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Selectivity of 2,4-D choline salt, glyphosate, glufosinate, and their mixtures for enlist E3™ soybeans

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To reduce the impacts caused by the constant use of the same active ingredient, a new genetically modified soybean cultivar has been developed for resistance to the herbicides 2,4-D choline salt, glyphosate, and glufosinate ammonium. Therefore, the objective of this study was to evaluate the effects of herbicides applied at the V3 and V6 stages on the development and productivity of Enlist E3™ soybeans over two growing seasons. The treatments were: 2,4-D choline salt (780 g a.e./ha^{-1}), glyphosate (820 g a.e./ha^{-1}), 2,4-D choline salt + glyphosate (1600 g a.e./ha^{-1}), glufosinate (400 g a.e./ha^{-1}), and glufosinate + 2,4-D choline salt (400 + 780 g a.e./ha^{-1}) and double the dose of each herbicide. Considering the results obtained in the 2017/2018 season, the treatments negatively affected the crop development only in terms of insertion height and number of pods, with the double doses negatively influencing grain yield and plant height. In the 2018/2019 season, the treatments negatively affected only plant height and productivity when double the recommended doses were used. Therefore, Enlist E3™ soybeans were not affected in most of the evaluated characteristics by the application of herbicides when the recommended doses for the crop were used.

Key words: *Glycine max*, herbicides, phytotoxicity, tolerance, productivity.

INTRODUCTION

Brazil is the world's largest soybean producer, accounting for 32% of global production (USDA, 2023). It is also the largest exporter, with approximately 362.947 million tons, and China is the main consumer (USDA, 2023; CONAB, 2021). Soybean production in Brazil could be even larger if not for the interference of external factors. These factors include environmental conditions such as water and heat stress, and anthropogenic factors such as the selection of cultivars and the management practices adopted by producers to control pests, diseases, and weeds. According to Song et al. (2017), the main reason for possible low soybean productivity is related to problems caused by inadequate weed management. Among the alternatives for weed control, the most widely adopted worldwide is chemical control because, according to Alvino et al. (2011), it has greater advantages since it is more economical and efficient than the others, especially in large areas of cultivation with

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high weed infestation.

Crop improvement through the creation of genetically modified plants has substantially increased soybean yield over the last 30 years (Oerke, 2006; Rincker et al., 2014). Specht et al. (2014) estimated that two-thirds of the gains in soybean yield were due to genetic improvements, and one-third resulted from agronomic improvements. With the inclusion of new transgenic herbicide-resistant cultivars, such as glyphosate-resistant RR (Roundup Ready®) soybeans and LL (Liberty Link®) soybeans, resistant to glufosinate ammonium, soybeans have become more competitive with weeds, partly due to increased seedling vigor, faster growth, and increased sowing density. However, the increased occurrence of glyphosate-resistant weed species, with consequent escape from control, can increase yield losses (Soltani et al., 2017).

Thus, MS Technologies and Dow AgroSciences LLC have developed a new genetically modified soybean cultivar to reduce the impacts caused by the constant use of the same active ingredient, especially the occurrence of resistant weeds (Fast et al., 2016). The new cultivar, Enlist E3™, was developed using *Agrobacterium*-mediated transformation to stably incorporate the AAD-12 gene from the bacterium *Delftia acidovorans*, the 2mEPSPS from *Zea mays*, and the PAT gene from the bacterium *Streptomyces viridochromogenes* into soybeans. The aim is to achieve resistance to the herbicides 2,4-dichlorophenoxyacetate (2,4-D) choline salt, glyphosate, and glufosinate ammonium, respectively (Papineni et al., 2017; Fast et al., 2016).

Enlist® soybeans combine high-performance genetics to improve weed and caterpillar management, allowing the producer to better control the operation and maximize the crop's productive potential (CORTEVA, 2021). Although selective for crops, herbicides can cause stress in plants, characterized by any negative effect on the normal growth and development of plant species (Agostinetto et al., 2016). Changes in metabolic pathways caused by herbicide application can lead to reduced plant growth and development, consequently reducing seed mass and affecting their physiological quality (Perboni et al., 2018). Thus, this study aimed to verify the effects of the herbicides 2,4-D choline salt, glyphosate, 2,4-D choline salt + glyphosate, glufosinate, and glufosinate + 2,4-D choline salt applied at the V3 and V6 stages on the development and productivity of Enlist E3™ soybeans in two crop years. Therefore, our hypotheses are that: 1) The plant will suffer greater action from the herbicide in stage V6 and 2) The application of twice the dose will affect the variables analyzed.

MATERIALS AND METHODS

The experiment was carried out in the 2017/2018 and 2018/2019 crop years in the municipality of Jaboticabal, SP (48º18'58"W; 21º15'22"S, and altitude of 570 m) with an average soil slope of 7%, in a Cwa climate (subtropical), according to the Köeppen classification. The soil in the experimental area is classified as a typical Eutroferric Red Latosol, with a very clayey texture, moderate A, kaolinitic, gently undulating relief (EMBRAPA, 2013). The soil was prepared using the conventional system, with two harrowings and leveling. Then, a composite sample was taken for routine chemical and physical characterization, which was carried out by Athenas Laboratory, Jaboticabal-SP. In both experiments, the seeds were sown on October 23rd, 18 seeds per meter at a spacing of 0.45 m and at a depth of 5 cm. For both, based on the results of the soil analysis and the crop's nutritional needs, sowing fertilization was applied with 300 kg ha^{-1} of the N-P-K 00:20:20 formulation. The cultivar used was Enlist E3™. As already mentioned, it is resistant to 2,4-D choline salt, glyphosate, and glufosinate. The seeds were previously treated with 120 ml of Biomax Premium Liquid inoculant per 100 kg of seed and 100 ml of Maxim XL fungicide per 100 kg of seed.

The experiments were conducted in a randomized block design, in a 5 × 2 + T factorial arrangement, with a total of 11 treatments. The two factors studied comprised five herbicides (2,4-D choline salt, glyphosate, glyphosate + 2,4-D choline salt, glufosinate, and glufosinate + 2,4-D choline salt) in two doses (X and 2X, where X corresponds to the recommended dose of each herbicide), in four replicates (Table 1). Each experimental unit had a useful area of 8 m^2 (2 × 4 m).

The herbicides were applied at the V3 (growth stage) and V6 (growth stage) stages using a constant pressure sprayer (CO_2) equipped with a boom with four AIXR 110.015 spray tips. The equipment was set at 2.2 bar pressure to distribute the equivalent of 150 L ha^{-1} of spray, moving at 1 m.s^{-1}, and with the boom at the height of 0.5 m from the target. In the 2017/2018 harvest, the application at the V3 stage was carried out in the late afternoon on 20 November 2017, with an average temperature of 29ºC, an average wind speed of 5 km/h, and 67.3% relative humidity. The application at the V6 stage was carried out on 04 de December 2017, with an average temperature of 28ºC, an average wind speed of 6 km/h, and 82.4% relative humidity. In the 18/19 harvest, the application at the V3 stage was carried out in the late afternoon on 20 November 2018, with an average temperature of 26ºC, an average wind speed of 2 km/h, and 74.3% relative humidity. The application at the V6 stage was carried out on 04 de December 2018, with an average temperature of 28ºC, an average wind speed of 6 km h^{-1}, and 60.7% relative humidity. Rainfall and average temperature data were recorded throughout the experimental period.

The selectivity of the products regarding soybeans (phytotoxicity) was assessed visually at 1, 3, 7, and 14 days after application of the treatments (DAA), using the scale proposed by the EWRC (1964). In this scale, a score of 1 represents 0 phytotoxicity, and a score of 9 represents the death of the soybean plants. At harvest time, 20 soybean plants were collected at random from the center area of the plots to assess plant height, insertion height of the first pod, number of pods per plant, and mass of 100 grains. The final grain productivity was estimated after the plots had been harvested, and the values extrapolated to kg ha^{-1}. The ideal harvest time was 13% grain moisture and the ripening time when 95% of the pods were ripe and had the cultivar's typical color (stage R8 on the scale of Fehr et al. (1971)). The data obtained underwent analysis of variance using the F test. Tukey's test was used to compare the means at the 1 or 5% probability level. The data were analyzed by means of the AGROSTAT statistical software.

RESULTS AND DISCUSSION

When analyzing the phytotoxicity scores in the soybean plants (Table 3), it is worth noting that in the 2017/2018 harvest, the doses used showed statistical differences, in

Table 1. Herbicides and their respective doses used in post-emergence on Enlist E3™ soybeans.

Herbicide	Doses			
	g a.e./ha		L c.p./ha	
	Dose X	Dose 2X	Dose X	Dose 2X
2,4-D choline salt	780	1560	1.71	3.42
Glyphosate	820	1640	1.71	3.42
Glyphosate + 2,4-D choline salt	1600	3200	4	8
Glufosinate	400	800	2	4
Glufosinate + 2,4-D choline salt	400 + 780	800 + 1560	2.00 + 1.71	4.00 + 3.42

which plants with twice the recommended dose showed higher phytotoxicity scores in all the evaluations carried out. However, the plants recovered over the course of the evaluations, with lower scores at 14 days, except for the treatment with 2,4-D choline salt + glyphosate, which had the highest phytotoxicity score at 14 DAA. In the 18/19 harvest, plants with twice the recommended dose showed higher phytotoxicity scores only in the first two evaluations carried out at 1 and 3 DAA. From 7 DAA onwards, the scores decreased. Finally, in the fourth evaluation carried out at 14 DAA, the plants showed scores close to one (no symptoms), except for the treatments with glyphosate + 2,4-D choline salt and glufosinate + 2,4-D choline salt, which still showed significant symptoms. There was an interaction between the factors for the phytotoxicity scores in the soybean plants (Table 4).

For the herbicides used in the 2017/2018 harvest (Table 4), the plants with 2,4-D choline salt and glyphosate alone showed no symptoms of phytotoxicity in all the evaluations carried out up to 14 DAA. However, the other treatments provided moderate phytotoxicity from 1 DAA when twice the recommended dose was used. The scores increased throughout the evaluations, with greater symptoms at 7 DAA and lower scores at 14 DAA, with a tendency to recover, except for the treatment with glyphosate + 2,4-D choline salt (Table 4).

The results obtained in the breakdown for the phytotoxicity scores in the 18/19 harvest were similar to those of the 2017/2018 harvest in the evaluations carried out at 1 and 3 DAA. In these evaluations, the treatments with 2,4-D choline salt and glyphosate alone did not cause phytotoxicity in the soybean plants in all the evaluations carried out. However, for the other treatments, the plants showed symptoms on the leaves from the first evaluation at 1 DAA, with the highest scores in the treatment of the combinations of 2,4-D choline salt + glyphosate and glufosinate + 2,4-D choline salt followed by glufosinate alone. As time went by, the plants showed recovery. From 7 DAA, the scores were close to 1, showing a tendency toward full recovery, with no interaction between the factors. Correia and Durigan (2007) and Amajioyi et al. (2022) obtained similar results. They tested eight glyphosate-based herbicides on CD 214 RR, M-SOY 8008 RR and Enlist E3 soybeans. They reported that the herbicides did not cause any phytotoxic effects that could be observed in the soybean plants of the two cultivars studied.

Krausz and Young (2001) reported that glyphosate caused more pronounced chlorosis on the leaves when applied at the R1 stage of RR soybeans, which intensified as the doses increased. Furthermore, the trimethylsulfonium salt formulation of glyphosate (commercial product Touchdown 5) caused leaf discoloration. However, the symptoms remained restricted to the leaves that received the product, as the new leaves were uninjured. In conclusion, they reported that the herbicides, regardless of their formulation, did not affect grain productivity (Zaccaro-Gruener et al., 2022; Shyam et al., 2021).

Typically, the direct damage caused by glyphosate on leaves is characterized by a chlorosis known as yellow flashing (Petter et al., 2016). This effect may be associated with the complexation of glyphosate with certain nutrients (Coutinho and Mazo, 2005), momentarily reducing the availability of these nutrients for metabolic reactions in the cells. Furthermore, the effects of glyphosate can extend beyond chlorosis, affecting physiological metabolism such as reducing the photosynthetic rate, transpiration, and stomatal conductance (Zobiole et al., 2010). As in the evaluation of the 2017/2018 harvest, the following year, 2018/2019, showed the most severe symptoms of phytotoxicity in treatments with a combination of glyphosate + 2,4-D choline salt, glufosinate + 2,4-D choline salt, and glufosinate alone.

In the last evaluation (14 DAA) in the 2017/2018 harvest, phytotoxicity levels were reduced in most of the treatments evaluated, except for the treatments with the herbicides glyphosate + 2,4-D choline salt and glufosinate + 2,4-D choline salt, which maintained a high percentage of phytotoxicity. However, the plants recovered from the symptoms by the end of the cycle. In the 18/19 harvest, the plants treated with the combinations of glyphosate + 2,4-D choline salt, glufosinate + 2,4-D choline salt, and glufosinate alone continued to show severe symptoms of poisoning. However, in the evaluation carried out at 7 DAA, the

Table 2. Average values for plant height (P.H.), insertion height of the first pod (I.H.), number of pods per plant (N.P.), mass of 100 grains (M.G.), and productivity (PROD.) of Enlist E3™ soybeans subjected to the application of herbicides in two doses in the 2017/2018 and 2018/2019 harvests.

Variable	P.H. (cm)	I.H. (cm)	N.P. (Un)	M.G. (g)	PROD. (kg/ha^{-1})
			2017/2018		
Herbicides					
2,4-D choline salt	76.60^{a}	11.13^{ab}	35.31^{b}	19.16^{a}	4764.5^{a}
Glyphosate	77.06^{a}	11.43^{ab}	37.83^{ab}	19.68^{a}	4576.1^{a}
Glyphosate + 2,4-D choline salt	75.71^{a}	11.55^{ab}	37.01^{b}	19.29^{a}	4664.2^{a}
Glufosinate	79.72^{a}	10.60^{b}	42.75^{a}	18.46^{a}	4651.4^{a}
Glufosinate + 2,4-D choline salt	78.68^{a}	12.18^{a}	39.37^{ab}	18.73^{a}	4800.1^{a}
Doses					
D1	78.98^{a}	11.15^{a}	38.66^{a}	19.31^{a}	4794.6^{a}
$D2_{(2x)}$	76.13^{b}	11.60^{a}	38.25^{a}	18.81^{a}	4588.0^{b}
Test	78.7	11.16	36.7	19.05	4894.3
Test × Fat	0.35^{NS}	0.15^{NS}	0.88^{NS}	0.00^{NS}	1.56^{NS}
F(herb)	1.56^{NS}	2.36^{NS}	4.97**	1.36^{NS}	0.68 ^{NS}
F(doses)	6.02*	1.77^{NS}	0.14^{NS}	1.84^{NS}	4.43 *
F(h × d)	0.77^{NS}	0.99^{NS}	1.31^{NS}	0.62^{NS}	0.60^{NS}
CV(%)	4.73	9.4	9.31	6.1	6.58
			2018/2019		
Herbicides					
2,4-D choline salt	86.15^{a}	11.45^{a}	67.02^{a}	20.47^{a}	3898.5^{a}
Glyphosate	83.19^{a}	11.65^{a}	64.37^{a}	19.99^{a}	3880.6^{a}
Glyphosate + 2,4-D choline salt	84.63^{a}	11.65^{a}	68.75^{a}	18.58^{a}	3815.8^{a}
Glufosinate	84.18^{a}	11.25^{a}	65.13^{a}	19.53^{a}	4016.1^{a}
Glufosinate + 2,4-D choline salt	83.42^{a}	10.12^{a}	70.88^{a}	19.09^{a}	3877.5^{a}
Doses					
D1	86.16^{a}	10.68^{a}	68.88^{a}	19.40^{a}	4065.3^{a}
$D2_{(2x)}$	82.47^{b}	11.77^{a}	65.58^{a}	19.40^{a}	3730.1^{b}
Test	89.17	12.27	72.05	19.59	4277.7
Test × Fat	3.69^{NS}	1.10^{NS}	0.91^{NS}	0.01 ^{NS}	7.19 *
F(herb)	0.48^{NS}	0.89^{NS}	0.61^{NS}	2.18 ^{NS}	0.59 ^{NS}
F(doses)	5.87*	3.25^{NS}	0.17^{NS}	0.34^{NS}	15.40**
F(h × d)	0.99^{NS}	0.48^{NS}	0.27^{NS}	0.74^{NS}	1.53^{NS}
CV(%)	5.68	16.89	14.23	7.26	6.86

Means followed by the same letter in the column do not differ by the Tukey test at 5% probability. * and **significant at 5 and 1% probability by the F test; NS: not significant.

scores were close to 1, showing recovery from symptom. Throughout the evaluations, the soybean plants showed an excellent ability to recover from the symptoms caused by the herbicides, which did not interfere with the development and productivity characteristics (Tables 2, 3, and 4).

Silva et al. (2021) found that Enlist E3 soybean plants showed a maximum of 3% phytotoxicity after applying 2,4-D choline, glyphosate, and glufosinate at the V4 stage. There were no differences compared to the control. Schryver et al. (2017) also assessed damage to the Enlist E3 soybean crop of almost 2% following the application of 2,4-D choline/glyphosate (838.5/881.5 g a.e. ha^{-1}), alone or in combination with pre-emergence herbicides. Robinson et al. (2015) assessed 3% crop damage in DAS68416 soybeans (transformed aad-12) after applying 2,4-D dimethylamine in different chemical management programs.

Regarding the variables analyzed in the soybean plants in the first crop year (2017/2018), there was no significant

Table 3. Average phytotoxicity scores of Enlist E3™ soybeans subjected to herbicide application at the V3 stage in the 2017/2018 and 2018/2019 harvests.

Variable	1 DAA	3 DAA	7 DAA	14 DAA
		2017/2018		
Herbicides				
2,4-D choline salt	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{c}
Glyphosate	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{c}
Glyphosate + 2,4-D choline salt	1.37^{ab}	2.50^{a}	2.62^{a}	2.75^{a}
Glufosinate	1.25^{ab}	2. 12^{a}	2.37^{a}	1.87^{b}
Glufosinate + 2,4-D choline salt	1.50^{a}	2.50^{a}	2.62^{a}	2.12^{b}
Doses				
D1	1.05^{b}	1.55^{b}	1.55^{b}	1.45^{b}
$D2_{(2x)}$	1.40^{a}	2.10^{a}	2.30^{a}	2.05^{a}
Test	1	1	1	1
Test × Fat	2.53^{NS}	18.56**	22.82**	16.67**
F(Herb)	5.50**	35.44**	42.44**	37.18**
F(Doses)	16.84**	22.69**	41.25**	29.33**
F(H × D)	6.53**	6.75**	8.71**	6.42**
CV (%)	22.38	20.86	20.05	20.83
		2018/2019		
Herbicides				
2,4-D choline salt	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}
Glyphosate	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}
Glyphosate + 2,4-D choline salt	3.25^{a}	3.25^{a}	2.75^{a}	1.50^{a}
Glufosinate	1.50^{b}	1.25^{b}	1.37^{b}	1.00^{b}
Glufosinate + 2,4-D choline salt	3.25^{a}	3.00^{a}	2.50^{a}	1.62^{a}
Doses				
D1	1.75^{b}	1.65^{b}	1.65^{a}	1.35^{a}
$D2_{(2x)}$	2.25^{a}	2.15^{a}	1.80^{a}	1.10^{b}
Test	1	1	1	1
Test × Fat	16.55**	14.84**	13.28**	1.79^{NS}
F(Herb)	48.93**	51.14**	39.25**	7.52**
F(Doses)	11.38**	12.70**	1.5^{NS}	6.07*
F(H × D)	7.97**	5.04**	0.69^{NS}	2.43^{NS}
CV (%)	24.55	24.5	22.86	26.64

Means followed by the same lowercase letter in the columns do not differ by the Tukey test at 5%. * and ** significant at 5 and 1%; NS: not significant; DAA: days after application.

effect of the interaction between the herbicides and doses or between these and the control. Except for productivity in the second crop year, when the factorial differed from the control. The herbicide treatments were applied sequentially at the V3 and V6 stages of the soybean crop. However, at the V6 stage, the phytophytotoxicity scores were lower, not causing much damage to the plants and allowing them to recover in a shorter period compared to the V3 stage, which is why we did not include the data from the phytophytotoxicity assessments carried out at the V6 stage.

For the insertion height of the first pod, number of pods, and mass of 100 grains, in the two crop years, there was no significant effect on the insertion height of the first pod, the number of pods per plant, and mass of 100 grains, nor was there a significant effect of all the herbicide combinations tested and double the doses. Significant differences were observed for plant height and productivity, with double the dose having a negative effect when these data were compared with the treatments with recommended doses (Table 2). When analyzing the effects of the herbicides on the plants, this

Table 4. Breakdown of the interaction between the effects of herbicides and doses for the phytotoxicity score in Enlist E3™ soybeans at stage V3, 1, 3, 7, and 14 DAA in the 2017/2018 and 2018/2019 harvests.

Herbicide	2017/2018					
	1 DAA			3 DAA		
	Doses			Doses		
	D1	D2$_{(2x)}$	F	D1	D2$_{(2x)}$	F
2,4-D choline salt	1.00^{Aa}	1.00^{Ca}	-	1.00^{Ba}	1.00^{Ca}	-
Glyphosate	1.00^{Aa}	1.00^{Ca}	-	1.00^{Ba}	1.00^{Ca}	-
Glyphosate + 2,4-D choline salt	1.00^{Ab}	1.75^{Aba}	15.47**	1.75^{Ab}	3.25^{Aa}	33.75**
Glufosinate	1.25^{Aa}	1.25^{BCa}	-	2.00^{Aa}	2.25^{Ba}	0.94^{NS}
Glufosinate + 2,4-D choline salt	1.00^{Ab}	2.00^{Aa}	27.50**	2.00^{Ab}	3.00^{Aa}	15.00**
F	0.69^{NS}	11.34**		7.88**	34.81**	
	7 D^{AA}			14 D^{AA}		
	D1	D2$_{(2x)}$	F	D1	D2$_{(2x)}$	F
2,4-D choline salt	1.00^{Ba}	1.00^{Ba}	-	1.00^{Ba}	1.00^{Ca}	-
Glyphosate	1.00^{Ba}	1.00^{Ba}	-	1.00^{Ba}	1.00^{Ca}	-
Glyphosate + 2,4-D choline salt	1.75^{ABb}	3.50^{Aa}	44.92**	2.00^{Ab}	3.50^{Aa}	36.67**
Glufosinate	2.00^{Ab}	2.75^{Aa}	8.25**	1.50^{ABb}	2.25^{Ba}	9.17**
Glufosinate + 2,4-D choline salt	2.00^{Ab}	3.25^{Aa}	22.92**	1.75^{Ab}	2.50^{Ba}	9.17**
F	7.70**	43.45**		6.52**	37.07**	
	2018/2019					
	1 D^{AA}			3 D^{AA}		
	Doses			Doses		
	D1	D2$_{(2x)}$	F	D1	D2$_{(2x)}$	F
2,4-D choline salt	1.00^{Ba}	1.00^{Ba}	-	1.00^{Ba}	1.00^{Ba}	-
Glyphosate	1.00^{Ba}	1.00^{Ba}	-	1.00^{Ba}	1.00^{Ba}	-
Glyphosate + 2,4-D choline salt	2.50^{Ab}	4.00^{Aa}	20.48**	2.50^{Ab}	4.00^{Aa}	22.67**
Glufosinate	1.75^{Aba}	1.25^{Ba}	2.28 NS	1.25^{Ba}	1.25^{Ba}	-
Glufosinate + 2,4-D choline salt	2.50^{Ab}	4.00^{Aa}	20.48**	2.50^{Ab}	3.50^{Aa}	10.08**
F	10.24**	46.66**		12.34**	43.83**	

study found that the insertion height of the first pod was significantly reduced with the treatment of the herbicide glufosinate in the 2017/2018 harvest. For the other treatments, the insertion height gradually increased, followed by 2,4-D choline salt, glyphosate, and the combinations of glyphosate + 2,4-D choline salt and glufosinate + 2,4-D choline salt, respectively, which can thus directly influence the height of the cutting base of the mechanized harvester. For the 2018/2019 harvest, there were no statistical differences between the herbicide treatments regarding pod insertion height.

For these variables, we must consider the effect related to cultivars, which completely influence the agronomic characteristics of soybeans, as observed by Soares et al. (2015) and Felisberto et al. (2015). They attribute these variations to differences in genetic potential, growth habit, and other intrinsic characteristics of each cultivar. The application of 2,4-D choline salt and some of its combinations with other herbicides significantly affected the number of pods per plant in the 2017/2018 harvest. The treatment with 2,4-D choline salt had the lowest number of pods per plant compared to the other treatments and the control. For the 2018/2019 harvest, the number of pods per plant showed no statistical differences between the treatments. Using twice the dose had a negative effect on the plant height variable in both the 2017/2018 and 2018/2019 harvests.

The results were similar for the two crop years (2017/2018 and 2018/2019), in which all the variables analyzed showed similar results, with significant differences only for plant height and yield, which was considerably reduced compared to the previous production year. It is worth noting that glyphosate application is recommended for RR soybeans up to the R1 stage (Rodrigues and Almeida, 2018). Meanwhile, for Enlist E3 soybeans, glyphosate application can be carried out up to the R2 stage (Full bloom and one open flower) (Chahal et al., 2015). For the mass of 100 grains

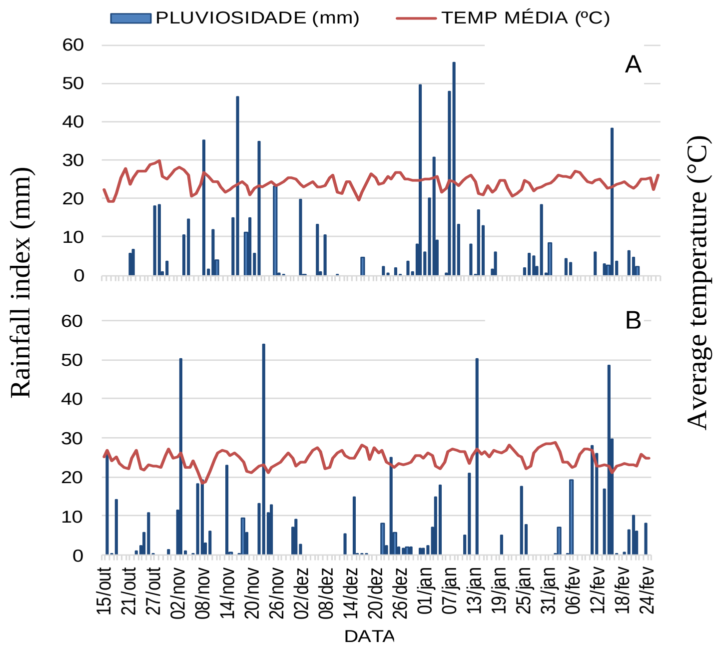

Figure 1. Rainfall and average temperature during the 2017/2018 (A) and 2018/2019 (B) soybean harvests.

in both the 2017/2018 and 2018/2019 harvests, there were no significant differences between the factors, regardless of the different herbicide treatments and their combinations (Table 2).

In an experiment by Miller and Norsworthy (2016), there were no yield reductions in Enlist E3 soybeans after applying 2,4-D choline, glyphosate, and glufosinate in different management programs. Frene et al. (2018) found a yield reduction of up to 23% in Enlist E3 soybeans with the application of glyphosate (1440 – 2280 g a.e. ha^{-1}) + 2,4-D choline (1440 – 2280 g a.e. ha^{-1}) in V3, while there were no yield reductions for symptoms that did not exceed 5%.

Kalsing et al. (2018) identified the tolerance of DAS-44406-6 and DAS44406-6 × DAS-81419-2 soybeans to the application of 2,4-D choline/glyphosate (1950/2050 g a.e. ha^{-1}) at the V3, V6, and R2 stages. Up to 13% phytotoxicity was observed at 7 DAA for the V6 stage, with no reduction in yield. When analyzing the yield of Enlist soybeans in ton/ha, this study found no significant difference between the treatments used when compared to the control, nor was there any interaction between the herbicide and dose factors, except when twice the recommended dose of the herbicides was used, which caused a decrease in yield. Thus, the crop is resistant to the application of the herbicides used when the recommended dose is used. It was possible to observe a drop in the productivity of the soybean crop in the second year of the experiment, which we can directly relate to the drought that occurred in the experiment region from October to February.

Water deficit is likely to occur throughout crop growth, and it has a major impact on productivity (Sinclair et al., 2007). From December to February, the crop was predominantly in the flowering and grain-filling stages (Figure 1), which are recognized as the periods most sensitive to water deficit and also when the crop normally consumes the most water (Berlato and Fontana, 1999; Matzenauer et al., 1998). Water deficit affects yield components differently depending on the period in which it occurs; when it occurs during the beginning of flowering, the number of pods per plant decreases. If stress occurs after flowering, grain filling and size may be lower, significantly reducing grain yield and its components (Oya et al., 2004).

According to the rainfall index data (Figure 1), the rainfall in 18/19 was lower, and there were also more consecutive days without rain during the flowering/grain-filling period compared to the previous harvest. A water deficit during the subperiod from the beginning of grain filling to the green grain stage can drastically reduce soybean yields, as almost half of the nutrients needed for grain formation come from the soil and biological nitrogen fixation (Hirakuri, 2010; Neumaier and Bonatto, 2000).

Conclusion

Enlist E3™ soybeans were not affected by the application of the herbicides 2,4-D choline salt, glyphosate, glyphosate + 2,4-D choline salt, glufosinate, and glufosinate + 2,4-D choline salt in most of their characteristics (especially productivity) when the recommended doses for the crop were used. Therefore, Enlist E3™ soybeans are tolerant to the herbicides used, even when applied the recommended dose. However, doubling the dose affects the productivity variable.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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