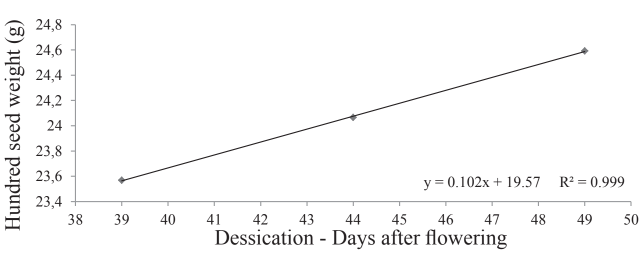

Figure 1. Effect of selective herbicides, applied at different times at pre-harvest, on hundred seed weight of off-season beans, cv. Gralha. (adjusted model is significant at $p < 0.05$ by the F-test).

The results in Table 2 show that herbicides and application times affected the physiological potential of seeds, confirming what was observed by other authors (Lacerda et al., 2005; Caierão and Acosta, 2007; Kappes et al., 2009; Daltro et al., 2010; Foloni et al., 2011; Kappes et al., 2012), whereas the position in the plant showed differences only for seed vigor.

Table 2. First count (FC), germination (GER), accelerated aging (AA), seedling dry biomass (DB), electrical conductivity (EC) and emergence speed rate (ESR) of seeds in off-season beans cv. Gralha, from two positions in the plant, desiccated with different herbicides at days after flowering (DAF).

Treatments	FC (%)	GER. (%)	AA (%)	DB (g)	EC (µS .cm^{-1}.g^{-1})	ESR
Herbicides - H						
Glyphosate	95.95	97.66	96.00	3.40	73.58	2.87
P. dichloride	98.00	99.13	96.83	3.28	65.42	3.04
G. ammonium	85.33	86.63	82.58	3.31	71.37	2.84
Control	98.13	98.50	89.75	3.39	75.66	2.95
F value[(1)]	151.90*	55.35*	136.56*	7.70*	17.30*	21.99*
Times - M						
39 DAF	87.42	90.08	86.58	3.34	76.59	2.81
44 DAF	95.58	96.54	93.08	3.32	66.57	2.91
49 DAF	96.79	97.26	95.75	3.32	67.21	2.98
F value	47.03*	31.18*	47.47*	0.13^{ns}	30.57*	15.46*
Positions - P						
Apex	92.69	94.17	90.17	3.30	66.27	2.91
Base	94.17	94.77	93.44	3.35	73.98	2.93
F value	0.91^{ns}	1.82^{ns}	17.20*	5.59*	43.35*	0.89^{ns}
Interaction			F value			
H x M	60.15*	27.29*	49.8*	7.10*	19.49*	11.12*
H x P	3.67*	0.44^{ns}	24.65*	0.53^{ns}	2.07^{ns}	1.61^{ns}
P x M	2.15^{ns}	1.12^{ns}	1.98^{ns}	1.41^{ns}	8.91*	0.27^{ns}
H x M x P	0.98^{ns}	1.93^{ns}	0.92^{ns}	0.98^{ns}	3.33^{ns}	3.64^{ns}
CV (%)	4.07	7.01	3.65	3.20	7.09	3.92

[1]F-test. *significant ($p < 0.05$).^{ns} non-significant ($p < 0.05$). DAF: Days after flowering. P. dichloride: Paraquat dichloride. - G. ammonium: Glufosinate ammonium.

For the interaction between herbicides and application times (Table 3), regardless of time of application, the lowest percentage of normal seedlings after the germination, accelerated aging and first count tests was observed in seed plants desiccated with the herbicide *glufosinate ammonium*. A comparison between application times in each herbicide showed that *glufosinate ammonium,* during the first germination count, was the only product that had a reduction in the number of normal seedlings compared with the control treatment, at all application times.

In the germination and accelerated aging tests, the herbicide *glufosinate ammonium* was the only one that showed a significant difference compared with the control when applied at 39 DAF, while the percentage of normal seedlings was 28.5 and 26.2% lower, respectively. Working with herbicides and application stages in soybean, Guimarães et al. (2012) also observed that *glufosinate ammonium*, when applied at stage R6, resulted in a greater number of abnormal plants compared with the control treatment, including a higher percentage of abnormal seedlings than *paraquat dichloride*, applied at the same time. The product *glufosinate ammonium* translocates more easily than *paraquat dichloride* (Lacerda et al., 2005); there may have been local absorption of the product, sprinkled directly over the pods, and further degradation of molecules and formation of secondary metabolites. This may have caused major damage to tissues when applied at 39 DAF, contrary to the results obtained by Penckowski (2005) and Kamikoga et al.(2009), who found that *glufosinate ammonium* did not affect the physiological quality of bean seeds, regardless of application time.

No differences were observed in germination percentage after the accelerated aging test. The lack of difference is indicative that plant desiccation does not favor the degradation process, as noted by other authors, who evaluated seeds from desiccated plants subjected to 8-month storage, for which they found lower values for germination and vigor compared with seeds that had not been desiccated (Penckowski, 2005).

Table 3. Values obtained in the first germination count, germination, accelerated aging and seedling dry biomass in off-season beans, depending on herbicides and application times.

Herbicide	Application times			Equations	R^2
	39	44	49		
First count (%)					
Glyphosate	98.75a	97.37a	96.87ab	$y = 0.017x^2 - 1.727x + 139.50$	0.86
P. dichloride	99.12a	99.50a	98.75a	non-significant	-
G. ammonium	72.37b*	92.75b*	94.75b*	non-significant	-
Control		98.00			
Germination (%)					
Glyphosate	97.87a	94.00b	99.00a	$y = 0.177x^2 - 15.507x + 432.69$	0.79
P. dichloride	96.12a	99.75a	98.12a	non-significant	-
G. ammonium	68.25b*	93.00b	94.75a	$y = - 0.460x^2 + 43.130x - 914.16$	0.76
Control		95.50			
Accelerated aging (%)					
Glyphosate	95.50a	94.75a	97.75a*	$y = 0.075x^2 - 6.375x + 230.05$	0.99
P. dichloride	98.00a*	95.00a	97.50a*	non-significant	-
G. ammonium	66.25b*	89.50b	92.00b	non-significant	-
Control		89.75			
Seedling dry biomass (g)					
Glyphosate	3.48a	3.26a*	3.45a	non-significant	-
P. dichloride	3.23b*	3.35a	3.26b	$y = - 0.004x^2 + 0.381x - 5.09$	1
G. ammonium	3.30ab	3.35a	3.27b	$y = - 0.003x^2 + 0.254 - 2.18$	0.90
Control		3.39			
EC ($\mu S.cm^{-1}.g^{-1}$)					
Glyphosate	79.22a	71.68a	69.82a	$y = 0.286x^2 - 24.57x + 588.22$	0.72
P. dichloride	64.84b*	60.65b*	70.77a	non-significant	-
G. ammonium	85.70a*	67.38ab	61.03b*	non-significant	-
Control		75.66			
Emergence Speed Rate					
Glyphosate	2.68b*	2.92b	3.01a	$y = - 0.005x^2 + 0.516x - 7.95$	0.70
P. dichloride	3.05a	3.14a*	2.93a	non-significant	-
G. ammonium	2.70b*	2.81b	2.99a	non-significant	-
Control		2.95			

Means followed by the same letters in the column do not differ by Tukey's test ($p < 0.05$). *Statistically different from the control treatment by Dunnett's test ($p < 0.05$). P. dichloride: Paraquat dichloride. - G. ammonium: Glufosinate ammonium.

The test for seedling dry biomass showed that the seeds of plants that received contact herbicides in applications made at 39 and 49 DAF had lower weight compared with plants whose seeds were desiccated with *glyphosate*, which showed no difference across application times. A similar response was found by Kappes at al. (2012) with the use of *paraquat dichloride*, where there were reductions in mass accumulation for applications of 285 $g.ha^{-1}$ and 305 $g.ha^{-1}$, respectively made at 35 and 40 DAF.

For application times, there was an increase in the percentage of normal seedlings in the germination test of seeds that had received *glufosinate ammonium*; such percentage increased with the delay of applications, an identical effect to that of *glyphosate* from the second application time onwards (Figure 2a). However, *glyphosate* showed lower germination percentage when the application was made at 44 DAF, which is contrary to the finding by Kamikoga et al. (2009), who found no difference between the applications made between 28 and 43 DAF. Otherwise, the seeds that received *glyphosate*, showed increase in vigor with the delay of product applications, as shown in the tests for accelerated aging (Figure 2b), electrical conductivity (Figure 2c) and emergence speed rate (Figure 2d).

Application times of *paraquat dichloride* did not affect seed germination percentage. Response to this herbicide was observed only in the vigor expressed by the seedling dry biomass test, where the greatest weight was obtained in seeds of plants desiccated at 44 DAF. Working with rates and application times in early Carioca bean cultivar, Kappes et al. (2012) found similar results. Seedlings had higher dry matter accumulation when desiccated at 40 DAF, although no difference was observed when compared with the application at 45 DAF. These results corroborate those found by Guimarães et al. (2012), who studied soybean, and found that the herbicide *paraquat dichloride* promoted positive rates of germination and seed vigor when used at development stages R6 and R7.2 of the crop. Santos et al. (2004) claimed that knowledge of the mode of action of the herbicide is as important for maximum yield of viable seeds as knowledge of the best time for product application in bean plants. Application time and mechanism of action of the herbicide directly affect seed quality, although yield may not always be affected (Kappes et al., 2009).

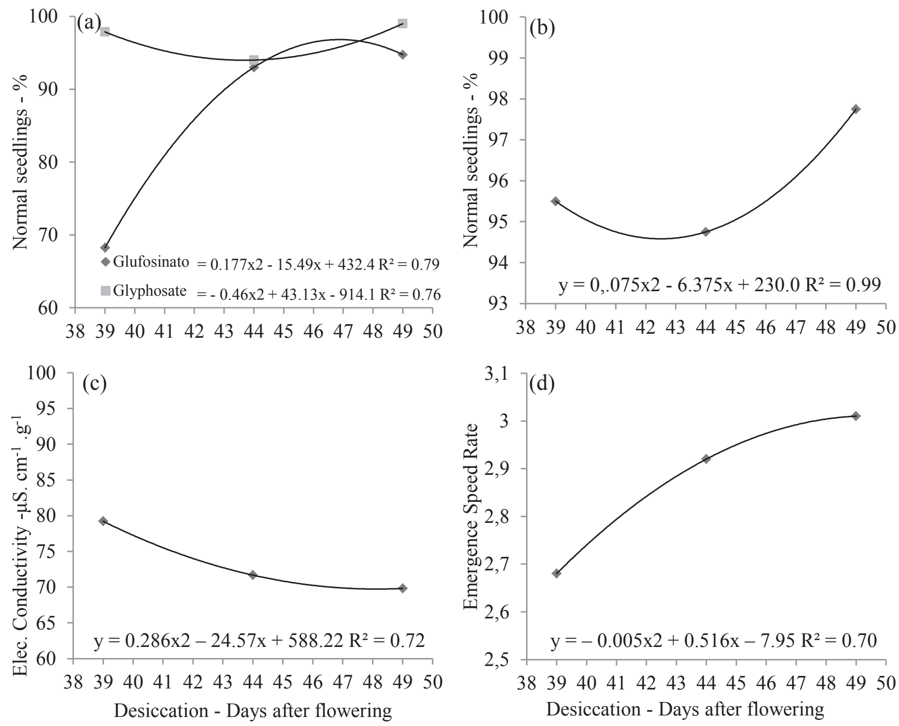

Figure 2. Regression analysis of the effect of *glyphosate* applied at different times after flowering in the germination (Figure 2a), accelerated aging (Figure 2b), electrical conductivity (Figure 2c) and emergence speed rate (Figure 2d) tests, for off-season bean seeds desiccated at pre-harvest. (the adjusted model is significant at $p < 0.05$ by the F-test).

For the interaction between herbicide and plant positions (Table 4), there was interference with the vigor expressed in the first count and accelerated aging tests. Regardless of position, seeds desiccated with *glufosinate ammonium* showed less vigor when compared with seeds obtained from plants desiccated with other products or with the control. Seeds from the upperportion showed less vigor when subjected to stress conditions for temperature and humidity, with reduction in the number of normal seedlings by 19.19 and 12.30% compared to the control comprised of seeds of the whole plant and seed from the lower portion, respectively. There are no clear distinction across developmental stages of bean plants, especially when plants with indeterminate habit are considered, although there is an overlap represented by the simultaneous emission of leaves, flowers and pods (Moura et al., 2012). This feature, coupled with greater ease of translocation of *glufosinate ammonium* (Lacerda et al., 2005), may have potentiated its action when it was sprayed directly on the pods, causing further damage to the tissues of the upper portion.

Interference effect was found in the electrical conductivity test (Table 5) for the interaction between application times and positions of the plant. No difference was observed between positions where the application of the product occurred at 39 DAF, while seeds in the upper position had higher vigor for applications carried out at 44 and 49 DAF. This result assumes that desiccation has acted upon the permeability of the seedcoat (Kikuti et al., 2006). When there is low release of solutes after sowing, there is lesser loss of cell compartmentalization, thus reducing the growth of microorganisms that are harmful to seedlings (Marcos-Filho, 2005). In addition, it was observed that the electrical conductivity test is effective in detecting differences between treatments, which agrees with the results obtained by Kikuti et al. (2006), Coelho et al. (2010) and Coelho et al. (2012).

Table 4. First germination count and accelerated aging of off-season bean seeds, depending on positions in the plant and different herbicides.

Position	Herbicide			
	Glyphosate	P. dichloride	G. ammonium	Control
	First count (%)			
Upper	98.83aA*	98.42aA	85.25aB**	98.75
Lower	99.41aA	96.92aA	88.00aB**	98.25
	Accelerated aging (%)			
Upper	95.33aA	98.00aA	77.17bB**	93.50
Lower	96.67aA	95.67aA	88.00aB**	96.00

Means followed by the same letters do not differ by Tukey's test ($p < 0.05$). Uppercase letters are compared in the row and lowercase letters are compared in the column. **Statistically different from the control treatment by Dunnett's test ($p < 0.05$). P. dichloride: Paraquat dichloride. - G. ammonium: Glufosinate ammonium.

Table 5. Electrical conductivity of off-season bean seeds, cv. IPR Gralha, depending on the position in the plant and application times of desiccant herbicides.

Position	Electrical Conductivity (µS. cm^{-1} .g^{-1})			Equations	R²
	39[1]	44	49		
Upper	76.07a	60.15b	62.59b	non-significant	-
Lower	77.10a	73.00a	71.83a	$y = 0.367x^2 – 33.666x + 830.48$	0.82

Means followed by the same letter in the column do not differ by Tukey's test ($p < 0.05$). [1]Days after flowering.

Conclusions

Seed yield is not influenced by herbicides and application times.

Regardless of application time, the lowest percentage of normal seedlings after the germination, first count and accelerated aging tests was shown by seeds from plants desiccated with the herbicide *glufosinate ammonium*.

The herbicide *glyphosate* reduces seedling vigor.

Application times of *paraquat dichloride* do not affect the germination and vigor of bean seeds.

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