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Research Article

Physiological attributes of Enlist E3™ soybean seed submitted to herbicides application

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INFORMATION ARTICLE

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The authors declare to not have conflict of interest.

HIGHLIGHTS

- New technologies are being developed to prevent resistance and ensure adequate control of weeds.
- Enlist E3™ cultivars contributes to the efficient management of weeds, especially those resistant to glyphosate.
- The application of herbicides may cause losses in the physiological quality of soybean seeds.

ABSTRACT

Background: Enlist E3™ soybean is tolerant to 2,4-D, glyphosate and ammonium-glufosinate and were developed for effective management of herbicide resistant weeds such as glyphosate Herbicides, even being selective, can cause damage to crop metabolism, affect growth and development and interfere with seed physiological quality.
Objective: The aim of this research was to evaluate the physiological quality of Enlist E3™ soybean seeds submitted to herbicide application.
Methods: The field trial was carried out in a randomized block design with four replications. The treatments tested were 2,4-D choline + glyphosate, 2,4-D choline, glyphosate + 2,4-D amine and ammonium-glufosinate; and control without application, sprayed at V6 soybean growth stage. After harvest, the seeds were submitted to first germination count, accelerated aging, germination speed index, shoot and root length, shoot and root dry mass and electrical conductivity tests.
Results: The management of Enlist E3 soybeans with Enlist herbicides (2,4-D choline + glyphosate and 2,4-D choline) as well as glyphosate + 2,4-D amine and glufosinate did not negatively impact soybean parameters such as: vigor, germination count, accelerated aging, germination speed, aerial part and primary root length, aerial part and root dry mass and electrical conductivity.
Conclusions: Those herbicides can be recommended to be used on post emergence (V6) of Enlist E3 soybean.

1 INTRODUCTION

Weed interference can cause significant losses in soybean productivity, depending on the population and weed species (Fleck et al., 2007). The damage to soybean productivity caused by the presence of weeds occurs due to competition for resources essential for development, release of allelopathic compounds and/or for being hosts of insects and diseases (Vasconcelos et al., 2012).

Chemical control has been the most widely used weed management tool due to its practicality and efficiency. However, the incorrect and indiscriminate use of herbicides has contributed to the increase in the selection of resistant weeds (Shaner, 2014). A classic example was the intensive and repeated use of the herbicide glyphosate after launching RR (Roundup Ready®) crops, introduced and commercialized from the 1990s, which favored the selection of several resistant species to the herbicide (Heap, 2018). Considering the increase in the number of resistant biotypes to glyphosate herbicide in soybean crop and its impact on agricultural production, new technologies and management strategies have been developed to prevent resistance and ensure adequate control of weeds.

Enlist E3™ cultivars are tolerant to 2,4-D, glyphosate and glufosinate herbicides, which contributes to the efficient management of weeds, especially those resistant to glyphosate. Soybean tolerance to 2,4-D herbicide was achieved by inserting a gene from *Delfia acidovorans* bacterium, which codes for the arylokyalkanoate dioxygenase isoenzyme (AAD), which metabolizes the 2,4-D to the non-herbicidal dichlorophenol metabolite (DCP) (Skelton et al., 2017). Considering the introduction of the herbicide tolerance the Enlist™ Duo Colex-D™ and Enlist™ Colex-D™ herbicides was also launched, which corresponds to the 2,4-D choline salta and mixture of glyphosate and 2,4-D choline, respectively. Both formulation featured Colex-D™ technology. These formulations has positive attributes, such as ultra-low volatility, reduced drift potential and less odor, compared to commercially available amine or ester formulations (Kalsing et al., 2018).

Although selective to crops, herbicide application can generate stresses, which are characterized as any negative effect on the growth and normal development of plant species (Agostinetto et al., 2016). Changes in the metabolic pathways caused by herbicides application can lead to reduced growth and development of plants and, consequently, reduce the mass of seeds, affecting their physiological quality (Perboni et al., 2018).

Seed quality is the sum of the genetic, physical, physiological and sanitary attributes of the seed, which are responsible for the ability to originate highly productive plants. Seed viability is mainly measured by germination in favorable conditions, which determines maximum germination, while vigor is determined under unfavorable conditions, measuring the decline of some biochemical or physiological function (Popinigis, 1985).

The knowledge of changes in the physiological quality of soybean seeds due to herbicides application, in advance of the technology commercial availability, will allow the adoption of preventive measures, aiming to avoid losses of seedling stand or vigor and consequent grain productivity. The objective of this study was to evaluate the physiological attributes of Enlist E3™ soybean seeds as a result of the herbicides application.

2 Material and Methods

The field experiment was conducted in a randomized block design with four replications, at Mogi Mirim Research Center of Corteva Agriscience, in the city of Mogi Mirim/SP, during the 2016/17 harvest. Soybeans sowing occurred in the second half of December, in rows spaced 0.5 m and population of 30 plants m^{-2}. The treatments consisted of herbicides application such as; ready mix 2,4-D choline salt + glyphosate dimethylamine salt (2000 g a.e. ha^{-1})), 2,4-D choline salt (975 g a.e.), glyphosate + 2,4-D amine salt (1,367 g a.e. + 975 g a.e.) and glufosinate (500 g a.i.), in addition to the control without application. The herbicides were applied at V6 soybean growth stage, using a pressurized CO_2 sprayer, equipped with AIXR 110.015 nozzle type spaced 0.5 m apart, calibrated for a flow rate of 100 L ha^{-1}.

At the end of soybean cycle, the seeds were harvested and stored in a cold chamber until the moment of the experiment assembly of physiological quality in the seed laboratory of the Faculty of Agronomy Eliseu Maciel/UFPel. The evaluations consisted of the first germination count (FGC), accelerated aging (AA), germination speed index (GSI), length of aerial part (LAP) and length of primary root (LR), root dry mass (RDM), dry mass of the aerial part (DMAP) and electrical conductivity (EC).

For the FGC, GSI, LAP, LR, DMAP and RDM tests, 50 soybean seeds per repetition were distributed on rolls of Germitest® paper previously moistened with distilled water in a proportion of three times the weight of the dry paper (Brasil, 2009). The experimental design was completely randomized with eight replications. The rolls were placed in a germinator at a temperature of 25 ºC (Brasil, 2009). The first germination count consisted of determining the percentage of seeds germinated five days after the test installation, according to the rules of seed analysis (Brasil, 2009).

Accelerated aging was carried out in a Gerbox type box with wire mesh. At the bottom of each box, 40 mL of distilled water were added and, on the wire mesh, the seeds of each treatment were evenly deposited, forming a single layer. Then, the boxes were covered and placed in a BOD incubator at 41 °C for 48 hours (Marcos Filho, 1999). After this period, the seeds were placed to germinate, according to the methodology described for the first germination count test, and evaluated on the fifth day, and the results expressed in percentage of normal seedlings.

The germination speed index was determined by counting the number of seeds germinated daily for eight days. For the GSI calculation, the formula was used: $GSI = \sum Ni/Di$, where Ni is the number of seedlings on day i, and Di, the number of days for germination. The lengths of aerial part and primary root (cm) of ten seedlings were measured with the aid of a millimeter ruler, according to the methodology described by Nakagawa (1999). The seedlings used in the analysis of LAP and LR were cut in the hypocotyl region, dried in an oven at a temperature of 60 °C until a constant mass was obtained and, later, weighed on a scale to determine the dry mass of the aerial part (DMAP) and the root dry mass (RDM).

For the electrical conductivity test, four replicates of 25 previously weighed soybean seeds were used. The seeds were placed in containers containing 75 mL of deionized water, which were kept in a germinator at a constant temperature of 20 °C for 24 hours. The EC was determined in a conductivity meter (Digimed CD-2), and the results, expressed in micro-siemens $cm^{-1}g^{-1}$ of seed (Krzyzanowski et al., 1991). The data obtained were analyzed for normality (Shapiro-Wilk test) and subsequently subjected to analysis of variance ($p \leq 0.05$). In the case of statistical significance, a comparison between means was performed using the Duncan test ($p \leq 0.05$).

3 Results and Discussion

The analysis of variance showed a significant effect for the first germination count (FGC), accelerated aging (AA), germination speed index (GSI), length of the aerial part (LAP), length of the root (LR), dry mass of the aerial part (DMAP) and root dry mass (RDM) (Tables 1, 2 and 3). For the variable electrical conductivity (EC), there was no statistical significance (data not shown). As for the variable FGC, which expresses the vigor of the seeds, it was observed that the highest values were obtained in the treatments with 2,4-D choline + glyphosate and glufosinate herbicides, compared to the other treatments and to the control (Table 1).

Table 1 - Germination after the accelerated aging test, first germination count (FGC) and germination speed index (GSI) of Enlist E3™ soybean seeds submitted to herbicide treatments

Treatment	Germination (%)	FGC (%)	GSI
Control	38 b	49 b	23.9 b
2,4-D choline + glyphosate	57 a	65 a	27.6 a
2,4-D Choline	31 b	44 b	22.8 c
Glyphosate + 2,4-D amine	39 b	51 b	24.5 bc
Glufosinate	38 b	62 a	26.0 ab
CV (%)	16.67	18.43	5.26

Averages followed by the same letters in the column do not differ by Duncan's test ($p \leq 0.05$).

Table 2 - Length of aerial part and root of Enlist E3™ soybean seedlings submitted to herbicide treatments

Treatment	LAP (cm)	LR (cm)
Control	3.6 b	4.2 b
2,4-D choline + glyphosate	4.5 a	8.1 a
2,4-D Choline	4.7 a	7.3 a
Glyphosate + 2,4-D amine	5.0 a	7.4 a
Glufosinate	4.9 a	7.3 a
CV (%)	10.12	12.53

Averages followed by the same letters in the column do not differ by Duncan's test ($p \leq 0.05$).

Table 3 - Dry mass of the aerial part and root of Enlist E3™ soybean seedlings submitted to herbicide treatments

Treatment	DMAP (mg)	RDM (mg)
Control	125.98 ab	5.72 b
2,4-D choline + glyphosate	131.96 ab	7.83 a
2,4-D Choline	141.01 a	7.90 a
Glyphosate + 2,4-D amine	122.75 b	6.60 ab
Glufosinate	120.37 b	7.92 a
CV (%)	7.13	14.48

Averages followed by the same letters in the column do not differ by Duncan's test ($p \leq 0.05$).

The degree of competition among weeds and crops can be changed according to the periods when the community is competing for a given resource. It is known that the competition established in the initial phases causes losses in production, and we must provide the establishment of the crop free of the presence of weeds to avoid such damages (Agostinetto et al., 2014). Therefore, results that demonstrate higher values of FGC can favor the crop in the initial stages of development, making it more competitive compared to weeds. In a study analyzing the RR soybean cultivar in soils with residual imazapyr + imazapic, a linear decrease of the first and second germination counts was observed as the herbicide doses increased (Agostinetto et al., 2018).

For the AA test, there was a higher germination value in the treatment with 2,4-D choline + glyphosate herbicide, and it differed from the control and other herbicides, while the other herbicide treatments did not differ from the control (Table 1). Accelerated aging consists of simulating adverse environmental factors, such as temperature and high relative humidity. Under these conditions, seeds with low physiological quality deteriorate more rapidly than more vigorous seeds, establishing differences in physiological potential (Guedes et al., 2011).

The emergence or growth of pea seedlings from seeds sprayed with glyphosate before harvest decreased when the application was made to plants with seeds containing high moisture content (Baig et al., 2003). Opposites results were verified when evaluating the effect of herbicides applied in pre-harvest of soybean, where the ammonium glufosinate (300 g a.i. ha^{-1}) did not affect the germination and the vigor of the seeds (Penckowski et al., 2005).

Low germination values obtained in this research may be related to the stress caused by the herbicide and/or also to the damage caused to the tegument during manual harvesting of the seeds, since the presence of cracks in a large number of seeds was observed. Regarding the GSI variable, the treatment that obtained the highest value was with the 2,4-D choline + glyphosate herbicide, which did not differ statistically from the treatment with the glufosinate herbicide (Table 1). The other treatments, including the glufosinate herbicide, did not differ from the control.

The seed vigor is proportional to its accumulation of dry matter. The maximum seed vigor is reached when it presents maximum weight of dry matter. From that point on, the evolution of this characteristic occurs in a similar way to that of germination, that is, it tends to remain at the same level, or decreases, depending on environmental factors and the manner and timing of the harvest (Carvalho and Nakagawa, 2012).

The highest GSI values in this study were achieved with 2,4-D choline + glyphosate and glufosinate herbicides, which corroborates studies using the glufosinate and 2,4-D + glyphosate desiccants in wheat (Perboni et al., 2018). The ammonium glufosinate herbicide can be used for the purpose of desiccant in the pre-harvest of soybean crops (Guimarães et al., 2012). However, studies demonstrate that the application of this herbicide reduces the germination and vigor of the seeds (Guimarães et al., 2012). The lowest GSI value of the control compared to herbicide treatments may be related to weed interference and the high humidity rate of the experimental unit. The rate of seed deterioration increases considerably due to exposure to adverse conditions of temperature and relative humidity, resulting in less vigor of the seeds.

When the LAP and LR were analyzed, it was found that all herbicide treatments did not differ among each other, but differed from the control (Table 2). Among the herbicide treatments, the highest value for LAP was obtained with the glyphosate + 2,4-D amine herbicide, while for the variable LR the highest was with the 2,4-D choline + glyphosate herbicide. The lowest values for the LAP and LR variables in the control treatment, regarding to the treatments with herbicides, may be due to the interference caused by the weeds and the lowest vigor of the seeds, as discussed. For herbicides that have the ability to translocate parts of plants with high metabolic activity, there is the possibility of translocating them to the grains (Cessna et al., 2002). Thus, it is hypothesized that, in the treatments that contain the 2,4-D molecule in the formulation, the herbicide may have accumulated in the tissues and, later, translocated to the soybean seeds, acting as a growth regulator and contributing to the highest values of the variables.

For the DMAP variable, higher values were found in treatments with the 2,4-D choline and 2,4-D choline + glyphosate herbicides, which did not differ from the control (Table 3). The glyphosate + 2,4-D amine and glufosinate herbicides had the lowest values for the variable DMAP, compared to the treatment with 2,4-D choline. However, as the others, the treatments glyphosate + 2,4-D amine and glufosinate did not also differ from the control.

For the RDM variable, all herbicide treatments differed from the control, except for the glyphosate + 2,4-D amine treatment (Table 3). Auxins occur naturally in plants and are important for growth, cell elongation and lateral growth control. However, when it is found in high concentrations, as with the application of auxinic herbicides, auxin becomes toxic, and, without homeostatic control the hormone can easily trigger irreversible damage (Peterson et al., 2016). Therefore, in low doses, auxinic herbicides have hormonal properties like those of natural auxin, and when the application rate increases, auxins cause various abnormalities in sensitive dicotyledons.

This may have been the reason why treatments containing the 2,4-D herbicide showed higher DMAP values, except for treatment with the glyphosate + 2,4-D amine mixture (Table 3). A similar result was observed with the RDM, in which 2,4-D choline + glyphosate and 2,4-D choline herbicides stimulated the development of the soybean root. It is hypothesized that 2,4-D metabolism may not have occurred completely, thus leaving some residue of the herbicide in the plant, which may have acted as a growth regulator. Given that, the plants would have greater growth compared to the control, causing greater availability of photoassimilates for the formation of seeds.

Further studies regarding the effect of the application of the Enlist system herbicides on the physiological quality of Enlist E3™ soybean seeds should be carried out, owing to the importance of this technology in the management of weeds in soybean crop.

4 Conclusions

The management of Enlist E3 soybeans with Enlist herbicides (2,4-D choline + glyphosate and 2,4-D choline) as well as glyphosate + 2,4-D amine and glufosinate did not negatively impact soybean parameters such as: vigor, germination count, accelerated aging, germination speed, aerial part and primary root length, aerial part and root dry mass and electrical conductivity. Those herbicides can be recommended to be used on post emergence (V6) of Enlist E3 soybean.

5 Contributions

Jéssica R. Garcia - Conducting experiments, analysis and writing. Andrés A. M. Vargas - Assistance in conducting the experiment and writing. Lais Tessari Perboni - Assistance in conducting the experiment and writing. Edna A. Souza - Assistance in conducting the experiment. Daniela Tessaro - Assistance in conducting the experiment. Felipe R. Lucio - Assistance in conducting the experiment. Dirceu Agostinetto - Creator of research, guidance in driving, analyzing and writing.

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